Short Communication

Comparative Antibacterial Study of Black Cumin Oil of Saudi and Syrian Origin Seeds with the Commercial Product

Mohammad Rashid[1], Majid A. Ganaie[1], Shamshir Khan[1], Naseem Akhtar[1], Makhmur Ahmad[1], Saiba Shams[2], Omer S. Hamid Bilal[3], Dheeraj Bisht[4]

[1]Department of Pharmaceutical Chemistry and Pharmacognosy, College of Dentistry and Pharmacy, Buraydah Colleges, Buraydah, Al-Qassim, Kingdom of Saudi Arabia, [2]Department of Pharmaceutics, School of Pharmacy, Glocal University, Saharanpur, Uttar Pradesh, India, [3]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Buraydah Colleges, Buraydah, Al-Qassim, Kingdom of Saudi Arabia, [4]Department of Pharmaceutical Sciences, Sir J. C. Bose Technical Campus Bhimtal, Kumaun University, Nainital, Uttarakhand, India

Abstract

Background: A literature survey showed that significant work has been done to evaluate antimicrobial activity of medicinal plants and their constituents. Thousands of phytoconstituents are tested against a wide range of microbial strains *in vitro, in vivo*, and clinically. Black cumin oil obtained from the seeds of *Nigella sativa* L. is used as carminative, stimulant, diuretic, emmenagogue, lactagogue, and anthelmintic. Seed oil is applied externally on skin as antiseptic, emollient, and to prevent cold symptoms. Many studies have displayed the antimicrobial activity of black seed oil against a variety of microorganisms including Gram-positive and Gram-negative bacteria. In the present study, a comparative antibacterial activity of black cumin oil of Saudi Arabian, Syrian seeds, and marketed/branded oil was undertaken. **Materials and Methods:** Black cumin oil (12%) is obtained from Saudi and Syrian originated seeds by the soxhlet extraction method. Agar disc diffusion method was applied for antibacterial activity of each oil and two marketed oils. Antibacterial activity of different black cumin oil samples has been evaluated against standard *Escherichia coli*, standard *Klebsiella pneumonia*, and standard *Staphylococcus aureus.* Phytochemical screening is also done to check the presence of phytoconstituents, which might be responsible for the activity. **Results:** All black cumin oil samples are found to be sensitive to *S. aureus* only. Black cumin of Saudi originated seeds showed higher activity than Syrian. Seeds oil of Syria had almost similar activity to one of the marketed oils (M1). Another marketed black cumin oil (M2) showed highest antibacterial activity among all types of oils. Phytochemical screening of these oils showed the presence of steroids and alkaloids, which might be responsible for the activity. Several factors that affect the phytochemical variations are environmental, geographical, agricultural, and extraction conditions, which result in differences in their antibacterial activity. **Conclusion:** The results of this study showed that all samples of black cumin oils have antibacterial activity against *S. aureus* (Gram-positive bacteria). Therefore, they might be considered as possible alternatives to antibiotics for the treatment of *S. aureus* infections.

Keywords: *Agar disc diffusion method, antibacterial activity, black cumin (Nigella sativa) seed oil, photochemical screening*

Introduction

Plants and their constituents have been used since thousands of years and continue to provide the latest remedies for human beings. Plants have importance as well as great economic status around the world and used for the treatment of various ailments, including infectious diseases.[1] Multiple drug resistance has occurred due to excessive use of antibacterial drugs, which results in insufficient disease control.[2] At the same time, patients experience certain adverse effects associated with antibiotics. This leads to the development of alternative plant medicines to prevent and treat the microbial infections.[3-6] Phytoconstituents had tremendous therapeutic ability and minimal side effects relative to synthetic drugs. Phytoconstituents such as alkaloids, tannins, polyphenols, quinines, flavonoids, coumarins, terpenoids, lectins, and polypeptides are responsible for various therapeutic activities.[7,8] Medicinal plants showed anticancer, antitumor, antimutagenic, antioxidant, hepato-protective, antiviral, antimalarial, antidysenteric, antiseptic, antistress, immunological, antibacterial, antifungal, and several other pharmacological activities.[9]

