TAJ December 2005; Volume 18 Number 2

ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

In vitro Antimicrobial Activity of the Volatile Oil of *Nigella Sativa* Linn Seeds

Nazma Ara[1], S A R Choudhury[2], Ruhul Amin[3]

Abstract

The in-vitro antimicrobial activity of the Volatile oils of Nigella Sativa Linn Seeds was tested against fifteen pathogenic microbial strains includung three gram-positive, eleven gram-negative and a yeast Candida albicans. The volatile oil showed strong sensitivity to all the organisms. The zone of inhibition was found 13-32mm at a dose of 600μg/disc. Minimum inhibitory concentration (MIC) of the volatile oil was also determined against Staph. Aureus ATCC and E.coli ATCC were found 187μg/ml and 375μg/ml respectively.

TAJ 2005; 18(2): 109-112

Introduction

Modern antibiotics are used against various types of infectious diseases caused by micro-organisms like bacteria fungus etc. But in rural areas different medicinal plants, seeds have been used successfully by our traditional practitioners. Bacterial diseases occupied major roles in our health problems. So, need for new antimicrobial agents have become apparent especially for the treatment of infectious diseases where microbial resistance to antibiotics has developed.

Previous work on Nigella Sativa seeds (Bengali name kalajira, family Ranunculacae) has shown about its various activities. A few antimicrobial work of the volatile oil of the seeds of Nigella sativa has been reported [1-5]. Nigella sativa seed volatile oils have some anti-oxytocic potential [6]. The volatile oil of the seeds has a calcium antagonistic effect [7]. The methanolic extract of the seeds has found promising anti-hypertensive activity both in normal and adrenaline induced hypertension[8]. Essential oils of the seeds are reputed to be used for both allergic and irritant contact dermatitis as herbal remedies [9].

In present study, a detailed anti-microbial activity of the volatile oil of seeds of Nigella sativa was done against a wide range of pathogenic micro-organism.

Materials and Methods

The seeds were purchased from local market and identified by the Department of Taxonomy, National Herbarium of Bangladesh, Dhaka.

The volatile oil was prepared by Clevenger's apparatus on steam distillation and the yield was .12%. It was dehydrated by passing through anhydrous sodium sulphite and stored in a refrigerator at 4^0C.

Test Organisms. Identified pure culture of test organisms were collected by sub-culture from stock cultures from the Department of

[1] Department of Pharmacology, Rajshahi Medical College, Rajshahi-6000
[2] Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University, Dhaka
[3] Department of Microbiology, Bangabandhu Sheikh Mujib Medical University, Dhaka

Microbiology, BSMMU and reference strains and Vibrios were collected from the Department of Microbiology, Bangladesh Institute of Child Health and from Laboratory Science Division, ICDDR'B, Dhaka. The organisms used are given in the Table-I

Sample preparation: 1200μg and 600μg per disc were prepared only by transferring 10μl and 5μl of the volatile oil to the blank filter paper disc collected from oxoid. But 300μg, 240μg, 120μg per disc were prepared by dissolving a measured amount of sample to the definite volume of ether and applied the dissolved amount to the previously mentioned blank filter paper disc. Five standard antibiotic disc were used for comparison i.e Ampicillin, Doxycycline, Gentamicin,Co-trimoxazole Nalidixic acid and Cephalexin.

Antimicrobial assays: The antimicrobial activity of various samples was determined by agar diffusion technique[10-12]. Microbial sensitivity testing was done on M-H agar media and Monsur's media was used for Vibrio cholerae. Freshly subcultured bacterial strains was inoculated in the

M-H agar plate and impregnated with disc of different strength which was mentioned previously and these plates were incubated at 37^0C for 18-24hrs. Finally the diameter of the zone of inhibition were recorded and expressed in mm. Minimum inhibitory concentration (MIC) of the sample were determined for Staph. aureus ATCC and E. coli ATCC using a broth micro dilution method.

Results

Shown in Table-I and II. The diameter of the zone of inhibition was proportional to the logarithm of disc drug content.

