Faculty of Veterinary Medicine,Cairo University (ISSN 1110-1423)
Accredited from national authority for Quality Assurance and Accreditation
Giza, 12211, Egypt

Antibacterial activity of *Spirulina platensis* and *Nigella sativa* extracts against some Fish pathogenic bacteria

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Abstract

This study was conducted to investigate the bactericidal effect of aqueous and ethanol, extracts of *Spirulina platensis* and *Nigella sativa* on some fish pathogenic bacterial Gram-negative bacteria *(Aeromonas hydrophila , A. sobria , Flavobacterium columnare , Vibrio paraheamoliticus,)* Gram-positive bacteria *(Enterococcus fecalis and Streptococcus dysgalactia)*. The results obtained revealed that aqueous extract of *Spirulina platensis* and *Nigella sativa* has no antibacterial effect on both Gram-positive and negative bacteria. Ethanol extract of Both *Spirulina platensis* and *Nigella sativa* have antibacterial effect *E. faecalis* and *S. dysgalactiae* as well as *V. parahemolyticus, F. columnare, A. hydrophila* and *A. sobria).* Besides *E. faecalis* was more sensitive to ethanol extract than other bacteria .

Key words: *Spirulina platensis*, Antibacterial activity, *Nigella Sativa*, Fish bacteria
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Introduction

Bacterial diseases result in major economic losses to fish production. Use of expensive chemotherapy and antibiotic for controlling disease has widely been criticized for their negative impact like residual accumulation in the tissue , development of the drug resistance and immunosuppression, thus resulting in reduced consumer preference for food fish treated with antibiotics **(Anderson, 1992).** Immunostimulants are valuable for the prevention and control of fish diseases in aquaculture **(Mukesh *et al* ., 2012).** *Spirulina platensis*, a filamentous cyanobacteria, possesses diverse biological and nutritional significant . It has the potentiality to produce large numbers of antimicrobial substance; therefore, it is considered a suitable candidate for exploitation as bio- control agent against pathogenic micro-organisms **(Ozdemir *et al.*, 2004).** *Spirulina platensis* is one of the most important micro-alga showing antimicrobial activity against many pathogenic bacteria and fungi **(Vinay *et al*,. 2013).** Also, **Ozdemir *et al.*, (2001),** found that extracts of spirulina obtained by differernt solvent exhibitd antimicrobial activity on both Gram-positive and Gram-negative organisms. **Ramamurthy and Raveendram(2012)**, revealed that ethanol extract of *Spirulina platensis* have antibacterial effect on *Vibrio alginolyticus, Pseudomonas fluorescence, P. aeruginosa ,Aeromonas hydrophila ,and A. salmonicida*. **Pradhan *et al.*, (2012),** cleared that ethanol, methanol and water extract of *Spirulina platensis* have antibacterial effect on some aquatic pathogens. Seeds of *Nigella sativa* L.(Ranunculaceae) commonly known as black seed are used in folk (herbal) medicine all over the world for the

ISSN 1110-1423

and prevention of a number of diseases and conditions. Recently, many biological activities of *Nigella sativa* seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial and antifungal ones, **(McCutcheon *et al.*, 1992)**. Several pharmacological effects have been attributed to active principles of *Nigella sativa* L. which includes thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine and alpha-hedrin **(Ali , 2003)**. So, the objective of this study was to evaluate the antibacterial effect of aqueous and ethanol extracts of *Spirulina platensis* and *Nigella sativa* in vitro against some Gram negative and positive pathogenic bacteria of *O. niloticus* which may play a future role by replacing or substituting antibiotics.

Material and Methods:

2.1 Spirulina platensis (SP)* algae**: commercial *Spirulina algae* powder was obtained from international center for vital energy. ***Nigella sativa (NS) seeds: Commercial *Nigella sativa* seed was obtained from the market in pure form free from debris and other plant seeds.

2.2 Bacterial strains: The well identified microorganisms used in antibacterial assay were kindly obtained from Microbiological Unit, Department of Fish Diseases, Animal Health Research Institute, Dokki. Four Gram-negative bacteria namely (***Aeromonas hydrophila , A. sobria , Flavobacterium columnare , Vibrio paraheamoliticus***,), two Gram-positive bacteria namely (***Enterococcus fecalis and Streptococcus dysgalactiae***).