Nigella sativa L. is a herbaceous plant and belongs to family Ranunculaceae. *Nigella* is commonly known as black cumin and indigenous to the Mediterranean region. It is also grown in Arab desert, Northern Africa, and some parts of Asia. Black cumin is used for the treatment of numerous ailments, including infectious diseases. The black seed

How to cite this article: Rashid M, Ganaie MA, Khan S, Akhtar N, Ahmad M, Shams S, *et al*. Comparative antibacterial study of black cumin oil of Saudi and Syrian origin seeds with the commercial product. J Rep Pharm Sci 2021;10:148-52.

Received: 27 Sept 2020
Accepted: 07 Apr 2021
Published: 31 May 2021

Address for correspondence:
Mr. Makhmur Ahmad, Department of Pharmaceutical Chemistry and Pharmacognosy, College of Dentistry and Pharmacy, Buraydah Colleges, Buraydah, Al-Qassim 31717, Kingdom of Saudi Arabia.
E-mail: makhmur@gmail.com

Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_118_20

Quick Response Code:

(*Nigella sativa*), commonly known as Habbat El Baraka in the Arab world and has been used for generations in various parts of the world, including most of the Arab population. Its seeds stimulate the body to recover from fatigue and also used in respiratory and gynecological diseases.[10-12] Black cumin oil is obtained from the seeds of *Nigella* and applied externally on skin to treat eczema, inflammation, rashes, abscesses, boils, and other topical infections.[13,14] Black cumin seeds have been used as various medical purposes from decades due to its high levels of antioxidants. The seed oil has been reported to have antitumor, antibacterial, anti-inflammatory, hypoglycemic, central nervous system depressant, antioxidant, and immunostimulatory activities.[15,16] These pharmacological activities attributed due to the presence of fixed oil, essential oil, or their constituents. The fixed oil is mainly composed of saturated and unsaturated fatty acids.[17] Several antimicrobial activities of black seed oil are reported against various species of microorganisms like *Escherichia coli*, *Salmonella, Shigella*, and *Vibrios*. The study was also extended to inhibit the spread of growth of phytopathogenic fungi.[18-20] In literature surveys, some other antimicrobial activity was also displayed against multiple antibiotics-resistant bacteria including *Staphylococcus aureus*.[21-24] Geographical and agricultural conditions determine the variation in percentage of phytoconstituents which leads to change in biological activities.[25] The present study was undertaken to evaluate and compare the antibacterial activity of black cumin oil from Saudi and Syria originated seeds with marketed/branded oil.

Antimicrobial resistance, which is currently causing destruction on global public health, has the ability to outnumber many communicable and non-communicable diseases in terms of attributable deaths. This is also due to shortcomings in medical practices and high healthcare prices. Inadequate and unnecessary antibiotic use in humans has been described as one of the major causes of antimicrobial resistance.

There are variations in antibiotic use between populations. Antibiotic use varies according to the healthcare industry. In European countries, antibiotic use is 10 times higher in the outpatient sector than in the hospital sector. In Germany, ambulatory care accounts for 85% of all antibiotic prescriptions for humans, demonstrating the outpatient sector's role as a major contributor to antibiotic resistance.[26]

Experimental Procedure

Selection of black cumin seed

Black cumin seed is easily obtained from the local market of Kingdom of Saudi Arabia and Syria. Black cumin is also important in traditional medicine in many places and is an esteemed herbal remedy for a wide variety of ailments. Among various medicinal plants, black cumin is emerging as a miracle herb with a rich historical and religious background as many researches revealed its wide spectrum of pharmacological potentials. It has been extensively studied for its biological activities and therapeutic potential and shown to possess a wide spectrum of actions, viz. as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintics, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective, and antioxidant properties. The seeds are widely used in the treatment of various diseases such as bronchitis, asthma, diarrhea, rheumatism, and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections and to support immune system.

