

# Anti-Microbial Potential of Panacea (Nigella Sativa)

Atika Liaquat, Liaquat Ali, Muhammad Awais Khalid, Faiqa Liaquat, Faiza Liaquat

## ABSTRACT

**Background:** In the existing scenario, increasing drug cost, toxic effects and drug resistance or the main motivating factors for researchers to exploit the natural bioactive sources for safe and natural therapeutic as an alternative to antibiotics. **Objectives:** The main objective of the study is to find out the antimicrobial potential, its spectrum and magnitude in N.sativa. **Study Design:** Experimental/in vitro study. **Duration:** September, 2016 – February, 2017. **Settings:** Different departments (of School of Pharmacy, Pathology and Bio-chemistry of UM & DC), The University of Faisalabad. **Methodology:** Antibiotics assay is performed by using the disc diffusion methods. N.sativa extracts are prepared and from these sensitivity discs are prepared. Standard size inoculum is also prepared. Then by using standard disc diffusion method the zones of inhibitions produced by N.sativa extracts are measured and recorded in mm and is compared with positive and negative controls. **Results:** Spice extract tested against test organisms revealed antimicrobial potency with diameter of zone of inhibition ( $27.17 \pm 0.98\text{mm}$  -  $31.33 \pm 0.21\text{mm}$ ) while positive control manifested zone of inhibition ( $19.33 \pm 0.21$  -  $29.17 \pm 0.17\text{mm}$ ). Ethanol extracts prepared by Soxhlet apparatus showed better results compared with extracts prepared by simple maceration process. Furthermore, overall ethanol extracts showed better results compared with the aqueous extracts. **Conclusion:** This study revealed the presence of natural bioactive compound(s) in N.sativa with highly significant broad spectrum antibacterial potential, even against multi resistant pathogens.

**Keywords:** Antimicrobial potential, antibiotic discs, Ciprofloxacin, Multi drug resistant (MDR) pathogens.

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## INTRODUCTION

The spread of multi-drugs resistant pathogens is emerging threatening problem worldwide.<sup>1</sup> It is estimated that about 70% of bacteria that caused ailments in hospitals, become insensitive to at least one of the drugs currently prescribed for treatment.<sup>2</sup> Continuous spread of multi drug resistance pathogens, and increasing side effects of current allopathic medicine, the scenario has paved the way for new and reemerging infectious diseases worldwide.<sup>3</sup> So, to address the problems, there is an urgent need to put attentions to natural sources (medicine plant, fruits and spices etc.), for cost effective safe and natural therapeutic agents as alternative to antibiotics.<sup>4</sup> Use of herbal medicine has amplified dramatically for various disease because natural remedies are supposed to have much less toxic effects as compared to synthetic medicine.<sup>5</sup> N.sativa in old Latin is called Panacea, meaning cure for all. where as in Islamic culture, these black seeds have been rated as cure for all diseases except death (Hadith). Black seeds are identified as the curative black cumin in the Holy Bible and also, the famous book of medicine by Ibne- Sina, "The Cannon of Medicine" (980-1037) revealed the historical importance of these black seeds. It has been used in traditional medicine for different ailments in Sub Continent. Currently therapeutic potentials of N.sativa are being actively probed. It has memory tonic<sup>5</sup> property, anti-inflammatory,<sup>6</sup> and hepato-protective effects.<sup>7</sup> Its seeds are evaluated to antimicrobial potential for these most common problematic pathogens, one from gram positive and other from

gram negative bacteria, are selected as test organisms. Salmonella typhi: Gram negative bacterium responsible for typhoid fever; which is still on rise, especially in high risk areas of the world.<sup>8,9</sup> Staphylococcus aureus: Gram positive bacterium is major pathogen for human that is rapidly acquiring multi drug resistance and can cause suppuration in any organ/system.<sup>10</sup>

**Objectives:** To investigate the anti-microbial potential of N.sativa that may lend more weight to general acceptability of plant products for therapeutic use in general and that may provide preliminary information(s) for further development of more potent broad spectrum antibiotics effective against multi resistant pathogens, particularly against salmonella (responsible for deadly typhoid fever), which is sensitive only to very few standard antibiotics presently.

## METHODOLOGY

**Study Design:** Experimental/in vitro study.

**Place of Study:** Different departments (of School of Pharmacy, Pathology and Bio-chemistry of UM & DC), The University of Faisalabad.

**Duration of Study:** September, 2016 – February, 2017.

**Methods:** Antibiotic assay is performed by using the disc diffusion methods.

**Extract Preparation:** the spice was grinded to make fine powder. Fine powder of spice, N.sativa, was taken. 30gram of spice powder was mixed with 100ml of ethanol and 100ml of

water separately. Extracts were prepared with the help of Soxhlet apparatus as well as simple maceration process. Then 10µl of each extract was used for anti-bio gram.