2.3 Preparation of extracts: *Spirulina platensis*: One gram of dried algal sample was extracted with 10 mL of the solvents (ethanol, water). The dried biomass was taken in sterile screw-capped bottles of 50 mL volume and was soaked in the solvents for 48 hr. The mixture was then centrifuged at 2000 rpm for 10 min at 4°C. The supernatants were filtered through a sterile funnel and sterile Whatman filter paper No 1. Filter. The extract obtained was used for screening of their antimicrobial potential, **(Bhakuni *et al.*, 1992).** This ratio of 10: 1, yielding 9.0mg extract/ml, i.e., 90 mg extracted from I g of dried algae material **(Eloff 1998)** .

Nigella sativa: Ethanol extract: the powdered seed 60 g was soaked in 300 ml of ethanol for 3 hours with stirring. The mixture was filtered using Whatman No.1 Filter paper, the final extract was evaporated by rotary evaporator, and obtained greenish colour yield 17.8%, **(Rooney and Ryan, 2005).** Aqueous extract: was carried according to **Samarakoon *et al.*, (2010).**

2.4 Preparation of bacterial suspension: The bacterial strains were inoculated on Tryptone Soya Agar (TSA) and incubated for 24h at 28C then one single colony bacteria was picked and inoculated into 5ml nutrient broth and incubated overnight and the concentration of the bacteria was standardized to (10^7 cuf/ml) based on the McFarland slandered. An amount of 300µl from bacteria suspension which was kept overnight was diluted into 10ml Muller Hinton Broth (Lab M Limited UK). **(Nor *et al.*, 2013).**

2.5 Antibacterial activity by Agar well Diffusion Assay: Antibacterial activity of different extracts was evaluated using the agar well diffusion assay **(Perez *et al.*, 1990 *and Nor et al., 2013).*** 25ml of Muller Hinton Agar (Lab M Limited UK) was poured into sterile petri dish. Media was allowed to solidify. A sterilized cotton bud was dipped into bacterial suspension prepared and spread evenly on the surface of

Muller Hinton Agar. Commercial antibiotic discs (Ciprofloxacine) was placed at the plates to serve as control positive. Plates were punched to make the well of 6mm diameter. Respective spirulina extracts (100µl) were pipetted into the well. Plates were incubated at 28°C for overnight. The plates were observed for the zone of inhibition and diameter of these zones was measured.

2.6 Determination of Minimum Inhibitory Concentration (MIC): Minimum inhibitory concentration of active crude extract(s) was determined by broth micro dilution method as recommended by ***NCCLS, (1997)***. The calculated amount present in the most diluted extract that produced a visible inhibition was defined as MIC.

Results:

Antibacterial activity and Minimum Inhibitory Concentration (MIC): antibacterial activity of aqueous and ethanol extract of either *Nigella sativa* or *Spirulina platensis* against tested bacteria represented in Tables (1 and 2), Figs (1and 2). The results cleared that the aqueous extract of both *NS* and *SP* showed no inhibition zone against all tested bacterial strains but the ethanol extract of either *SP* or *NS* gave the highest biological activities against ***E. faecalis(40 ,45mm)*** inhibition zone followed by ***S. dysgalactiae (20,23mm), V. parahemolyticus (20,22mm), F.columnare (16,16mm), A. hydrophila (16, 12mm)*** and ***A. Sobria(12 , 21mm).***

Table (1): Antibacterial activities of different extracts of *Spirulina platensis* and *Nigella Sativa*

Bacterial strains	Diameter of inhibition zone (mm)				
	Ethanol extract of *Spirulina*	Aqueous extract of *Spirulina*	Ethanol extract of *N. sativa*	Aqueous extract of *N.sativa*	Control Antibiotic (ciprofloxacine)
A.hydrophila	16		12		25
A. sobria	12	–	21	–	28
V. parahaemolyticus	20	–	22	–	18
F. Columnare	16	–	16	–	10
E. faecalis	40	–	45	–	-
S. dysgalactiae	20	–	23	–	-

Table (2): Minimum inhibitory concentrations of ethanol extract of *Spirulina platensis* and *Nigella sativa*

Tested microorganism	Minimum inhibitory concentration (in µg/ ml)	
	Ethanol extract of *Spirulina platensis*	Ethanol extract of *Nigella sativa*
A. hydrophila	1µg/ml	2µg/ml
A.sobria	1µg/ml	2µg/ml
V. parahemolyticus	8µg/ml	4µg/ml
F. Columnare	2µg/ml	4µg/ml
E. faecalis	512µg/ml	8µg/ml