Preparation of black cumin oil

The dried seeds of black cumin were completely crushed for 4-5 min in a stainless steel grinder. The crushed black cumin seeds are making coarsely powdered and extracted exhaustively with hexane for 72 h. Hexane (solvent) was evaporated under reduced pressure using a rotary evaporator and traces of solvent were removed under a stream of nitrogen to get black cumin oil.[27]

Preparation of test samples

Antibacterial activity of extracted black cumin seed oil (*Nigella sativa*) was evaluated against the *E. coli, Klebsiella pneumoniae*, and *S. aureus*. All *Nigella sativa* oil samples (90% v/v) were prepared in dimethyl sulfoxide (DMSO, 0.1% v/v in PBS).[20]

Antibacterial assay by agar diffusion method

The agar disc diffusion method[28,29] was applied for antibacterial testing of each oil extracted from the Saudi and Syrian Black seeds and compares the activity with marketed oil. The test organisms used in this study were both Gram-positive and Gram-negative bacteria including *E. coli*, *K. pneumoniae*, and *S. aureus*. Gentamycin (10 mcg) and DMSO discs (10 µL) were used as positive and negative controls, respectively, for all microorganisms. All tests were performed in triplicate.

The agar diffusion method was developed as a practical alternative to the agar and broth tube dilution procedures. In the agar diffusion method, plant extracts (extracted seed oil Saudi and Syrian Black origin) and standard antibiotics (Gentamycin) are passed through a hole in the agar plate. During the incubation period, the tested material diffuses from the hole into the agar medium seeded with the test microorganism. The active antimicrobial extracts result in zones of inhibition around the hole, which gives information about the value of the minimum inhibitory concentration (MIC). Factors influencing the size of inhibition zones include the size of the filter paper disk or hole, the amount of compound placed onto the disk or into the hole, the type and concentration of the agar, the thickness and pH of the medium, the microbial strain tested, and the incubation temperature.[30]

In this method, the test agar plate is swabbed with a standardized concentration of the test organism, and then paper disks containing a defined antibiotic concentration are placed on the lawn of bacteria. After overnight incubation,

the diameter of the zone of inhibited growth around the disk is measured. This zone is influenced by a number of variables, including the susceptibility test medium, the concentration of the test organism, the rate of growth of the test organism, the concentration of antibiotic in the disk, the diffusion of the antibiotic in the agar, and the susceptibility of the organism to the antibiotic. The first five variables are standardized by CLSI; therefore, if the test is properly performed, the size of the zone of inhibited growth is directly related to the susceptibility of the organism — the larger the zone, the more susceptible the organism is to the antibiotic. As would be expected, the results of the dilution tests and diffusion tests are related. There is an inverse linear relationship between the size of the zone and the MIC value — the larger the zone of inhibited growth (more susceptible the organism to the antibiotic), the smaller the MIC value. Thus, it is possible to extrapolate from the measured size of the inhibitory zone to the corresponding MIC value.[31]

Phytochemical screening

Phytochemical screening refers to the extraction, screening, and identification of the medicinally active substances found in plants. Photochemical are chemical compounds produced by plants, generally to help them resist fungi, bacteria, and plant virus infections and also consumption by insects and other animals. The presence of various phytochemicals is confirmed by phytochemical screening. Each black cumin oil is evaluated for the presence of major classes of phytoconstituents like steroids, saponins, terpenoids, flavonoids, and alkaloids.

