

Effect of Black Seed Alkaloids Against some Pathogenic Bacteria

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ABSTRACT

Alkaloids were separated from black seed by using two type of solvents; ethanol and chloroform, we obtained two fractions of alkaloid (A1 and A2). Separated fractions were tested in different concentrations 50, 75, 100, 125 and 150 mg/ml against four types of bacteria

(*Staphylococcus aureus* , *Bacillus cereus*, *E. coli* and *Pseudomonas* spp.). The results showed that both fractions of alkaloid have antibacterial activity against tested bacteria but the effect of A1 was more than A2 and Gram positive bacteria were more sensitive than Gram-negative bacteria, the inhibition zone increased by increasing concentration of alkaloid in all tested bacteria.

Keywords: Alkaloid, Antibacterial, *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, *Pseudomonas* spp.

125 75,100 50

A2

A1

³ / 150

Staphylococcus aureus , *Bacillus cereus*, *E. coli* and *Pseudomonas* spp. -:

A2

A1

INTRODUCTION

Plant extracts and essential oil showed a broad range of pharmacological effects such as anti diabetic (Al-Awadi *et al.*, 1991 ; Farah *et al.*, 2002). The extracts of the plant also showed anti microbial effects (Salomin *et al.*, 1992 ; Chakravaty, 1993). Seeds of *Nigella sativa* L. (*Ranunculaceae*) commonly known as black seed or black cumine, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhea and dyslipidaemia (Ali and Blundea, 2003). Seeds of *Nigella sativa*, have been employed for thousands of years as a spice and food preservative; The oil and seed constituents, in particular thymoquinone, have shown potential medicinal properties in traditional medicine (Salem, 2005). The proteins fractionated from the plant showed immune modulator effect using non-activated or mitogen activated cells (Salim and Fukushima, 2003). The crude and ethanol extract of the seed showed hepatoprotective activity (Worther *et al.*, 1998 ; Daba and Abdel-Rahman, 1998). The addition of black seed to feed of pigeons could act as an immunoprotective agent when chronic administration of antibiotics are considered (Al-Ankari, 2005).

Seed extracts from six species of the genus *Nigella* (Family *Ranunculaceae*)-*Nigella arvensis*, *Nigella damascena*, *Nigella hispanica*, *Nigella nigellastrum*, *Nigella orientalis* and *Nigella sativa* obtained by successive extraction with n-hexane, chloroform, and methanol, tested for their antimicrobial activity against 10 strains of pathogenic bacteria and yeast, anti-inflammatory screening revealed that *N. sativa*, *N. orientalis*, *N. hispanica*, *N. arvensis* n-hexane extracts, and *N. hispanica* chloroform extracts had strong inhibitory activity (Landa *et al.*, 2009). Hanafy and Hatem studied the antimicrobial activity of black seed extract on certain pathogenic Gram positive and Gram negative bacteria, but they noticed that black seed has no effect on *Salmonella typhimurium* (Hanafy and Hatem, 1991). *Nigella sativa* is a herbaceous plant, whose seeds (black seed) have been used as a spice and condiment in foods in the Middle East (Zaha *et al.*, 2008). The antiyeast activity of the black cumin seed *Nigella sativa* quinones dithymoquinone, thymohydroquinone and thymoquinone are active antiyeast agents that could be used in the dairy industry as chemical preservatives (Halamova *et al.*, 2010). *Nigella sativa* oil reduced *Hymenolepis nana* eggs starting from second day of the treatment until necropsy day during 5 days due to its stimulating immune system.(Ayaz *et al.*, 2007). Thymoquinone inhibited 80-100% of the fungal growth which support its use in folk medicine for the treatment of fungal skin infections (Aljabre *et al.*, 2005). The steam –distilled essential oil of Iranian black cumin seed was investigated for its composition and analgesic and anti inflammatory properties after oil analysis, (20) compounds were identified among them para- cymene and thymoquinone has an important role in pharmacological effects (Hajhashemi *et al.*, 2004).

In the current work, the inhibitory effect of alkaloid extracts from *Nigella sativa* L. seeds were studied against the growth of *Staphylococcus aureus*, *Bacillus cereus*, *E. coli* and *Pseudomonas* spp., to demonstrate its antibacterial effects, bearing in mind that natural products may play a future role by replacing or substituting antibiotics that face the great threat of over all resistance.

