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Original Research

The inhibition effect of *Nigella sativa* oil extract on the growth of some gram positive and negative bacteria

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ABSTRACT:

In this study, the inhibition activity of *Nigella Sativa* oil extracted Clevenger apparatus by the process the hydro distillation was examined against seven species of pathogenic bacteria *Staphylococcus aureus, Micrococcus* sp*, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Salmonella* sp. Agar well diffusion method was used to estimate the inhibiting activity of the extracted oil. The results of this study revealed that the oil extracts of *Nigella Sativa* is good at inhibiting Grams negative and Gram positive bacteria. The highest inhibition percentage were at 50% concentrate of the oils extracted and showed 33.80, 33.94, 26.61, 25.32, 32.23, 23.32 and 32.37 % against *Staphylococcus aureus, Micrococcus* sp*, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Salmonella* sp.

Keywords:
Nigella sativa, Oil extract, Agar diffusion, Bacterial inhibition.

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INTRODUCTION

Many recent researches were proving that the plants are rich in distinct secondary products were characterized by their biological activity and their Physiological effects as drugs against many diseases, in human and animals (Ahmed *et al*. 2013). Because of the randomly usage of antibiotics for a long time, side effects appear on the human health, in addition to create new resistant strains of pathogens Roberts (2002) the very thinking of alternate treatment was started by using medical plants and popularly is known as Natural Pharmacy.

In popular medicine, many plants were used as drugs to treat different diseases, one of the most important plants was the black grain *Nigella sativa* seeds, which belong to the family Ranunculaceae. It is indigenous to Mediterranean and the most important cultivars were India, Pakistan, Iraq, Iran, America and Egypt**.** *N. sativa* is an annual plant with fine leaves and punctuate fruits with black seeds, the flowers are white and some are blue in color (Ahmad *et al*., 2013; Warrier and Nambiar, 2004). The oil and powder of this grain has many medicinal and useful characters such as stimulant, analeptic, digestant, uretic, milk secretagogue, appetent and worm repellent (Yarnell and Abascal, 2011; Khader and Eckl, 2014). The black grain seeds contained stable (30-38)% and volatile oils (0.4-0.5)% Mehta *et al*. (2009), and many types of medically active compounds such as thymoquinone and it is derivatives (dithymoquinone, thymohydroquinone) which acts as an antimicrobial, in addition to it cymene, carbonyl and limonene were having anti- carcinogenic effect on some types of human cancer (Al-Ali *et al*., 2008; Yarnell and Abascal, 2011). This study was aimed to determine the inhibitory efficiency of black grains oil extracted against a number of pathogenic bacteria included Gram positive and negative bacteria.

MATERIAL AND METHODS

Isolation and identification of bacteria

Isolates of grams positive and negative bacteria were obtained from the Central Health Laboratory of Baghdad. Mostly, these microbes were isolated from the cases of diarrhea, tonsillitis and urinary tract infection, according to Collee *et al*. (1996) and Cruickshnak *et al*. (1975).

Plant oil extraction

Nigella sativa seeds were obtained from the local herb shops at Baghdad. These seeds were powdered by electrical pound and conserved in polyethylene bags until it is used. In order to extract the seed oil, hydrodistillation method by Clevenger apparatus was used according to the methodology followed by Bankole and Joda (2004).

Preparation of oil extracted concentrations

Concentrations of 3.12, 6.25, 12.5, 25, 50, 100% of seed oil were prepared by dissolving 30.20, 60.25 , 125 , 250 , 500 and 0ml of oil respectively with the organic solvent Dimethyl Sulphoxide (DMSO) to reach to one ml solution.

Estimation of inhibitory efficiency of the extracted oil on bacterial growth

Agar well diffusion method was used to determine the inhibitory activity of the oil extracted on the bacterial growth. The solid Muller-Hinton medium was inoculated with the bacterial suspension (1.5x10 cell/ ml) and compared with 0.5 McFarland standrad, by using sterile cotton swap.

Wells were made in the center of the inculcated media by using cork borer, then the prepared concentrations of the extracted oil were placed in the wells at 0.1ml of each concentration for each of the wells, in addition to the control well (only DMSO), the plates were kept at the room temperature for 20 min, then incubated at 37°C for 24h, three replicates were used for each of the bacterial isolate.

The efficiency of the oil extracted was deter-

Table 1. Effect of different concentrations of *N. sativa* oil in the diameter of various colonies (MM)

S. No	Bacterial isolates	Concentration (%) 6.25	12.5	25	50	100	Control	LSD P<0.05
1	*S. aureus*	20.66 ± 0.82	21.66 ± 0.94	22.00 ± 0.76	23.66 ± 0.88	0.0	0.0	2.408 *
2	*Micrococcus* sp	12.00 ± 0.38	17.66 ± 0.69	21.66 ± 0.55	23.66 ± 0.91	0.0	0.0	2.054 *
3	*E. coli*	14.33 ± 0.52	14.66 ± 0.47	15.66 ± 0.47	16.10 ± 0.63	0.0	0.0	2.218 *
4	*P. aeruginosa*	12.33 ± 0.36	14.33 ± 0.38	16.00 ± 0.51	17.66 ± 0.82	0.0	0.0	2.408 *
5	*K. pneumoniae*	17.66 ± 0.71	19.66 ± 0.65	22.00 ± 0.75	22.66 ± 0.71	0.0	0.0	3.703 *
6	*S. marcescens*	12.33 ± 0.42	16.00 ± 0.57	18.33 ± 0.61	19.33 ± 0.85	0.0	0.0	1.966 *
7	*Salmonella* sp	12.33 ± 0.33	17.33 ± 0.71	20.66 ± 0.55	22.66 ± 0.73	0.0	0.0	1.966 *
	LSD p<0.05	2.260 *	3.989 *	2.591 *	2.642 *	0.00 NS	0.00 NS	---

