ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* LIN.

BY

MONA ABDEL EMONIEM[1], ELAMIN IBRAHIM ELNIMA[2]

AND SAMIA AHMAD GUMMAA[3]

ABSTRACT

The antibacterial activity of *Nigella sativa* seed extracts:n-hexane, chloroform, 70%MeOH, volatile oil, fixed oil and crude oil was investigated against ten pathogenic bacterial strains: *Escherichia coli, Pseudomonas aeruginosa, Straphylococcus aureus. Bacillus subtilis, Shigella species, Klebsiella species, Proteus species, Neisseria catarrhalis, Beta-haemolytic Streptococci, Brucella species.* The volatile oil reflects a strong activity against all strains tested at a concentration of 200µgLm1 followed by hexane and chloroform extracts while the rude oil and 70%MeOH extract showed weak activity against more than five strains at the same concentration. The fixed oil exhibited no activity against any of the tested strains.

الملخص:

نبات الحبة السوداء من عائلة (Ranunclaceae) وتسمى محليا بحبة البركة أو الكمون الأسود. تم استخلاص جميع المواد الموجودة في بذرة هذا النبات في حمام مائي باستعمال محاليل عضويه هي: الهكسين - الكلوروفورم - الكحول المائي (70%). في الوقت نفسه لقد تم استخلاص الزيت من البذرة بطرق مختلفة. وهذا الزيت تم تقطيره لفصل الزيت الطيار من الزيت الثابت. تم اختبار النشاط البيولوجي

[1] Faculty of Pharmacy, Omdurman Islamic University..

[2] Faculty of Pharmacy, University of Khartoum.

[3] Faculty of Medicine., University of Khartoum

لهذه المستخلصات تحت ثلاثة تراكيز مختلفة: 100، 150، 200 ميكروجرام/مل ضد عشرة أنواع ممرضة من البكتريا. عكست نتائج اختبار حساسية البكتريا ان الزيت الطيار له تأثير مثبط واضح ضد كل الأنواع البكتيرية المستعملة في هذا الاختبار عند تركيز 200 ميكروجرام. بينما الزيت الثابت لم يظهر أي نشاط واضح ضد أي من الأنواع البكتيرية المستعملة. لقد عكست نتائج اختبار حساسية البكتريا ان الزيت الطيار له تأثير مثبط واضح ضد كل الأنواع البكتيرية المستعملة في هذا الاختبار تركيز 200 ميكروجرام. بينما الزيت الثابت لم يظهر أي نشاط واضح ضد أي من الأنواع البكتيرية المستعملة.

INTRODUCTION

Increased utilization of indigenous plant medicines in developing countries becomes a World Health Organization policy in the 1970s. There is evidence that the use of plants as medicines dated from the earliest years of man's evolution. People in widely separated cultures. With no obvious means of communication, are known to have used the same plants for similar medical problems (Fransworth, 1984).

The major part of the reported investigations was concerned with lower plants, with special attention being paid to different species of Streptomyces and some fungi. However, a number of reports indicated new source of antimicrobial agents by higher plants (Mitscher *et al*., 1978).

Nigella sativa (N. sativa), commonly known as black seed, belong to the botanical family of Ranunculaceae. It has been in use in many Middle Eastern and Far Eastern countries as a natural remedy for over 2000 years. *N. sativa* seeds are commonly eaten alone or with honey and in many food preparations.

Nao and Nigam (178) reported a strong antibacterial activity of the volatile oil of *N. sativa* seed against *Shigella flexeneri* 2A, *Shigella flexeneri* 3A, *E. coli*, and *Vibrio cholerae* at a MIC of 100mg/ml.

Naovi et al. (1991) found that 10 mg/ml f a different organic extract of *N. sativa* seed exhibited no activity against Corynebacterium diphtheriae and Diplococcus pneumoniae, but weak activity was observed against *S.aureus; S. pyogenes* and *S. viridans*. On the

other hand Tanira *et al.* (1994) reported that the Pet. Ether and EtOH extracts of N. sativa seed at a concentration of 3mg/ml had no activity against *P.aeruginosa, E.cloacae, E. coli K.pneumoniae, P.vulgaris, Serratia marcescens, S. aureas* and *S. faecalis*. Also a strong antimutagenic activity of the volatile oil of the black seeds has been shown in S. *typhimurium* TA100 and S. *typhimurium* TA 98 at 100µg/disc compared with ethylmethane sulphonate (Badria, 1994). Previous work on N.Sativa has also shown that the seed extracts inhibited the growth of *E. coli, B. subtilis* and *Streptococus feacatis* (Saxena and Vyas, 1986).