Table 1: Antibacterial activity by black cumin oil samples

Organism	Sample S	Sample Y	M1	M2
Escherichia coli	−ve	−ve	−ve	−ve
Klebsiella pneumonia	−ve	−ve	−ve	−ve
Staphylococcus aureus	+ve	+ve	+ve	+ve

Results and Discussion

Antibacterial activity of different black cumin oil samples has been evaluated against *E. coli*, *K. pneumoniae*, and *S. aureus* [Table 1]. All black cumin oil samples are sensitive to only *S. aureus* strains [Table 1 and Figure 1]. Zones of inhibition of all black cumin samples were detected and compared with two marketed black cumin oils [Table 2 and Figures 1–3]. Black cumin oil isolated from Saudi originated seeds showed almost similar antibacterial to positive control (Gentamycin) and higher activity than oil isolated from Syrian originated seeds. Oil isolated from Syrian seeds had almost similar activity to one of the marketed oils (M1). Both types of oil showed higher activity than one marketed black cumin oil (M1). Another marketed black cumin oil (M2) showed highest antibacterial activity among all types of oils. Phytochemical screening of all black cumin oil samples is displayed in Table 3, which represents that oil isolated from Saudi and Syrian originated seeds had alkaloids whereas steroid was present in all oil samples. It is also reported that black seed oil has 17% saturated fatty acids and 82.9% unsaturated fatty acids.[17] Therefore, antibacterial activity of black cumin oil samples might be attributed to the presence of alkaloids, steroids, and fatty acids. Differences in antibacterial activity might be due to various factors such as extraction method, drying, storage, geographical, agriculture and harvesting conditions, etc., which result in chemical variation.[25]

Conclusion

In conclusion, the results of this study showed that all samples of black cumin oils have antibacterial activity against *S. aureus* (Gram-positive bacteria). Therefore, they might be considered as possible alternatives to antibiotics for the treatment of *S. aureus* infections. The comparable activity of Saudi and Syrian black cumin oil seeds to certain antibiotics can help discover new

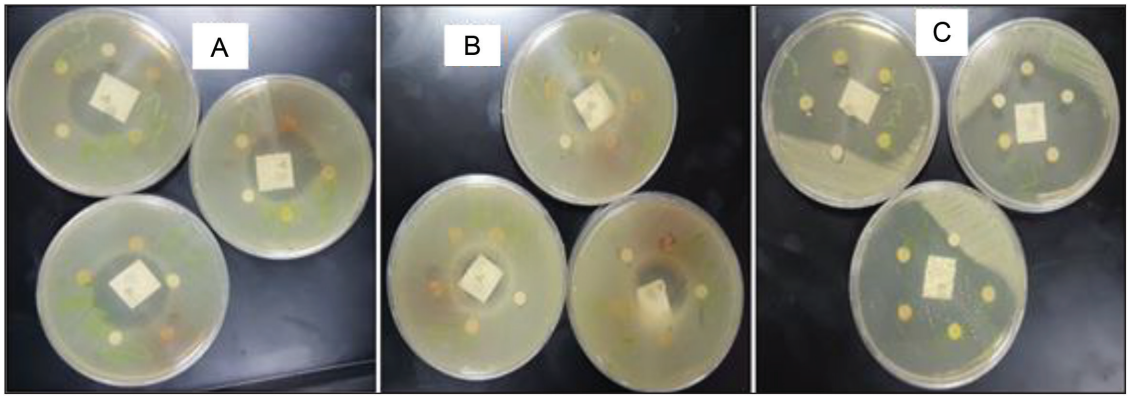

Figure 1: Antibacterial activity of black cumin oil samples against (A) *E. coli*, (B) *K. pneumonia*, and (C) *S. aureus*

Table 2: Zone of inhibition in mm

Organism	−ve control (DMSO)	+ve control (Gentamycin)	Black cumin oil (Saudi)	Black cumin oil (Syria)	Marketed black cumin oil (M1)	Marketed black cumin oil (M2)
S. aureus	0	25	24	15	14	63

