

Antibacterial and Antifungal Activity Assessment of *Nigella Sativa* Essential Oils

Entela Haloci, Stefano Manfredini, Vilma Toska, Silvia Vertuani, Paola Ziosi, Irma Topi, Henri Kolani

Abstract—Antifungal activities of ether and methanolic extracts of volatiles oils of *Nigella Sativa* seeds were tested against pathogenic bacteria and fungus strains. The volatile oil was found to have significant antifungal and antibacterial activities compared to tetracycline, cefuroxime and ciprofloxacin positive controls. The ether and methanolic extracts were compared to each other for antifungal and antibacterial activities and ether extracts showed stronger activity than methanolic one.

Keywords—Antifungal, antibacterial, essential oils, extraction, *Nigella Sativa*.

I. INTRODUCTION

A LARGE number of medicinal plants have therapeutic potentials. Seeds of *Nigella sativa* L. (Ranunculaceae), known commonly as “black cumin” have been employed for thousands years as a spice and food preservative. The oil and seed constituents have shown potential medicinal properties in traditional medicine. Recently, many biological activities of *Nigella sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial and antifungal ones. [2]

Several pharmacological effects have been attributed to active principles of *Nigella sativa* L. which includes thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellidine, nigellimine-x-oxide, nigellidine and alpha-hedrin (Aljabre et al. 2005). [3],[7],[11] *Nigella sativa* L. seed extract inhibits fungal strains. In our study we have tested the antifungal and antibacterial properties of *Nigella Sativa*.

II. EXPERIMENTS

A. Extraction of the Essential Oils

25 g seeds were crushed and extracted with petroleum ether for 4 h in a Soxhlet apparatus. After extraction, the solvents were removed by rotary vacuum and dried in a vacuum oven at 30°C for. The same method was repeated by using methanol as extract agent.

E. Haloci, Pharmaceutical Department, Ferrara University, Ferrara, Italy and Pharmaceutical Department, Aldent University, Albania

S. Manfredini, Head of Pharmaceutical Department, Ferrara University Ferrara, Italy

V. Toska, Pharmaceutical Tirana University, Department, Rr. Dibres, Tirane, Albania

S. Vertuani, Pharmaceutical Department, Ferrara University, Ferrara, Italy

P. Ziosi, Pharmaceutical Department, Ferrara University, Ferrara, Italy

I. Topi, National Laboratory of Drug Control, Head of Antimicrobial Laboratory Analyses

H. Kolani, Klinika nr 1, Pavioni i Kirurgjise, QSUT, Tirane, Albania

B. Materials and Methods

Staphylococcus Aerus ATCC 29737 Lot 58312397, *Proteus Vulgaris* ATCC 1978 Lot 0876523C, *Escherichia coli*, ATCC 8456 LOT 6543109, *Candida Albicans* ATCC 2091 Lot 7051869, Mueller–Hinton agar (Lot 685C2S, Code 060098), Dimethylsulfoxide (DMSO), Cefuroxime 30ug lot 1A320, Tetracycline 30ug lot OD3313, Ciprofloxacin 5 ug Lot OM3189

C. Antimicrobial and Antifungal Activity of Essential Oil

The essential oil of samples M1- Ether extract and M2- methanol extract were tested for antibacterial activity by the disc diffusion method using 100µL of suspension of the tested microorganisms, containing 2.0 x 10⁶ colony forming units (cfu mL⁻¹) for bacteria and 2.0x10⁵ spore mL⁻¹ for fungal strains. [11]