MATERIALS AND METHODS

Materials

1- Bacterial strains :-

Staphylococcus aureus, *E. coli* and *Pseudomonas* spp. obtained from Hawler teaching hospital laboratory, *Bacillus cereus* isolated from food sample in the college of Science-Salahaddin university. All strains identified by biochemical tests (Barrow and Feltham, 1993).

2- Nutrient agar and Nutrient broth.

3- Pepton water 0.1% .

4- black seed (From local market).

5- Ethanol, chloroform, silica gel 60.

Methods

Isolation of alkaloids

Eighty gram of *Nigella sativa* seed were ground and defatted with petroleum ether extracted with ethanol 96% using soxhlet apparatus, the extract then evaporated by rotary evaporator.

The ethanol extract was acidified with 2% hydrochloric acid, filtered, made basic with concentrated ammonium hydroxide and extracted with chloroform. The chloroform extract were evaporated by rotary evaporator to afford a total alkaloid. Total alkaloid were chromatographed on an open column packed with silica gel 60. Elution with chloroform followed by increasingly polar solvent chloroform and ethanol. After evaporation of solvents the fractions from chloroform only (A₁) and the fractions from ethanol only (A₂) were monitored by thin layer chromatography and ultra violet chromatography (Akbar *et al.*, 1988).

Antibacterial activity

With a sterile wire loop four to five colonies from each bacteria were touched and transferred to 10 ml nutrient broth, the tubes were incubated for 12 –18 hours at 37°C, a volume of 0.1 ml of the suspension was transferred to 9.9 ml broth to obtain 1/100 dilution..

The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish Muller Hinton agar. 50 µL (50, 75, 100, 125 and 150 mg/mL from fractions) of extracts were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, 20 mm apart from each other) with three replications for each concentration. The systems were incubated for 24 h at 37°C under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition zone of the bacterial growth was measured in mm (Cleidson *et al.*, 2007).

Statistical analysis

One-way duncan test was used to analyze the obtained data.

RESULTS AND DISCUSSION

The two alkaloid fractions (A1 and A2), the ultra violet chromatograph of both fractions shown in Fig. (1) and Fig. (2), these fractions were extracted from ethanol extraction of *Nigella sativa* seeds, A1 obtained by using chloroform and A2 obtained by using methanol as a solvent (Akbar *et al.*, 1988). The effect of different concentrations of both alkaloid fractions (A1 and A2) were shown in Table (1) and Table (2), as shown, both alkaloid fractions had antibacterial activity against each of *Staphylococcus aureus*, *Bacillus cereus*, *E. coli* and *Pseudomonas* spp. but alkaloid A1 was more effective than alkaloid A2 toward all tested bacteria [may be due to differences of both type of alkaloids in their sensitivity because different solvents gives different type of alkaloids (Akbar *et al.*, 1988)] and we observed increasing the concentration in both fraction increased the inhibition zone against all bacteria but some of them have no significant differences, the Gram-positive bacteria were more sensitive than Gram-negative bacteria [the reasons may be due to differences in cell envelop of both type of bacteria because the cell envelop of Gram-negative bacteria is more complex than Gram-positive bacteria therefore the Gram-negative bacteria was more resistance than Gram-positive bacteria (Ryan and ray, 2004)].

Our results are in agreement with Karib and Mawlood which reported that black seed oil has antimicrobial activity against *Staphylococcus aureus* (Karib and Mawlood, 2001). Also with Alberezhy which reported that the oil of *Nigella sativa* has antimicrobial activity against *Bacillus cereus* (Alberezhy, 2002).

Nigella sativa L. has inhibitory effect on methicillin resistant *Staphylococcus aureus* (MRSA) this finding warrants necessity of further investigation of this product of folk medicine (Hanan *et al.*, 2008). The black cumin or *Nigella sativa* L. seeds have many acclaimed medicinal properties such as bronchodilatory, hypotensive, antibacterial, antifungal, analgesic, anti-inflammatory and immunopotentiating and are universally accepted as a panacea (Khan, 1999). It has been reported that *Nigella sativa* oil have protective effect against murine cytomegalovirus infection (Salem and Hossain, 2000).

Nigella sativa seeds possess clinically useful anti-*H. pylori* activity, comparable to triple therapy clarithromycin, amoxicillin, omeprazole (Salem *et al.*, 2010). *Nigella sativa* L. seed essential oils obtained by hydrodistillation, dry steam distillation, steam distillation of crude oils obtained by solvent extraction and supercritical fluid extraction were tested for their antibacterial activities, all oil samples were significantly more active against Gram-positive than against Gram-negative bacteria. Thymoquinone exhibited potent growth-inhibiting activity against Gram-positive bacteria, with MICs ranging from 8 to 64 microg/ml (Kokoska *et al.*, 2008).