**Table 1: Botanical information of the spices used**

Sr . #	Botanica l Name of Plant	Commo n Name (English)	Commo n Name (Urdu)	Family	Part Used.
1.	Nigella sativa L	black cumin	Kalonji	Ranunculacea e	Seed s

**Inoculum Preparation:** The test organism of each strain was sub cultured on nutrient agar medium (by incubating at 37 degree overnight), and from this fresh culture, standard size (10<sup>8</sup>to10<sup>9</sup> CFU per ml) inoculum is prepared and used.

**Preparation of Disc:** What Mann No. 1.6mm filtered paper antibiotic (extract) discs were prepared and sterilized by autoclaving.

**Inoculum and Testing:** Antimicrobial activity of the extracts was tested using the disc diffusion method. 10µl of each extract was impregnated into empty sterilized antibiotic disc. Each Muller Hinton agar plate was inoculated with the standard inoculum suspension by soaking a swab and rotating it over the agar plate. The paper antibiotic discs were placed over the inoculated agar. After 24 hours of incubation at 37°C, zones of an inhibition were measured and recorded.

Ciprofloxacin 5µg of composed disc was used as positive control and Ethanol & Distilled water were used as negative control are used as reference drugs.

**Antimicrobial Bioassay:** Antimicrobial activities of different extracts were determined by Agar Diffusion method.<sup>11</sup> For this, nutrient agar was used as culture media, Cotton swabs were dipped in the standard size inoculum and were swabbed on the solidified media surface. Discs were placed aseptically over the standard size inoculum on the nutrient agar plates along with positive and negative controls and incubated at 37°C for 24 hours. All treated petri plates were immediately placed in incubator at 37°C. Sterile, blank paper discs impregnated with only sterile water and ethanol were used as negative control each time. Standard Ciprofloxacin was used as positive control for comparison of antibacterial activity. After 24 hours incubation, all the plates having discs for alcoholic extracts and water extracts were observed for their zone of inhibition Fig 3.3-3.5. The zone of inhibition around the discs were measured (in mm) by venire caliper. The data of zone of inhibition of spice extract, having two types of solvent extracts (water and ethanol) and a control against two bacterial pathogens was recorded with six times repeats to confirm the reproducible results of plant extracts. Since all the observations in negative control were zero, therefore data in negative control was not used for statistical analysis.

## RESULTS

It was observed that Nigella sativa revealed more antimicrobial activity (29.25±0.79mm) than ciprofloxacin (24.25±1.49mm).

Nigella sativa was more effective against Salmonella typhi (31.33±0.21mm) than Staphylococcus aureus (27.17±0.98mm). Alcoholic extracts revealed more antibacterial activity (31.33±0.21mm) than water extracts (17.17±0.53mm). Extract prepared by Soxhlet method revealed more antimicrobial activity (29.25±0.79mm) than the extracts prepared by maceration method (26.67±0.92mm).

**Table 2: Analysis of variance table**

Source	DF	SS	MS	F	P
Organism (O)	1	378.13	378.13	311.50**	0.000
Treatment (T)	1	62.35	62.35	51.36**	0.000
Extract (E)	2	822.69	411.35	338.87**	0.000
O x T	1	3.13	3.13	2.57 <sup>NS</sup>	0.114
O x E	2	57.25	28.62	23.58**	0.000
T x E	2	239.19	119.60	98.52**	0.000
O x T x E	2	56.08	28.04	23.10**	0.000
Error	60	72.83	1.21		
Total	71	1691.65			

NS = Non-significant (P>0.05); \* = Significant (P<0.05); \*\* = Highly significant (P<0.01)

**Table 3: Comparison of Antimicrobial activity (in mm) of Alcoholic crude extract (by Soxhlet extraction) of Nigella sativa spice with ciprofloxacin (standard drug) against test organisms**

Organism	Treatment		Mean
	Plant extract(P)	Ciprofloxacin	
S. aureus	27.17±0.98c	19.33±0.21d	23.25±1.27B
S. typhi	31.33±0.21a	29.17±0.17b	30.25±0.35A
Mean	29.25±0.79A	24.25±1.49B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Table 4: Comparison of Antimicrobial activity (in mm) of Alcoholic crude extract (by maceration extraction) of Nigella sativa spice with ciprofloxacin (standard drug) against test organisms**

Organism	Treatment		Mean
	Plant extract	Ciprofloxacin	
S. aureus	24.00±0.45b	21.50±0.22c	22.75±0.45B
S. typhi	29.33±0.80a	24.17±0.17b	26.75±0.87A
Mean	26.67±0.92A	22.83±0.42B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Table 5: Comparison of Antimicrobial activity (in mm) of aqueous crude extract (by maceration extraction) of *Nigella sativa* spice with ciprofloxacin (standard drug) against test organisms**

