Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(8):9-11

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial activity of aqueous, ethanolic, methanolic, cyclohexanic extracts and essential oil of *Nigella sativa* seeds

Kamal Benlafya, Khalid Karrouchi*, Yassine Charkaoui, Miloud El Karbane and Youssef Ramli

Laboratoire National de Contrôle des Médicaments, Direction du Médicament et de la Pharmacie, Ministère de la Santé, Rabat, Maroc

---

ABSTRACT

Most of the bacterial pathogens are resistant to existing synthetic antibacterial agents demanding an increasing effort to seek effective phytochemicals as antibacterial agents against such pathogens. Nigella sativa L. (black cumin) seeds play an important role in folk medicine and some of its major constituents are reported to be pharmacologically active. In this present work, Nigellasativa seed extracts were obtained using maceration extraction by various solvents (water, ethanol, methanol, and cyclohexan). The antibacterial and antifungal effects of different extracts and essential oil of seeds were investigated by the agar diffusion method against two gram positive (Bacillus subtilis CIP52.62 ,Staphylococcus aureus CIP4.83) and three gram negative strains of bacteria (Escherichia coli CIP53.126 ,Pseudomonas aeroginosa CIP82.118, Salmonella abony CIP80.39) and one strain of fungi (Candida albicans CIP48.72). All the extracts and essential oil showed varying degree of inhibition.

Keywords: Nigella sativa, Black cumin, antibacterial activity, antifungal activity.

INTRODUCTION

The problem of microbial resistance and degenerative diseases are growing and the outlook for the use of synthetic drugs without adverse effects in the future is still uncertain. Synthetic drugs also block receptor sites and hence attempts are being made to control the use of synthetic drugs and develop new drugs from natural resources like medicinal plants. Medicinal plants are important therapeutic aids for various ailments and the use of those that are native to Morocco in various traditional system of medicine are awe inspiring.

Nigella sativa (*N. sativa*) seed, called as 'Black Seed' in English language, 'Al-Habba Al-Sauda' or 'Al-Habba Al-Barakah' in Arabic and '*Kalvanji*' in Urdu and some local languages in the Indian Subcontinent, is well known in the Middle East, Middle Asia and Far East as a natural remedy for many ailments and as a flavouring agent in bread and prickles. An authentic saying of the Prophet Muhammad (Peace Be Upon Him) about black seed is also quoted in Al-Bukhari1: *Abu Huraira (Allah be pleased from him) narrated thatAllah's Apostle (peace be upon him) said "Use the black seed, which is a healing for all diseases except 'As-Sam" and As-Sam is Death* [1].

Different pharmacological effects such as isulinotropic[2], hypoglycemic [3], anticancer [4,5], antinociceptive, anti-inflammatory[3,6,7], hepatoprotective[8], neuroprotective[9], antihistamine, antiulcer [10] and bronchodilator [11] activities have been reported for this plant. Black cumin has been traditionally used in the Arabian countries, Indian continent and Europe for culinary and medicinal purposes as a natural remedy for a number of illnesses and conditions that include asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza. The seeds or its oil are used as a carminative, diuretic, lactagogue and vermifuge[12].

N. sativa seeds contain 36%–38% fixed oils, proteins, alkaloids, saponin and 0.4%–2.5% essential oil. The fixed oil is composed mainly of unsaturated fatty acids. The essential oil was analyzed using GC-MS. Many components were characterized, but the major ones were thymoquinone (27.8%–57.0%), ρ-cymene (7.1%–15.5%), carvacrol

(5.8%-11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2.0%–6.6%) and longifoline (1.0%–8.0%). Thymoquinone readily dimerizes to form dithymoquinone. Four alkaloids have been reported as constituents of *N. sativa* seeds. Two, nigellicine and nigellidine have an indazole nucleus, whereas nigellimine and its N-oxide are isoquinolines[12]. As the public becomes more interested in herbal medicine and bacterial pathogens become more resistant to commercial antibiotics, scientists are increasingly investigating the antibacterial properties of plant extracts and fractions [13-16]. Antimicrobial activities have been reported for the extractsof *N. sativa* seed *in vitro*[17,18].The objective of this study is to investigate the antimicrobial activity of *Nigella sativa* seed various extracts obtained by maceration extraction and essential oil against different bacterial and fungi pathogens.