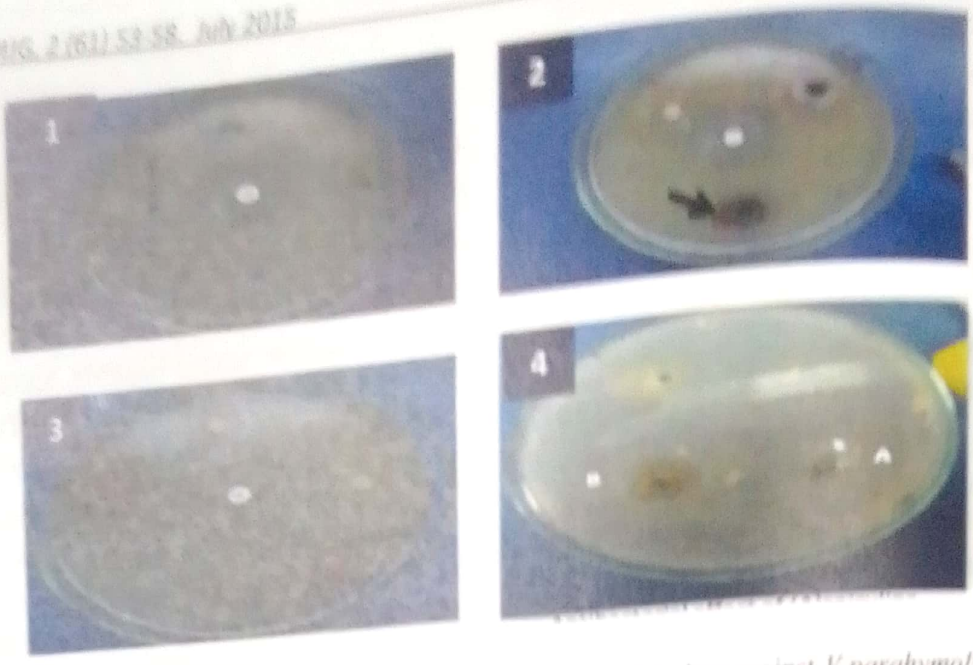
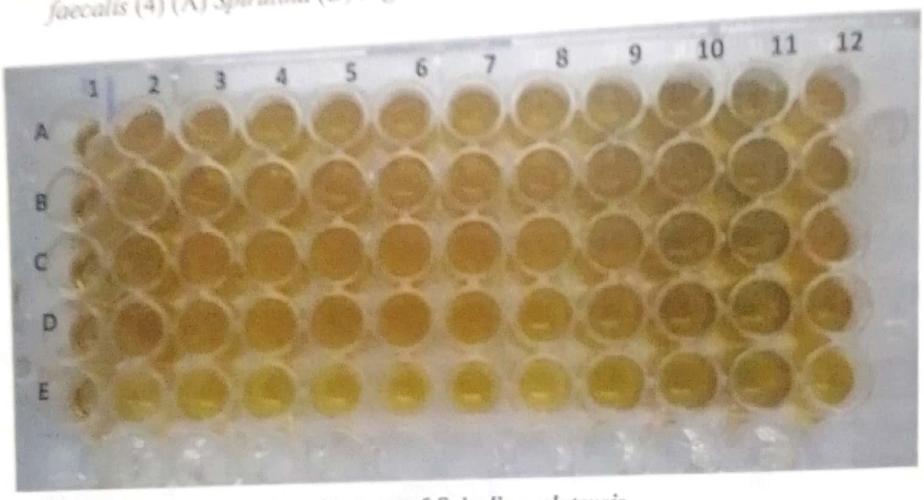
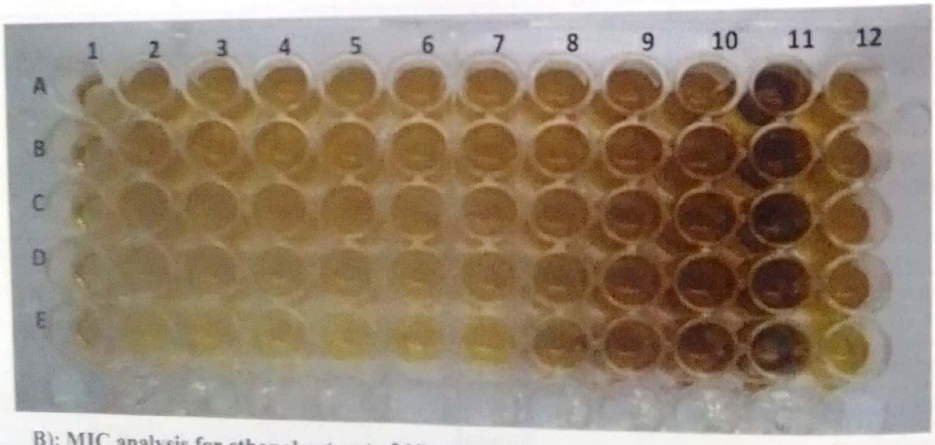


Fig (1): Zone of inhibition exhibited by ethanol extract of (1) *Spirulina* against *V parahymoliticus*, (2) *Nigella sativa* against *V. parahymolyticus*., (3) (A) *Spirulina* (B) *Nigella sativa* against *E. faecalis* (4) (A) *Spirulina* (B) *Nigella sativa* against *S. dysgalactia*.



