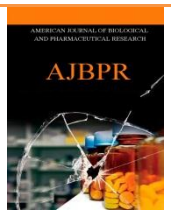
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



DETERMINATION OF ANTIBACTERIAL ACTIVITY OF NIGELLA SATIVA SEED EXTRACT AGAINST SOME HUMAN PATHOGENIC BACTERIA

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Article Info

Received 29/07/2016
Revised 16/08/2016
Accepted 19/08/2016

Key words: -
Antimicrobial activity; disk diffusion method; methanol extract; Nigella sativa; pathogenic bacteria.

ABSTRACT

Nigella Sativa (NS) seeds have been used for medicinal purposes for centuries both as herbs and its seed oil. In Islam it is regarded as one of the greatest forms of healing medicine included in the medicine of Prophet Mohammed (SAW). Pathogenic bacterial infections have become a major health problem worldwide. New antimicrobial agents are urgently needed to overcome this problem. In this study, antibacterial activity of *Nigella sativa* seed extract against some pathogenic bacterial strains gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter* was evaluated. This study show that methanolic fruits extract of Nigella sativa Linn inhibits the growth of micro organism dose dependently. A significant correlation was observed between zone of inhibition and concentration of extract. These results confirm the antibacterial activity of *Nigella sativa* seed and support the traditional use of the plant in therapy of bacterial infection. These promising findings suggest the presence of antibacterial activity in the tested plant material, exhibited by its bioactive compounds, and serving them as an alternative antimicrobial agent.

INTRODUCTION

The use of synthetic drugs containing microbes those are biochemically and genetically modified as a treatment of common infectious disease are not reliable due to many controversial issues. Synthetic drugs are not only expensive and inadequate but also often had issues with adulterations and side effects. Customers are more concerned about the pathogenicity and the high mortality rate of the product they used. Therefore, with the advancement of the technology, scientists are challenged to come out with new ideas of alternative and novel drugs to overcome the usage of microbial resistant drugs. Since ancient civilization, natural sources especially plants are used as medicinal therapy because they contain several components which are believed to cure various infectious diseases. The biodiversity of plants provides an important source of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal and anticancer activities [1]. Nigella sativa is a herbaceous plant which is better known as black seed, a habitat of Southest Asia and Mediterranean countries. Indian folks used this plant as a food preservative as well as a protective and curative treatment for numerous disorders [2]. The black seeds contain 36–38% fixed oil, with proteins, alkaloids, saponins and essential oils making up the rest of the composition [3]. Although black seed extract

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or oil has been reported to possess antimicrobial activity [4], antioxidant activity [3], antitumor activity [5] and a stimulatory effect on the immune system [6], its full potential as an antimicrobial agent has not been exploited. This current study was conducted to investigate the antibacterial activity of the seed extract of *Nigella sativa* against pathogenic isolates of bacteria. The results of this study may further strengthen the recommendation for the use of ethno medicine in the treatment and control of microbial infections.

MATERIALS AND METHOD

Extract preparation: *Nigella sativa* **(**100 g**)** was defatted with petroleum ether (1000 ml) and the residue was extracted in 50% methanol with the help of soxhlet extraction unit. The sample was collected and concentrated in water bath at 40-50^{o}C and dried in hot air oven at 40^{o}C. The dried powder was kept in air tied box.

Microorganism: The test organism included the gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter.* All the bacterial strain was obtained from National Chemical Laboratory (NCL), Pune, india. The bacteria were grown in the nutrient broth at 37^{o}C and maintained on nutrient agar slant at 4^{o}C.

Antibacterial Assay:

Antibacterial test was done by preparing bacterial suspension followed by the disc diffusion test. Antibacterial activity of Nigella sativa extract was determined by agar disk diffusion method at four different concentrations i.e., 100, 75, 50 and 25 mg/ml. Muller Hinton agar was prepared according to the manufactur's instruction and the plates were seeded with appropriate micro organism *(*Gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter).* Discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and sterilized. The discs were than impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (TE- Tetracycline, OF- Ofloxacin, AZ- Azithromucin and PC- Pipracillin) and Gram negative (Fu- Nitrofurantoin, GM- Gentamicine, CX- Cefotaxime and NF- Norfloxaci,5 µl/disc) bacteria were used as standard. The plates were incubated at 37^{o}C for 24 hrs and the zone of inhibition was measured with measuring scale. This experiment was carried out in triplicate for their confirmation.