In this work the antibacterial activity of *N. sativa* seed extracts: n-hexane, choloroform, 70% MeOH extracts, crude oil and fixed oil was examined against ten pathogenic bacterial strains: *Escherichia coli, Pseudomonas aeruginosa, Staphyloccus aureus, Bacillus subtilis, Shigella species, Klebsiella species, Proteus species, Neisseria catarrhalis, Beta-haemolytic Streptcocci, and Brucella species.*

2. MATERIALS AND METHODS:

2.1.PREAPERATION OF PLANT EXTRACTS

One kg of *N sativa* Lin. Dry seed was powdered and extracted in a soxhlet unit with different organic solvents: n-hexane, chloroform and 70% MeOH (photochemical methods). The oils of this seed were extracted according to modified method by Gad *et al* (1963). Each of the extracts obtained was evaporated to dryness under reduced pressure. One mg of each extract was dissolved in 1ml of de-ionized distilled water. Three concentrations were prepared: 100, 150 and 200µg/ml.

2.2 ORGANISMS

Ten pathogenic bacterial strains were collected from National Health Laboratory (NHL.) while the standard strains were obtained from National Collection of Type Culture (N.C.T.C.) Nutrient agar and nutrient broth were used as general media and tryptone Soya diphasic medium. The tested organisms were cultured as described by Cheesbrough (1984)

2.3 Sensitivity Tests:

These tests were carried out using the cup plate agar diffusion technique. Five hundred ml of molten nutrient agar, maintained at 45°C - 50°C, were inoculated with 2.5 ml of fresh brothe bacterial culture (18-20 hours) and gently mixed to ensure uniform distribution of the test organism. After thorough mixing, the inoculated agar was distributed in 20ml into sterile Petri dishes. These were allowed to solidify on a leveled surface for 30 minutes at room temperature. A sterile cork borer (7 m m in diameter) was used to make four cups in each of the inoculated and solidified agar plates. These plates were kept in the inverted position in the refrigerator until used. The cups were filled with either the solution of the antibiotic or the and crude extract. The crude extracts were examined at concentrations 200, 100 and 150µg/ml. Each concentration was tested in four replicates. The plate was allowed to stand at room temperature for 2 hours and then incubated in the up-right position at 37°C for 18 hours. The diameter of the inhibition zone was measured by viewing the plates against a suitable background using Gallenkamp colony counter.

3. RESULTS AND DISCUSSION

The drugs, which are active against microbial growth, are of two types: those produced by microorganisms, classed as antibiotics and those that are synthetic antibiotics. Antibiotics contribution to drug therapy in the past half-century represents a period characterized by unprecedented advancements in health care. These groups of drugs provide effective control of many human microbial infections that previously caused prolonged incapacitating diseases or death without appreciable regard for age, economic status or physical fitness.

Tables 1,2,3 and figures 1,2,3 display the antibacterial activity of 6 extracts of *N. sativa* seeds against ten bacterial species: *Staphylococcus aureus, Bacillus subtilis, Pseudomnas aeruginosa, Escherichia coli, Shigella species , Klebsiella species, Proteus species, Neisseria catarrhalis, Streptococus faecalis, Brucella species* . The antibacterial profiles of volatile oil and hexane extracts

Volatile oil was more pronounced than the rest of the investigated extracts. It seems that its antibacterial activity is due to the presence of triterpene and the antibacterial activity of triterpenes was established by Menounos et al. (1986).

It is evident that the fixed oil extract of this plant showed no significant activity against the ten examined organisms, while the chloroform and the methanol extracts examined exhibited inhibitory activity against five or more of the tested organisms.

CONCLUSION

Though, lower plants (Actimycetes, moulds and bacteria) constitute an important source of antibiotics, higher plants may offer and equally important source of antimicrobial source.