Organism	Treatment		Mean
	Plant extract	Ciprofloxacin	
<i>S. aureus</i>	15.67±0.42	19.17±0.17	17.42±0.57B
<i>S. typhi</i>	18.67±0.42	21.67±0.21	20.17±0.51A
Mean	17.17±0.53B	20.42±0.40A	

**Table 6: Overall Comparison of Antimicrobial activity (in mm) of Ethanolic extract (by Soxhlet), Ethanolic extract (by maceration) & water extract (by maceration) of *Nigella sativa* spice with ciprofloxacin (standard drug)**

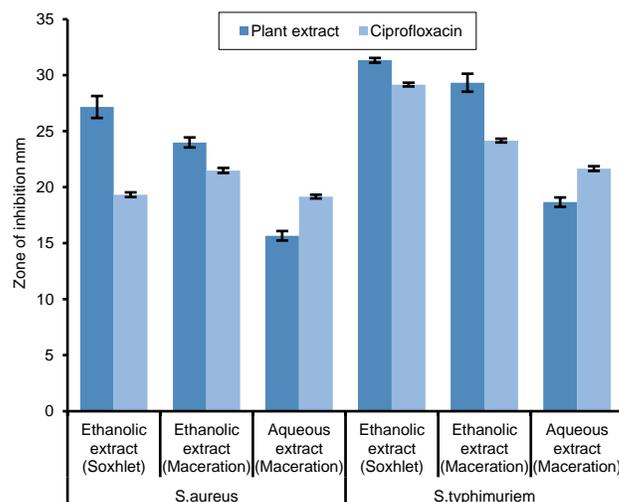
Extract	Treatment		Mean
	Plant extract	Ciprofloxacin	
Ethanolic extract (Soxhlet)	29.25±0.79 a	24.25±1.49 c	26.75±0.97 A
Ethanolic extract (Maceration)	26.67±0.92 b	22.83±0.42 d	24.75±0.63 B
Aqueous extract (Maceration)	17.17±0.53 f	20.42±0.40 e	18.79±0.47 C

Means sharing similar letter in a row or in a column are statistically non-significant ( $P>0.05$ ). Small letters represent comparison among interaction means and capital letters are used for overall mean

**Table 7: Overall Comparison of Antimicrobial activity (in mm) of Ethanolic extract (by Soxhlet), Ethanolic extract (by maceration) & water extract (by maceration) of *Nigella sativa* spice with ciprofloxacin (standard drug) against test organisms**

Organism	Extract	Treatment		Mean
		Plant extract	Ciprofloxacin	
<i>S. aureus</i>	Ethanolic (Soxhlet)	27.17±0.9 8c	19.33±0.2 1f	23.25±1.27C
	Ethanolic (Maceration)	24.00±0.4 5d	21.50±0.2 2e	22.75±0.45C
	Aqueous (Maceration)	15.67±0.4 2g	19.17±0.1 7f	17.42±0.57E
<i>S. typhimurium</i>	Ethanolic (Soxhlet)	31.33±0.2 1a	29.17±0.1 7b	30.25±0.35A
	Ethanolic (Maceration)	29.33±0.8 0b	24.17±0.1 7d	26.75±0.87B
	Aqueous (Maceration)	18.67±0.4 2f	21.67±0.2 1e	20.17±0.51D

Means sharing similar letter in a row or in a column are statistically non-significant ( $P>0.05$ ). Small letters represent comparison among interaction means and capital letters are used for overall mean.



**Figure 1: Overall Comparison of Antimicrobial activity (in mm) of Ethanolic extract (by Soxhlet), Ethanolic extract (by maceration) & water extract (by maceration) of *Nigella sativa* spice with ciprofloxacin (standard drug) against test organisms**

**Ciprofloxacin** (reference drug) showed zone of inhibition ranging from 19.17±0.17mm (*S. aureus*) to 29.17±0.17mm (*S. typhi*).

**Plant extracts:** All extracts manifested variation but show significant degree of activity against two tested bacteria.

**Solvents:** Among the solvent's ethanol showed better extraction power than distilled water.

**Methods:** Soxhlet method proved more effective.

**Bacteria:** Both bacteria tested proved susceptible to the plant extracts evaluated. Among these tested organisms, *S. typhi* showed highest zone of inhibition 31.33±0.21mm.

## DISCUSSION

The important findings of present study are: All spice extracts showed significant antimicrobial activity against both clinically important pathogens. Magnitude of antimicrobial activity of different extracts varied significantly according to the solvent used, as well as method of extraction applied. Highly significant finding was, that spice extract exhibited better antimicrobial activity than the Ciprofloxacin (positive control).