EXPERIMENTAL SECTION

Preparation of plant sample

The *Nigella sativa*seeds were purchased from a local herbal shop in Morocco The seeds were dried at 40°C overnight and were ground to powder in a grinder.

Preparation of different extracts:

30 g of the dried black cumin seed powder was taken in a 500 ml conical flask and 300 ml of different solvents (water, ethanol, methanol, cyclohexan) was added to the powder, which was kept at room temperature for 72 h in a dark place. The suspensions were filtrated through a sterile filter paper (Whatman-1) and the filtrate was centrifuged at 4000 rpm for 15 min. The final concentration of different extracts was fixed at 100 mg/ml.

Antibacterial test

The different extracts and essential oil of seeds were first tested *in vitro* for their activity against three bacterial strains (*Sataphylococcusaureus CIP 4.83, Escherichia coli CIP53.126, Pseudomonas aeruginosa CIP 82-118, Salmonella abony CIP80.39 and Bacillus subtilis CIP 52-62*) and against fungal strains (*Candida albicans CIP 48.72*). Tetracyclin was used as control drugs. The activities were determined by the agar diffusion technique as previously described [19]. 18 mL of Muller-Hinton agar medium were poured into petri dishes as a first layer, a suspension of each test bacterium was diluted with an appropriate volume of Muller-Hinton agar medium to contain about 10^6 CFU/ml and 8 mL of it was poured on of the first layer of each petri dish. The petri dishes were kept 15 min at room temperature and then at 4°C. Cylindrical cavities were punched in the agar with a suitable device. 50µl volume with concentration 100mg/ml of each extracts was loaded into the cavities prepared in the agar. All the plates were incubated at 37 °C for 48h in case of bacteria and after 72h in case of fungi at 25°C.

RESULTS AND DISCUSSION

This study reports the antimicrobial activity of various extracts of *Nigella sativa* seed and essential oil against three bacterial strains (*Sataphylococcusaureus CIP 4.83, Escherichia coli CIP53.126, Pseudomonas aeruginosa CIP 82-118, Salmonella abony CIP80.39 and Bacillus subtilis CIP 52-62*) and against fungal strains (*Candida albicans CIP 48.72*)..The antimicrobial activity has been assessed quantitatively by the presence or absence of inhibition zones. The results of the antimicrobial activity of the investigated extracts and essential oil are shown in Tables 1. These results suggest that the aqueous extract did not show any activity against *B. subtilis, S. aureus, P. aeroginosa, S. abony* and *C.albicans*. However ethanolic extract showed some activity against this particular strain, whereas *B.subtilis*and*C. albicansat* 100mg/ml (Table 1). Methanolic extract gave higher inhibition zone than aqueous against *E.coli*, but against *S.aureus* and *B.subtilis* water extract showed higher inhibition zones.Essential oil gave higher inhibition zone against *S. aureus, B.subtilis and C. albicans,* but against *S. abony, P. aeroginosa and E. coli* showed no inhibition. Against fungal strain i.e*C. albicans*aqueous, methanolic and cyclohexanic extracts did not show any inhibition.

Table 1. Antimicrobial activity of aqueous, ethanolic, methalonic and cyclohexanic extracts and essential oil

Test	*E. Coli*	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*S. abony*	*C. albicans*
Aquoeusextract	++	--	--	--	--	--
Ethanol extract	--	--	--	++	--	++
Methanolextract	--	++	--	++	--	--
Cyclohexanextract	--	--	--	++	--	--
Essential oil	--	++	--	++	--	++

--: No inhibition zone.