−ve, no zone of inhibition; +ve, zone of inhibition appears

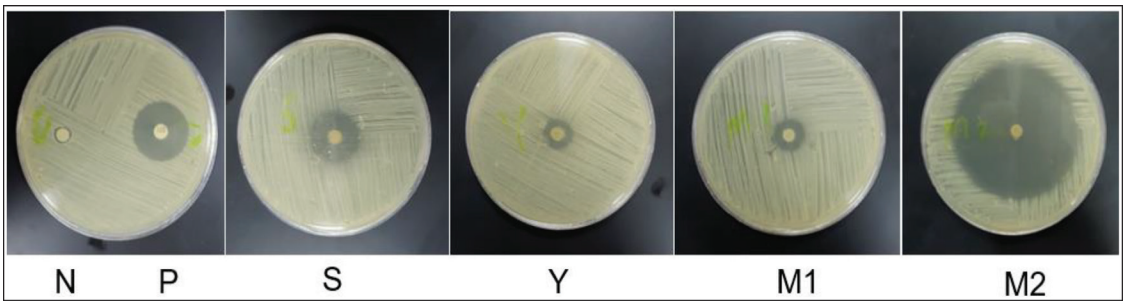

Figure 2: Antibacterial activity of black cumin oil samples against *S. aureus*. S = oil isolated from Saudi originated black cumin seeds, Y = oil isolated from Syrian originated black cumin seeds, M1 = marketed black cumin oil 1, M2 = marketed black cumin oil 2, N = negative control, P = positive control

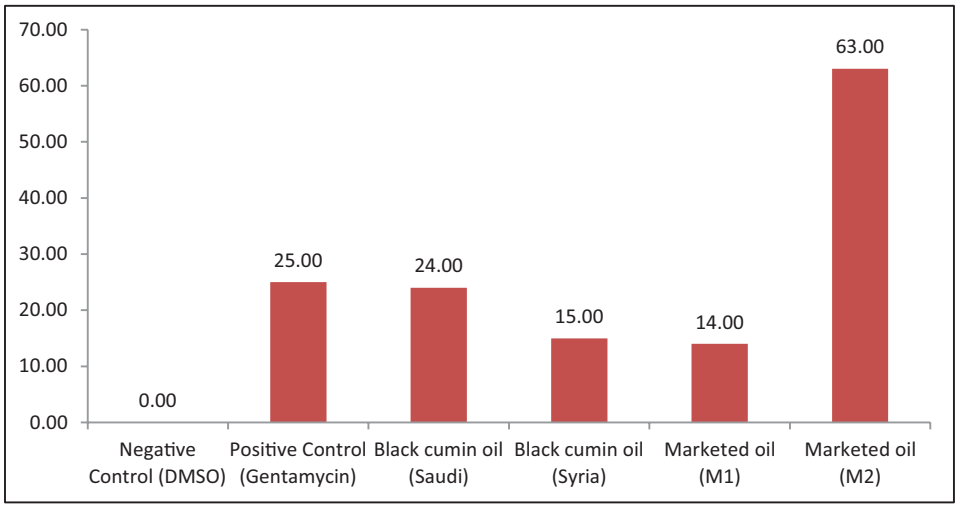

Figure 3: Antibacterial activity of black cumin oil samples

Table 3: Phytochemical screening of black cumin oil samples

Chemical class	Sample S	Sample Y	M1	M2
Steroids	+ve	+ve	+ve	+ve
Terpenoids	−ve	−ve	−ve	−ve
Flavanoids	−ve	−ve	−ve	−ve
Saponins	−ve	−ve	−ve	−ve
Alkaloids	+ve	+ve	−ve	−ve

chemical classes of antimicrobials that can be used to treat topical disease against the microbial strains tested. Furthermore, more work on the phytochemicals and medicinal quality of the above-tested plants should be highlighted. It is also recommended to find the antiviral activity of black cumin oil against life-threatening viral diseases, e.g. HCV, HBV, and dengue.

Ethics Approval and Consent to Participate

Not applicable.

Human and Animal Rights

No animals/humans were used for studies that are the basis of this research.

Acknowledgements

Authors are thankful to research unit of College of Dentistry and Pharmacy, Buraydah Colleges, Buraydah, Al-Qassim, Kingdom of Saudi Arabia for supporting and providing necessary facility for compiling research work.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

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