*= Significant differences (P<0.05), N.S.= Non-significant

mined by measuring the diameter of inhibition zone (mm) around the well, the mean of the three replicates were calculated according to the method followed by Stahl (1969) and Vandepitte *et al*. (1991).

Statistical analysis

The Statistical Analysis System (SAS, 2012) program was used to determine the effect of difference factors (bacterial isolates and concentrations) in the study parameters. Least Significant Difference – LSD test was used to significantly compare between means in this study.

RESULTS AND DISCUSSION

The results of this study revealed the existence of inhibitory effect for all concentrations of the extracted oil of black grain seed on the pathogenic bacteria used in this study, except 100% concentration which showed no effects on any type of these bacteria, the reason of this case is due to the heavy oil that persisted in the well and was not diffused on the medium surface.

The results showed significant differences between different concentrations for the same bacterial species, and the inhibitory ability of the extracted oil was increased with increasing concentration. The highest inhibitory effect was with 50% concentration of oil extracted, where the diameters of inhibition zones reached to 23.66mm (Table 1) for each of *Staphylococcus aureus* and *Micrococcus* sp with the

Table 2. Effect of different concentrations of *N. sativa* oil in inhibition percentage of bacterial colonies

S. No	Bacterial isolates	Concentrations 6.25	12.5	25	50	100	Control	LSD P<0.05
1	*S. aureus*	29.52 ± 1.27	30.94 ± 1.38	31.42 ± 1.08	33.80 ± 1.42	0.0	0.0	3.437 *
2	*Micrococcus* sp	17.14 ± 0.64	25.23 ± 1.07	30.94 ± 1.26	33.80 ± 1.28	0.0	0.0	2.935 *
3	*E. coli*	20.54 ± 0.78	20.94 ± 0.86	22.47 ± 0.97	26.61 ± 1.06	0.0	0.0	3.133 *
4	*P. aeruginosa*	17.61 ± 0.72	20.54 ± 0.79	23.60 ± 0.85	25.23 ± 0.94	0.0	0.0	2.442 *
5	*K. pneumoniae*	25.23 ± 1.09	28.93 ± 1.36	31.42 ± 1.19	32.23 ± 1.52	0.0	0.0	5.291 *
6	*S. marcescens*	17.61 ± 0.68	23.60 ± 0.74	26.61 ± 0.97	28.61 ± 1.05	0.0	0.0	3.442 *
7	*Salmonella* sp	17.61 ± 0.75	24.75 ± 0.92	29.52 ± 1.26	32.23 ± 1.25	0.0	0.0	2.808 *
	LSD p<0.05	4.823 *	3.955 *	3.078 *	4.227 *	0.00 NS	0.00 NS	---

*= significant differences (P<0.01), N.S.= Non-significant

inhibition percentage as 33.80 and 33.94% respectively, (Table2). Where as the diameters of the zone of inhibition (*P. aeruginosa, E. coli, K. pneumoniae, Serratia marcescens and Salmonella sp*) were (17.66, 16.10, 22.66, 19.33 and 22.66) mm respectively, (Table 1) and with inhibition percentage (25.23, 26.61, 32.23, 28.61, 23.32 and 32.37)% respectively, (Table 2).

These results were in agreement with the Haloci *et al*. (2012); Gharibi *et al*. (2012); Vishal *et al*. (2012), where the oil of *N. sativa* has inhibitory effect on the Gram positive bacteria was greater than Gram negative bacteria, in addition to its effect on the yeast- *Candida albicans* and molds such as *Aspergillus flavus* and *Penicillium* sp and the lowest effect was on *E. coli*. On the other hand, Al-Sayed *et al*. (2001) proved that the oil of *N. sativa* has similar effect of antibiotics like gentamycine, inrofloxacine and streptomycine against *Staphylococcus* sp and *Listeria*, and using this oil with antibiotics like Amoxicillin and Neomycin lead to the additional inhibitory effect (synergism) against pathogens. The researchers were recommended to use this oil as natural conservative material in the food products to prevent the contamination.

The efficiency of the *N. sativa* oil against the pathogens was due to some active materials such as thymol, thymoquinone, dithymoquinone which are present in it. The mechanism of Thymoquinone differs with varying types of pathogens (Kundu *et al*., 2014; Mouhajer *et al*., 1999), where this compound is involved in blocking the enzyme receptors especially the respiratory enzymes which contains (S-H) group that are substituted with (c=o) group. So, the presence of thymoquinone is limited and is subsequently converted to thymohydroquinone, this reaction also reciprocally (opposite) occurred which lead to increasing of its toxicity on microorganisms, on the other hand, the inhibitory action of thymoquinone may be due to increasing in oxidation – reduction potential of some microorganisms and the poisoning would happened by accumulation inside the cell.

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