Table 1: Mean \pm standard error of inhibition zone diameter with mm of alkaloid A1 against tested bacteria .

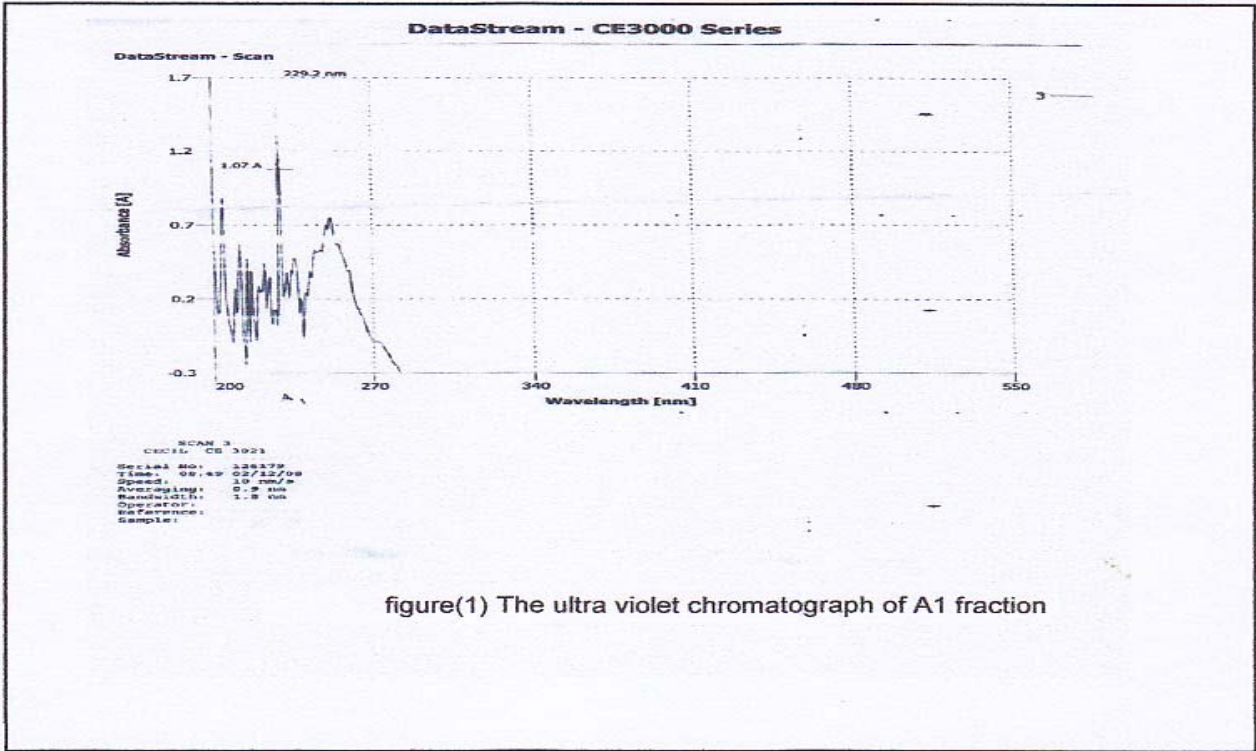
Types of bacteria	Concentrations of A1 mg/ml				
	50	75	100	125	150
<i>Staphylococcus aureus</i>	22.33 \pm 0.33 ^a	25.33 \pm 0.33 ^b	30.0 \pm 0.57 ^c	32.66 \pm 0.33 ^d	35.33 \pm 0.33 ^e
<i>Bacillus cereus</i>	23.0 \pm 0.00 ^a	27.66 \pm 0.33 ^b	30.66 \pm 0.66 ^c	33.0 \pm 0.57 ^d	35.0 \pm 0.57 ^e
<i>E. coli</i>	18.66 \pm 0.33 ^a	22.33 \pm 0.33 ^b	26.0 \pm 0.57 ^c	28.33 \pm 0.33 ^d	28.66 \pm 0.33 ^d
<i>Pseudomonas spp.</i>	17.66 \pm 0.33 ^a	21.66 \pm 0.33 ^b	24.0 \pm 0.57 ^c	26.66 \pm 0.33 ^d	27.66 \pm 0.66 ^d