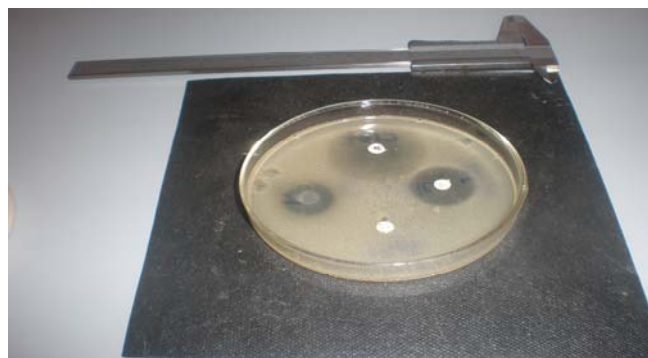


Fig.1 Essential Oils Inhibition Zone And Positive Controls vs *C. Albicans*

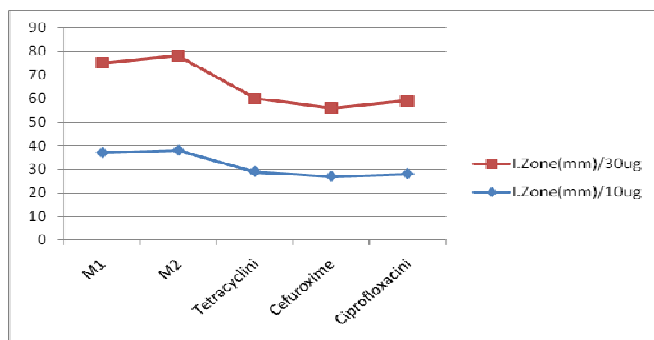
Mueller–Hinton agar and dextrose agar were distributed to sterilized Petri dishes with a diameter of 9 cm.

The filter paper discs (6 mm in diameter) were individually impregnated with 10µL and 30µL of the essential oils dissolved in dimethylsulfoxide (DMSO). (Fig 1, Fig 2)

The Petri dishes were kept at 4°C for 2 h. The plates inoculated with bacteria incubated at 37°C for 24 h and at 30 °C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimetres. Negative controls were set up with equivalent quantities of DMSO. Studies were performed in triplicate. In addition, positive controls antibiotic discs such as Cefuroxime, Ciprofloxacin, Tetracycline and Nystatin were used for comparison.

II. RESULTS AND DISCUSSION

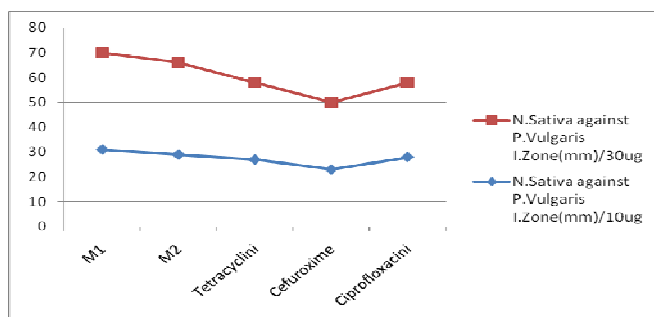
The results of disc diffusion assay are demonstrated on table I.



Graph .1 Essential Oils Inhibition Zone and Positive Controls vs / S.Aureus



Fig .2 Essential Oils Inhibition Zone And Positive Controls Vs /C. Albicans

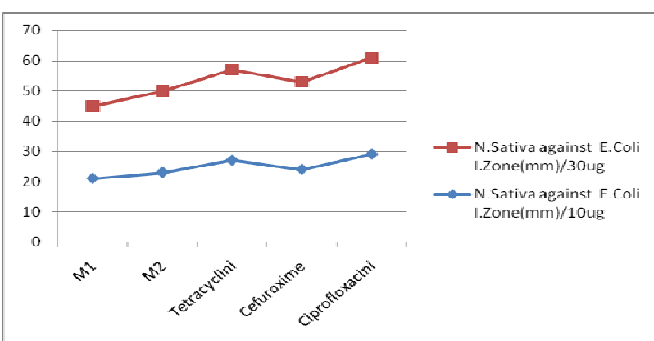


Graph. 2 Essential Oils Inhibition Zone and Positive Controls vs /P. Vulgaris

III. DISCUSSION

Nigella Sativa essential oils are more sensible against gram positive bacteria. (Graph 1) then those gram - negative ones. (Graph 2,3)