++: Presence of inhibition zone.

Due to the increased resistance of microorganisms against the currently used antibiotics and the high cost of production of synthetic drugs, pharmaceutical companies are now searching for alternatives. Medicinal plants could be one approach because most of them are safe with little side effects, if any, are of low cost and affect a wide range

of antibiotic resistant microorganisms[20]. The present findings demonstrated that the plant material has been blessed with many of the natural phytoconstituents. These phytoconstituents as well as plant extracts are responsible for antimicrobial activity.

CONCLUSION

Based on the results obtained in this study, it may be concluded that plant extracts of *N. sativa* have a stronger and broader spectrum of antimicrobial activity against a number of food borne bacteria and the extracts may be used to discover bioactive natural products that may serve as basic source for the development of new antimicrobial compounds to overcome the problem of increasing resistance to known traditional antibiotics.

REFERENCES

[1] Al-Bukhari. MI. Division (71) on medicine. In Sahi Al-Bukhari, the collection of authentic sayings of Prophet Mohammad (peace be upon him). 2nd edition.HilalYayinlari, Ankara, Turkey, **1976**.
[2] KM Fararh;Y Atoji;Y Shimizu;T Takewaki,*Res Vet Sci*.,**2002**, 73, 279-82.
[3] M El-Dakhakhny;NMady;NLembert;HP Ammon,*Planta Med*.,**2002**, 68, 465-466.
[4] GM Mabrouk;SS MoselhySF Zohny;E Ali;TE Helal;AA Amin;AA Khalifa,*J ExpClin Cancer Res*.,**2002**, 21, 341-346.
[5] NJ Salomi;SC Nair;KK Jayawardhanan;CD Varghese;KR Panikkar,*Cancer Lett*.,**1992**, 63, 41-6.
[6] AM Abdel-Fattah;K Matsumoto;H Watanabe;*Eur J Pharmacol*.,**2000**, 400, 89-97.
[7] A Ghannadi; V Hajhashemi; H Jafarabadi,*J Med Food*. **2005**, 8, 488-493.
[8] MR Mahmoud;HS El-Abhar;S Saleh,*J Ethnopharmacol*.,**2002**, 79, 1-11.
[9] M Kanter;O Coskun;M Kalayci;S Buyukbas;F Cagavi, *Hum ExpToxicol*.,**2006**, 25, 127-133.
[10] M Kanter;O Coskun;H Uysal, *ArchToxicol.*,**2006**, 80, 217-224.
[11] AH Gilani;N Aziz;IM Khurram;KS Chaudhary;A Iqbal,*J Pak Med Assoc*.,**2001**, 51, 115-120.
[12] BH Ali;G Blunden,*Phytother Res*.,**2003**, 17, 299-305.
[13] BS FazlyBazzaz;H Parsaei;G Haririzadeh;AN Shoshtari,*Daru*.,**1997**, 7, 1-22.
[14] MK Hassanzadeh;M Rahimizadeh;BS FazlyBazzaz; SAEmami;J Assili, *Pharmac Biol.*,**2001**, 39, 388-390. 12.
[15] R Taskova;M Mitova;H Najdenski;I Tzvetkova;H Duddeck,*Fitoterapia*.,**2002**, 73, 540-543.
[16] RM Kariba,*Fitoterapia*.,**2002**, 73, 523-525.
[17] MS Hanafy;ME Hatem,*J Ethnopharmacol*.,**1991**, 34, 275-278.
[18] NM Morsi,*ActaMicrobiol Pol*.,**2000**, 49, 63-74.
[19] European Pharmacopeia 8.2, Chapter 2.7.2 (Microbiological Assay of antibiotics).
[20] S Maleki; SM Seyyednejad; NM Damabi; H Motamedi, *J Biol Sci*., **2008**, 11, 1286-1289.