RESULTS

Antimicrobial Activity

The initiation of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study shows that antibacterial activity of 50 % methanolic extract of Nigella sativa against *B. subtilis* is best in 100 % concentration after 12 hrs. (18.00 ± 1.00 mm zone of inhibition). Although 75% concentration is having mild effect as 14.00 mm zone of inhibition. In *Citrobacter* 100% concentration of extract is having good antibacterial activity at maximum zone of inhibition 16.66 mm. On the other hand 75 % is showing static activity from, with zone of inhibition of 12.00 mm. For E. coli 100% concentration of extract show maximum zone of inhibition 12.66 mm. Although the same effect of 75% concentration of extract is also revealing as showing zone of inhibition 11.33 mm. In the case of *Acetobacter* 75% and 100% concentration of extract show good activity with zone of inhibition of 14.66 mm and 13.33 mm respectively, and *Citrobacter* shows the inhibition 16.66 mm and 12.00 mm for 75% and 100 % concentration respectively.

The above observations suggest that different concentration (50 %, 75 % & 100 %) were having good antibacterial activity against *Acetobacter, Citrobacter, E. coli, B. subtilis, S. aureus and Enterococcus.* Thus the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. But extract is not potent than erythromycin and amoxicillin in these conditions (Table 1 and 2).

Table 1: The study of anti-bacterial activities of standard antibiotics using disk diffusion method

Sl	Name of Bacteria	Zone of Inhibition (In MM)			
1	**Gram Negative (-)**	**TE10**	**OF5**	**AZ15**	**PC5**
	Acetobacter	17.00	15.00	14.00	11.00
	Citrobacter	15.00	17.00	19.00	16.00
	E. coli	12.00	12.00	17.00	12.00
2.	***Gram Positive (+)***	**FU10**	**GM5**	**CX15**	**NF5**
	Bacillus subtilis	11.00	12.00	09.00	14.00
	S. aureus	16.00	14.00	19.00	17.00
	Enterobacter	16.00	16.00	14.00	18.00

Table 2: The study of anti-bacterial activities of *Nigella sativa* extracts using Disk Diffusion method third observation table (Mean ± SE).

Sl	Bacterial Stain	Bacteria Use	Zone of Inhibition (In MM)			
			25%	50%	75%	100%
1	Gram Negative (-)	*Acetobacter*	10.66 ± 0.57	13.00 ± 1.00	13.33 ± 1.52	14.66 ± 0.33
		Citrobacter	09.33 ± 1.34	07.66 ± 1.45	12.00 ± 1.00	16.66 ± 1.66
		E. coli	08.66 ± 1.17	10.33 ± 1.19	11.33 ± 0.66	12.66 ± 0.81
2	Gram Positive (+)	*B. subtilis*	12.00 ± 1.00	12.66 ± 0.97	14.00 ± 1.50	18.00 ± 1.00
		S. aureus	05.00 ± 1.00	04.66 ± 0.33	13.66 ± 0.87	14.66 ± 0.33
		Enterobacter	04.00 ± 0.57	08.00 ± 1.24	05.66 ± 0.66	11.15 ± 2.72

Figure 1. Antibacterial Activity of Standard Antibiotic

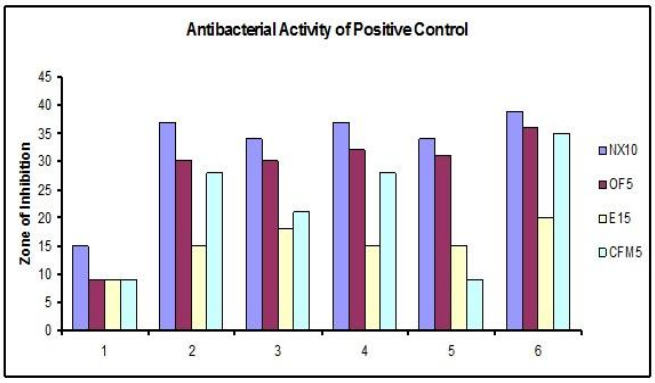


Figure 2. Antibacterial Activity of *Nigella sativa* seed extract using some Gram Negative Bacteria