Table 1. The antibacterial activity of *Nigella sativa* L. extracts

Type of extract at concentration 200µl/ml	Sa	B.s.	P.a	E.c	Sh. Sp	K.p	Pr.sp.	N. g	St.f.	Br.sp
Hexane	24 19	9 8	10 15	17 18	20 18	8 8	10 12	9 8	11 10	8 7
Chloroform	20 17	13 10	9 7	16 12	0 0	0 0	8 8	0 0	8 8	0 0
MeOH (70%)	26 19	20 10	15 14	19 16	19 20	9 9	10 9	8 7	11 10	0 0
Volatile oil	28 20	26 19	20 21	18 19	26 25	20 20	1 14	10 11	17 15	18 10
Fixws oil	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Crude oil	20 19	27 19	19 20	20 16	11 0	19 11	8 7	0 0	0 0	0 0

Table 2. The antibacterial activity of *Nigella sativa* L. extracts

Type of extract at concentration 200µl/ml	Sa	B.s.	P.a	E.c	Sh. Sp	K.p	Pr.s p.	N.g	St.f.	Br.s p
Hexane	14	0	8	12	18	0	9	9	11	0
	19	0	8	11	17	0	8	9	10	0
Chloroform	17	10	11	12	12	0	0	0	8	0
	10	9	8	10	10	0	0	0	5	0
MeOH (70%)	20	16	10	15	15	7	10	8	9	0
	19	16	9	14	14	7	8	6	6	0
Volatile oil	24	21	0	19	20	16	12	8	11	13
	17	15	15	17	18	12	12	5	9	13
Fixed oil	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Crude oil	18	21	20	20	8	10	0	0	0	0
	18	20	20	11	0	7	0	0	0	0

Table 3. The antibacterial activity of *Nigella sativa* L. extracts

Type of extract at concentration 200µl/ml	Sa	B.s.	P.a	E.c	Sh.S p	K.p	Pr.s p.	N.g	St. f.	Br.s p
Hexane	10	0	0	8	10	0	0	0	9	0
	9	0	0	7	10	0	0	0	8	0
Chloroform	11	9	7	6	9	0	0	0	8	0
	10	9	6	6	9	0	0	0	5	0
MeOH (70%)	15	12	6	10	11	0	6	0	0	0
	15	11	6	10	10	0	4	0	0	0
Volatile oil	19	18	10	11	18	12	9	0	9	10
	17	15	10	11	18	12	8	0	9	10
Fixed oil	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Crude oil	10	16	14	13	0	8	0	0	0	0
	9	10	12	11	0	5	0	0	0	0

Key:

S.a Staphylococcus aureus, B.S: Bacillus sutilis, P.a: Pseudomonas aeruginosa,

E.c: Escherichia coli, Sh. Sp.: Shigella species, p: Klebsiella species, Pr.sp.: Proteus species,

N.g: Neisseria catarrhalis, St.f: Streptococcus faecalis, Br. Sp.: Brusella species

Mean Diameter of Growth Inhibition Zones in mm. Average of four replicates

Standard Strains : Isloated strains (25 isolates)

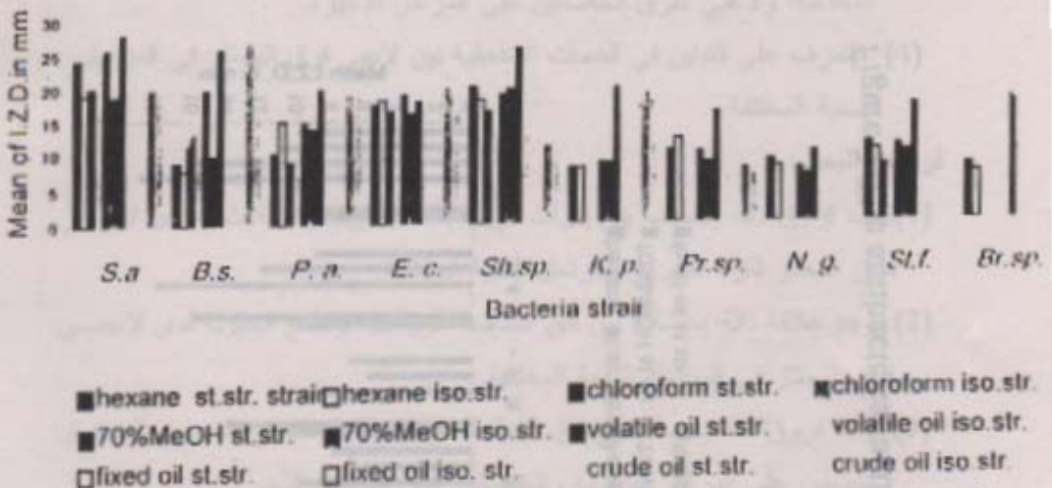

Figure 1: Antibacterial activity of *Nigella sativa* extracts at 200µg/ml.