Same letter in the same row means non significant at ($\alpha = 0.05$)

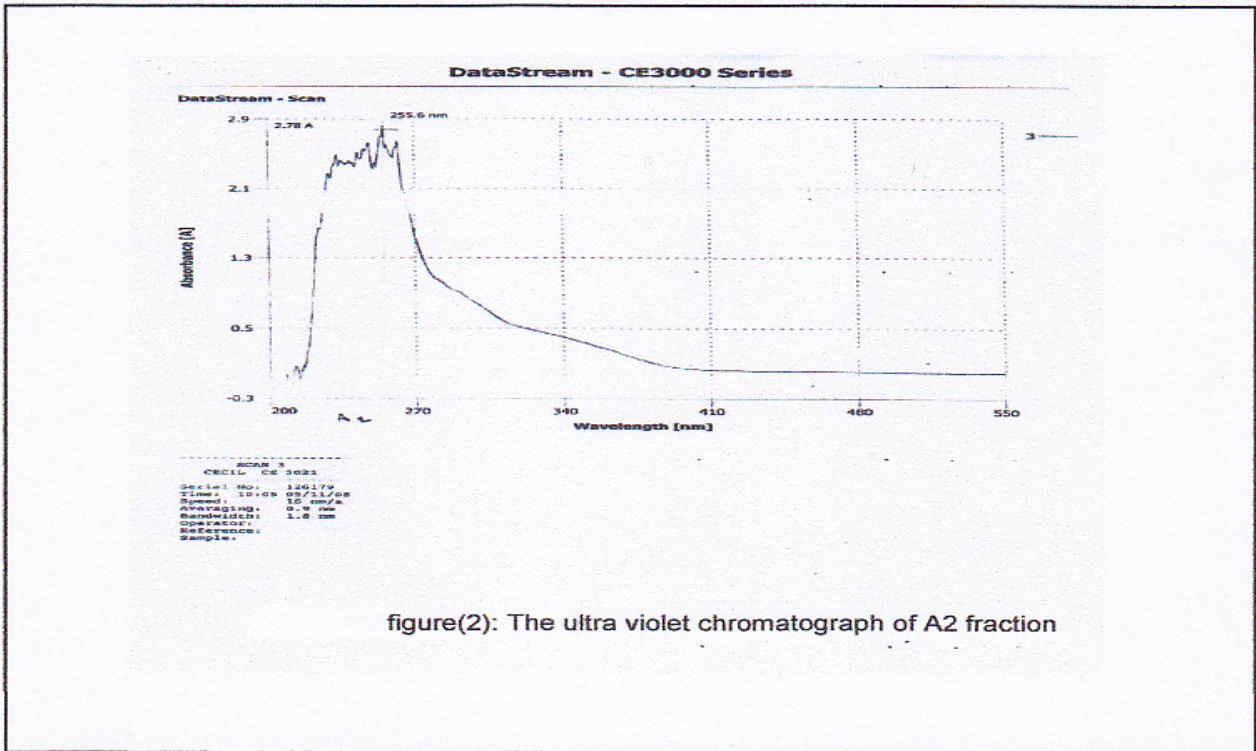
Table 2: Mean \pm standard error of inhibition zone diameter with mm of alkaloid A2 against tested bacteria .

Types of bacteria	Concentrations of A2 mg/ml				
	50	75	100	125	150
<i>Staphylococcus aureus</i>	19.33 \pm 0.33 ^a	23.33 \pm 0.33 ^b	28.3 \pm 0.66 ^c	30.00 \pm 0.00 ^d	32.66 \pm 0.57 ^e
<i>Bacillus cereus</i>	21.0 \pm 0.00 ^a	24.66 \pm 0.33 ^b	26.66 \pm 0.66 ^c	29.33 \pm 0.66 ^d	32.33 \pm 0.33 ^e
<i>E. coli</i>	16.0 \pm 0.00 ^a	20.66 \pm 0.881 ^b	23.66 \pm 0.33 ^c	24.66 \pm 0.33 ^{cd}	25.66 \pm 0.33 ^d
<i>Pseudomonas spp.</i>	15.00 \pm 0.00 ^a	21.33 \pm 0.33 ^b	22.66 \pm 0.66 ^b	26.33 \pm 0.33 ^c	28.66 \pm 0.66 ^d

Same letter in the same row means non significant at ($\alpha = 0.05$)



figure(1) The ultra violet chromatograph of A1 fraction



figure(2): The ultra violet chromatograph of A2 fraction

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