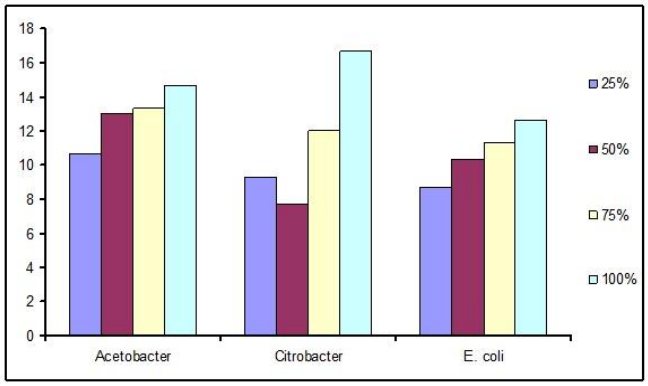
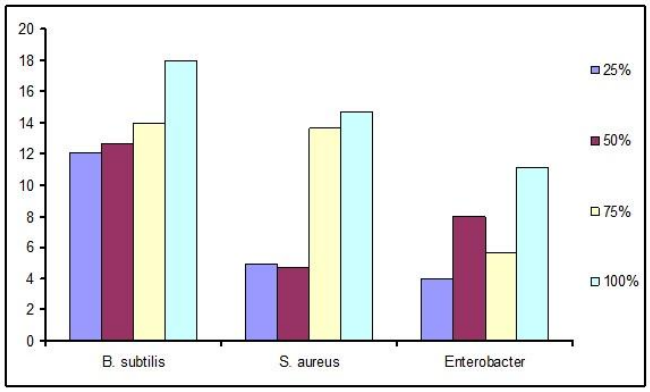


Figure 3. Antibacterial Activity of *Nigella sativa* seed extract using some Gram Positive Bacteria



DISCUSSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc.

The antibacterial study suggest that different concentration (50 %, 75 % & 100 %) of plant extract were having good antibacterial activity against gram negative bacteria; *Acetobactor, Citrobactor and E. coli* and gram positive bacteria; *Bacillus subtilis, S. aureus and Enterobacter*. Thus the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Gram positive (TE- Tetracycline, OF- Ofloxacin, AZ- Azithromucin and PC- Pipracillin) and Gram negative (Fu- Nitrofurantoin, GM- Gentamicine, CX- Cefotaxime and NF- Norfloxaci,5 µl/disc) in these conditions. This finding is interesting, because in the traditional method of treating a microbial infection, decoction of the plant parts or boiling the plant in water was employed. Whereas, according to the present study, preparing an extract with an organic solvent (acetone

and ethanol) shows a better antimicrobial activity [7, 8]. On the basis of the above results, it showed that methanol extract of N. sativa exhibited a greater inhibition compared with aqueous extract. Parekh et al. (2006) reported that most of the antimicrobial active compounds were soluble in polar solvent such as methanol instead of water [9]. This result is comparable to the study by de Souza et al. (2004) using methanol extract of L. sibiricus that showed effective antibacterial activity on Bacillus subtilis [10]. Bajwa and Shafique (2008) showed that methanol fraction of A. rabiei exhibited more promising results in suppressing the fungal growth rather than aqueous extract [11]. This was also reported by Zafar et al. (2002), where chloroform extract of Melia azedarch leaves was active against Fusarium chamdosporum while water extract of the leaves did not show any positive results [12]. By referring to Tables 1 and 2, the extracts were found to be more effective on Gram positive than Gram negative bacteria, which is in conformity with a number of earlier studies where compounds derived from plants often show considerable activity against Gram positive bacteria but not against Gram negative species [13]. Gram negative bacteria have effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphiphatic compounds and multidrug resistance pumps that extrude toxins across this barrier [13]. It is possible that the apparent ineffectiveness of the plant antimicrobial activity is largely due to this permeability barrier. Results of the study indicate that black seed extract showed a dose of dependent inhibition against concentration. This finding is in agreement with results reported by Hannan et al. (2008) using the same genus of plant tested [14].

The antibacterial study was also done which shows a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and shown antibacterial susceptibility to Nigella sativa seed extracts with clear zone of inhibition. So, in future it can be used as an alternate to antibiotics. This work provides an insight to understanding some molecular basis of therapeutic properties of Nigella sativa in traditional medicine. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as in vivo assays will be necessary in discovering new biological antibiotic agent.

CONCLUSION

Plant–derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in them as sources of agents to fight microbial diseases. The development of multiple antibiotic resistance organisms has constituted a global problem as far as treatment of some infectious diseases is concerned. Vehicle of transmission of this etiologic agent are mainly food and water. Many disease-causing organisms of medical importance have developed resistance to antibiotics. The use of plant extracts and phytochemicals both with known and unknown antimicrobial properties can be of great significance in therapeutic treatments. The seed oil of some *Nigella* species has powerful germicidal and antibacterial properties.

It may be concluded from this study that Nigella sativa seed extract exhibits some degree of antibacterial activity towards *Bacillus subtilis* and *Citobactor*. Thus, it shows that Nigella sativa has a great potential as an effective antimicrobial agent for medicinal purposes.

ACKNOWLEDGMENT

The authors are thankful to Prof. M.M. Hambarde, Director General, Chhattisgarh Council of Science and Technology, Raipur (Chhattisgarh) India, for providing facility and technical support to carry out the above work.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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