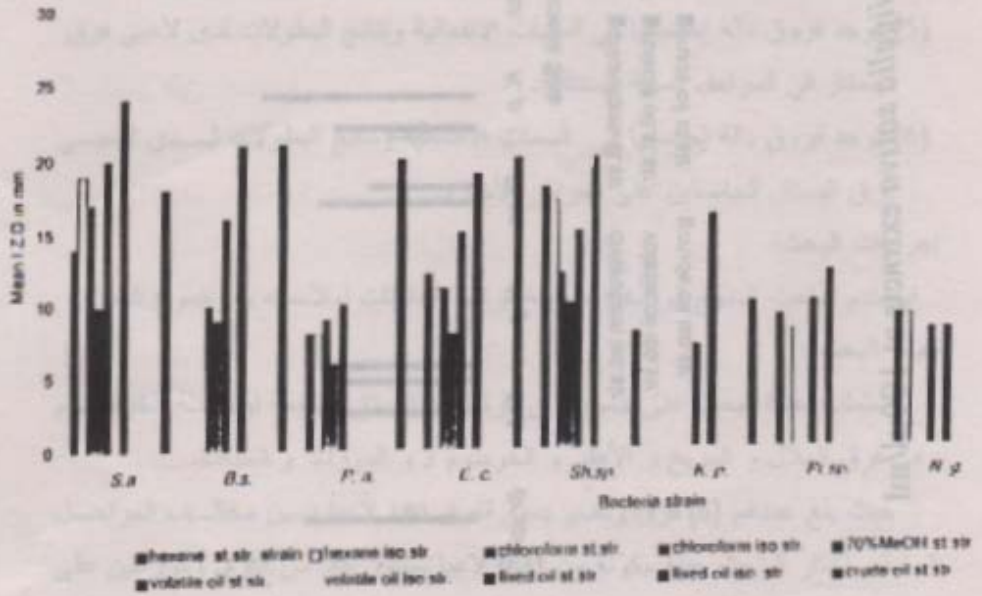

Figure 2: Antibacterial activity of *Nigella sativa* extracts at 150µg/ml

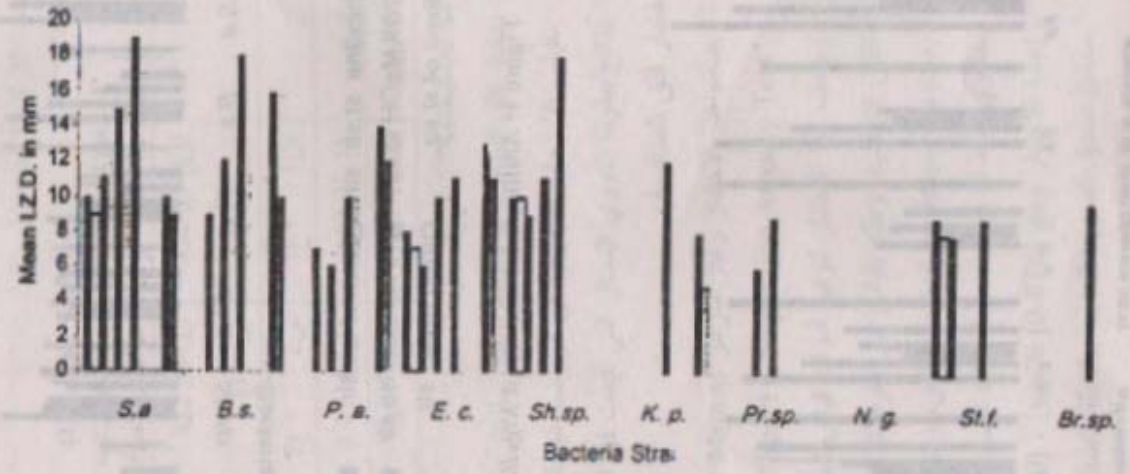

Figure 3:The antibacterial activity of *Nigella sativa* extracts at 100µl/ml

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