Discussion

The volatile oil of Nigella sativa seeds were tested against both gram-positive and gram-negative organisms showed in Table-I and II. Comparism between mean diameter of zone of inhibition

Table-I: Results of antimicrobial activityof the volatile oil of Nigella sativa seeds against both gram-positive and gram-negative organisms.

Organisms	Drug Disc content in μg and zone of inhibition in mm				
	200	600	300	240	120
Gram-positive:					
S. aureusATCC	36	31	27	19	16
S.aureus(lab. Isolates)	32	24	21	15	09
B. sublitis	38	32	18	14	09
Gram-negative:					
E.coliATCC	29	24	20	14	08
E.coli (lab. Isolates)	27	22	13	10	08
S.typhi	24	19	16	11	10
S.paratyphiA	23	16	12	09	07
Klebsiella	18	13	12	09	08
Sh. Flexneri	31	23	17	15	11
Sh. Dysentriae	32	26	18	13	09
Pseudomonus	23	20	16	12	08
Proteus	18	15	12	08	_
Vibrio cholerae classical	22	19	18	16	12
Vibrio cholerae 0139	25	21	19	17	14
Candida	24	20	18	12	09

"_"indicates no inhibition.

Table-II: Comparison between mean diameter of zone of inhibition around discs containing N.sativa seeds oil and commercial antibiotic discs on Muller-Hinton agar plates against B. subtilis, S.aureus, E.coli, S.typhi & Pseudomonus.

Drug	Disc content(μg)	Zone of inhibition in mm				
		B.subtilis	S. aureus	E. coli	S. typhi	Pseudomonus
N. sativa v. oil	1200	38	32	27	24	23
	600	32	24	22	19	20
	300	18	21	13	16	16
	240	11	15	10	11	12
	120	07	09	08	09	08
Ampicillin	25	29	31	17	11	--
Co-trimoxazole	25	12	29	28	22	09
Doxycycline	30	24	23	21	20	17
Gentamicin	10	11	25	24	17	18
Cephalexin	30	27	35	22	15	09

"_" indicates no inhibition.

of disc containing Volatile oil of N.sativa seeds with commercial antibiotic disc on M-H agar plate against Staph. aureus, E. coli, B. subtilis, Salmonella typhi and Pseudomonas appears to be significant in compared to that of standard antibiotics which was shown in Table-II. The volatile oil of kalajira seeds was more active against a large numbers of pathogenic gram-negative organism than pathogenic gram-positive groups, though sensitivity is more in case of gram-positive organism than gram-negative organism i.e in case of 16-gm-+ve 16-32mm and in gm-ve 7-32mm. Five different concentrations 1200, 600, 300, 240, 120 micro gram per disc were used against most of the micro-organisms. Amoung them 600μg/disc and 300μg/disc were found more sensitive against all organisms. Statistically significant sensitivity were found taking sensitivity pattern of reference strain as standard for both gram-positive and gram-negative bacterial groups with that of the other strains of the corresponding group. Minimum inhibitory concentration (MIC) were determined against Staph. aureus ATCC and E. Coli ATCC. The MIC value were found 187μg/ml and 375μg/ml correspondingly.

Lastly it may be inferred that antimicrobial active principle reside in the volatile oil of kalajira seeds and further microbiological investigations for detection of active principle are recommended to substantiate our encouraging results of investigations.

Acknowledgements

The authors wish to thank Prof. Ruhul Amin, chairman and Head of the department of Microbiology, BSMMU and Dr. S.K.Saha, Associate professor Department of microbiology, Bangladesh Institute of Child Health, Dhaka in collecting reference strains and sincere advice for determination of MIC value of the oily sample and also Dr. Motiur Rahman, Assistant Scientist, ICDDR'B for their help in collecting reference strains and vibrios. We are grateful to the staff of microbiology Department, BSMMU for their co-operation.

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All Correspondence to :
Nazma Ara
Department of Pharmacology,
Rajshahi Medical College.