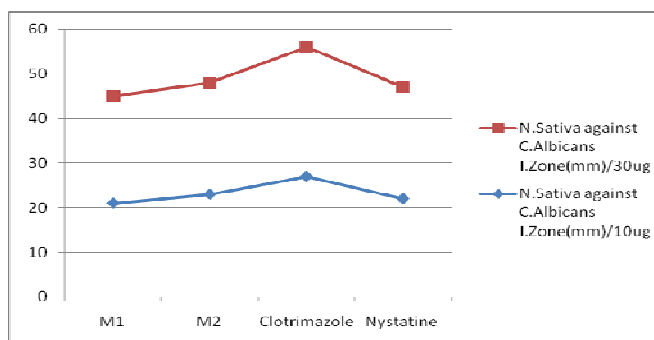
Nigella Sativa essential oil have stronger antibacterial properties compare to Cefuroxime, Tetracycline, (graph 1,2) and about the same strength compare to (graph 3) Ciprofloxacin and antifungal properties compare to Clomatrizol. (Graph 4)



Graph .3 Essential Oils Inhibition Zone and Positive Controls vs /E. Coli

Nigella Sativa essential oils with high concentration of (30 ug) carvacrol and thymol are more sensible against bacteria then those with lower concentrations (10 ug) maybe because they are responsible of the antibacterial activity. (Graph 1,2,3,4 and fig 1,2)

Nigella Sativa essential oils Methanol extracts have more antibacterial and antifungal properties than etheric ones.



Graph .4 Essential Oils Inhibition Zone and Positive Controls vs /C. Albicans

IV. CONCLUSION

It may be concluded from this study that N. sativa seed extract has antimicrobial activity against Staphylococcus Aerus, Proteus Vulgaris, Escherichia Coli, Candida Albicans. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

REFERENCES

- [1] Iraz M., 2005 — Antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazolinduced kindling in mice.
- [2] McCutcheon, A.R., Ellis, S.M., Hancock, R.E. and Towers, G.H., 1992 — Antibiotic screening of medicinal plants of the British Colombian native people. Journal of Ethnopharmacology 37, 212-223.
- [3] Swamy S.M.K. and Tan B.K.H., 2000—Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa L. seeds. Journal of Ethnopharmacology, 70: 1-7. Thabrew M.I., Mity R.R., Morsy M.A. and
- [4] Hughes R.D., 2005 — Cytotoxic effects of a decoction of Nigella sativa, Hemidesmus indicus and Smilax glabra on human hepatoma HepG2 cells. Life Sciences, 77: 1319-1330.

- [5] [5] -Zaoui A., Cherrah Y., Mahassini N., Alaoui K., Amarouch H. and Hassar M., 2002 — Acute and chronic toxicity of *Nigella sativa* fixed oil. *Phytomedicine*, 9: 69-74
- [6] Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahn DF, Nathwani D. Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. *Int J Antimicrob Agents*. 2003;22:406-19.
- [7] Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res*. 2003;17:299-305.
- [8] Salem ML. Immunomodulatory and therapeutic properties of *Nigella sativa* L. Seed. *Int Immunopharmacol*. 2005;5:1749-70.
- [9] Dadgar T, Asmar M, Saifi A, Mazandarani M, Bayat H, Moradi A, Bazueri M. Antibacterial Activity of Certain Iranian Medicinal Plants Against Methicillin-Resistant and Sensitive *Staphylococcus aureus*. *Asian J Plant Sci*. 2006;5:861-6.
- [10] Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res*. 2005;36:464-7.
- [11] Mashhadian NV, Rakhshandeh H. Antibacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus*, *P. aeruginosa* and *C. albicans*. *Pak J Med Sci*. 2005;21
- [12] Salem M.L., 2005—Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *International Immunopharmacology*, 5: 1749-1770.

TABLE I BACTERIAS AND FUNGI MEAN OF INHIBITION ZONE OF ETHER AND METHANOLIC EXTRACTS

Bacteria and Fungi	Ether extracts mean inhibition zone (mm)	Methanolic extracts mean inhibition zone. (mm)
<i>Staphylococcus Aerus</i> ATCC 29737 Lot 58312397	37±0.1	38±0.3
<i>Proteus Vulgaris</i> , ATCC 1978 Lot 0876523C	31±0.8	29±0.6
<i>Escherichia Coli</i> , ATCC 8456 LOT 6543109	21±0.7	23±0.4
<i>Candida Albicans</i> , ATCC 2091 Lot 7051869	21±0.5	24±0.8