(A): MIC analysis for ethanol extract of *Spirulina platensis*



B): MIC analysis for ethanol extract of *Nigella sativa*

Fig (2).A ninety six well microtiter plate showing the inhibition of growth.

Rows:- A- *A. hydrophila* B- *A. sobria* C- *F. Columnare* D- *Vibrio parahymolitcis* E- *Enterococcus faecalis*. Column:- 1-negative control (MHB) [2-11dilution of extract range from 512µg/ml-1µg/ml] 2- 512µg/ml, 3-256µg/ml, 4- 128µg/ml, 5-64µg/ml, 6- 32µg/ml, 7- 16µg/ml, 8- 8µg/ml, 9-4µg/ml, 10-2µg/ml, 11- 1µg/ml. 12-positive control (MHB+culture).

Discussion

The results obtained from the present study concerning the antibacterial activity of aqueous and ethanol extract of either *NS* or *SP* against different species of bacteria are recorded that the aqueous extract of either *NS* or *SP* showed no inhibition zones against all tested bacterial strains. This result was supported by **Mashhadian and Rakhshandeh (2005)** who found that aqueous extract of *NS* had no inhibitory effect against *Staph. aureus* and *P. aeruginosa*. Also **Arun *et al.*, (2012)** who found that aqueous extract of *SP* was not effective against any selected pathogenic microbes. These results may due to that active principle which responsible for antibacterial effect were oil in case *Nigella sativa* (thymoquinone) which not soluble in water and **Kumar *et al.*,(2011)** observed that mostly fatty acid compounds are present in crude extract which are associated with the antibacterial properties. On the other hand, the ethanol extract of either NS or *SP* showed antibacterial effect against Gram negative (*V. parahemolyticus, F.columnare , A.hydrophila* and *A. sobria)* and Gram positive (*E. fecalis , S. dysgalactiae*). These results agree with **Mashhadian and Rakhshandeh, (2005) Kumar *et al.*, (2011) Pradhan *et al.*, (2012) Ramamurthy and Raveendram, (2012)** and **EL-Sheekh *et al.*, (2014)**. Also, the results showed that ethanol extract of both of *SP* and *NS* had antibacterial effect against gram positive bacteria more than gram negative bacteria. These results supported by the finding of minimal inhibitory concentration MIC in this study which revealed that the lowest concentration of ethanol extract of *SP* or *NS* make inhibition to the bacteria were against *E. fecalis* gram positive bacteria which were (512µg/ml in case of *SP* extract) and (8µg/ml in case of *NS*), which mean that 0.017mg of *Spirulina* extract and 0.02ml *Nigella sativa* extract this result may be due to Gram-negative bacteria differ from Gram-positive bacteria in having a smaller cell wall peptidoglycan layer, but possessing an outer membrane in addition to the common cytoplasmic membrane. Generally speaking, the possession of an outer membrane that functions as a size-selective, sieve-like permeability barrier, in conjunction with secondary Protective mechanisms such as active antibiotic makes Gram negative bacteria, as a class, more difficult to target new antibacterial agents towards, and more intrinsically resistant to most antibiotics **(Hancock1997)**. These results agree with **Arun *et al.*, (2012) and EL-Sheekh *et al.*, (2014).**

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الملخص العربى

اجريت الدراسة بهدف تقييم التأثير المضاد للبكتيريا فى المختبر للمستخلص المائى والايثانولى لكل من طحلب السبيرولينا وحبة البركة على بعض البكتيريا الممرضة للاسماك السالبة الجرام مثل (الايروموناس هيدروفيلا, الايروموناس سوبريا, فلافوبكتيريم كولمينارس وفيبريوباراهيموليتكس) والموجبة الجرام مثل (انتيروكوكس فيكالس و ستريبتوكوكس ديس جالاكتيا) وقد اظهرت النتائج ان المستخلص المائى لكلا من طحلب السبيرولينا وحبة البركة لم يظهر اى تاثير مضادللبكتيريا سواء الموجبة الجرام أو السالبة الجرام بينما المستخلص الايثانولى اظهر تاثير مضاد للبكتيريا المستخدمة ولكن البكتيريا الموجبة الجرام وبالاخص (انتيروكوكس فيكالس) كانت اكثر حساسية للمستخلص بالمقارنة بالبكتيريا السالبة الجرام.