Extensive survey of literature revealed that *Nigella sativa* is highly regarded as universal panacea in the herbal medicine with diverse pharmacological activities spectrum. In present study *N. sativa* revealed highest antimicrobial activity against both G negative and G positive organisms, which was even better than (the reference drug) Ciprofloxacin. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible for various activities of the plant. Hence extensive investigation/ research is needed to exploit their therapeutic utilities to combat diseases. A drug development program should be undertaken to develop modern drugs with the compounds isolated from *N. sativa*. Although crude extracts from the seeds of this plant have medicinal

applications/uses from time immemorial, modern medicine/drug can be developed after proper/intensive research work of its bioactivity, mechanism of action pharmaco-therapeutics and toxicity after proper standardization and clinical trials. Recently, crude extracts of *N. sativa* have revealed a remarkable plasmid curing activity,<sup>12</sup> and this may prove it to be a real “Canon of Medicine” against spread of antibiotic resistance plasmid in the eco system. *N. sativa* imbuing a tremendous potential deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its various medicinal uses. Further, extensive multidimensional research work is needed to explore the concealed areas and their practical clinical applications/uses, which can be exploited for welfare of the mankind.

Soxhlet method proved to be better because different conditioning factors (temperature, pressure and pH etc.) can properly/effectively be controlled by this apparatus.<sup>13</sup>

But in other studies, different extracting solvents proved to be more effective for different plants. Methanol proved to be a better extractor for *Psidium guajava*, *nigella sativa*, *citrus sinensis*, *valerian jatamansi* and *cucurbita papa*.<sup>14</sup> Other extractors; Chloroform for *Foeniculum vulgare* Mill,<sup>15</sup> distilled water for *Law suniainternis* Limm,<sup>16</sup> and petroleum ether for *Trigonella foenum- graecum*<sup>17</sup> proved more effective than other solvents. Differential extraction ability/power of solvents may be because of different solubility of different active principles of different plants in different solvents. So, it appears to be related with differential solubility of active principles of plants for different solvents. It is thus concluded that in preparation of extracts, the solvents used are very important factor. So, different solvents should be probed to find out the best solvent for particular plant.

Generally a plant is consider to be active/effective against pathogenic microbes when the zone of inhibition is greater than 6mm.<sup>18</sup> Indicated that spice (*N.sativa*) extracts tested, significantly inhibited the growth of two tested pathogen bacteria at varying degrees. The maximum zone of inhibition ( $31.33\pm 0.21$ ) was found with ethanol extract of spice (against *S.typhi*) and the minimum zone of inhibition ( $15.67\pm 0.42$ ) was found with aqueous extract of spice (against *S.aureous*). Results obtained from in vitro antimicrobial activity showed that all plant extracts exhibited a substantial / significant inhibitory effect against two tested bacteria. Ethanol extracts of plant extract from soxhlet method proved superior in suppressing the bacterial growth. This was followed by water extracts. Ethanol extracts exhibited promising antibacterial activity against two tested bacteria (both gram negative and positive), may be due to presence of phenols and flavonoids (active principles/ingredients) which are better extracted with ethanol.<sup>19</sup> The lesser activity seen with other solvent (water) may be due to low solubility of active constituents/principles in this solvent.<sup>19</sup> Varying degrees of antimicrobial activities may be due to different;

(i) active principle(s) within the different plant (spice) extracts

- (ii) solubility of active principles in different solvents
- (iii) conditioning factors (temperature, pressure and pH etc.) during processing

In present study, plant extracts revealed significant antimicrobial activity against both Gram negative and Gram-positive microbes, proving their broad-spectrum potential. Studies all over the world are acknowledging the different properties/activities of medicinal plants, and it seems that very soon the Plant Kingdom is going to be the Veritable Universal Natural Source of all type of desired drugs. Regarding the susceptibility; *S.typhi* proved to be most sensitive organism to reference drug and also against the plant extract compared to the *Staphylococcus aureus*.

### Academic Value of study

This study provides;

- i. Scientific prove/base to traditional healer's claim.
- ii. Bases for a full-scale investigation of the therapeutic potential of this medicinal plant.

This may help to establish parameters for further development of more effective broad-spectrum antimicrobial (against gram negative as well as Gram positive) agents, more potent antimicrobial agent against multi drug resistance strains of microbes and other pathogens with higher resistance, and also more effective and safer anti-microbial for deadly typhoid fever, against which limited anti-biotic are available at present.

### CONCLUSION

Present study indicated the presence of natural bioactive compounds in *N.sativa*, with highly significant broad spectrum antimicrobial (including *S. typhi*) potential.

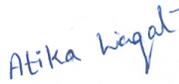
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