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Antibacterial Activity of Thymoquinone and Thymohydroquinone of *Nigella sativa* L. and Their Interaction with Some Antibiotics

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Abstract: The two main components of black seed essential oil, thymoquinone (TQ) and thymohydroquinone (THQ) were investigated for their antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Salmonella* Typhimurium*, Salmonella* Enteritidis *and Staphylococcus aureus.* Both TQ and THQ exerted antibacterial activity against gram-positive and gram-negative bacteria regardless to their susceptibility to antibiotics. *S. aureus*, was highly susceptible to TQ, since, three and 6 µg/ml were enough to inhibit and kill the bacteria respectively. On the contrary the concentration of THQ required to inhibit and kill *S. aureus* was 400 and 800 µg/ml respectively which is 100 times more than that of TQ. Gram-negative bacteria were less susceptible to both TQ and THQ and their MIC and MBC ranged between 200 and 1600 µg/ml. Combination of TQ and THQ with antibiotics (ampicillin, cephalexin, chloramphenicol, tetracycline, gentamicin, and ciprofloxacin) exerted synergism in *S. aureus*. On the other hand, in gram-negative bacteria, synergism, antagonism and indifferent effects were detected in 28.9%, 23.6% and 47.5 of the tested combinations respectively. This study demonstrated that both TQ and THQ have antibacterial activity and their activity could be potentiated by antibiotics especially in case of *S. aureus*.

Key words: Thymoquinone · Thymohydroquinone · *Nigella sativa* · Antibacterial activity

INTRODUCTION

One of the central themes of success in human therapeutics in the 20th century was the discovery and development of antibiotics and antibacterial agents, for the treatment of bacterial infections. A huge array of antibacterial agents have been introduced and antibiotics can be used effectively to treat major infectious diseases [1].

However, the usage of antibiotics and antibacterial chemotherapeutics is becoming more and more restricted, because: (i) bacteria is capable of developing resistance to antibiotics soon after their introduction [2], (ii) most antibiotics have side effects. Therefore, it becomes essential to search for newer drugs with lesser rate of resistance development and lesser toxicity [2,3].

Nigella sativa Linneaus is a herbal plant which is popularly called black cumen, black seed and the seed of blessing (Habatul-barakah in Arabic countries).The seeds have traditionally been used for thousands of years in the Middle East, Far East and Asia as a food additive and as a herbal health aid [4]. The seed or its oil has been used as a carminative, diuretic, lactagogue and vermifuge. It has also been used in the treatment of fever, common cold, headache, asthma, rheumatic diseases, warts, and stings of scorpions and bites of snake [5-10].

Recently it was possible to demonstrate that black seed, its oil and extracts act as antimicrobial, immune stimulant [11], hypotensive [12], anti-inflammatory [13,14], anti-cancer [15,16], anti-oxidant [17,18], hypoglycemic [19,20], spasmolytic and bronchodilator [21,22].

N. sativa oil [23], extracts and thymoquinone and thymohydroquinone, isolated from the volatile oil were found to have inhibitory activity against gram-positive and gram-negative bacteria [24]. Diethyl ether extract of *N. sativa* had a synergistic effect with streptomycin and gentamicin and additive effect with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and co-trimoxazole [25]. Furthermore, both antibiotic sensitive and multi-drug resistant gram-positive and gram-negative bacterial isolates were susceptible to *N. sativa* extracts [26,27].

In this study we report the antibacterial activity of TQ and THQ alone and in combination with different antibiotics.

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MATERIALS AND METHODS

Bacteria: Tree standard strains and 4 clinical bacterial strains were used in this study (Table 1).

Synergism Between Antibiotics and TQ and THQ: Disc diffusion technique, as described by National Committee for Clinical laboratory Standards (28) was used to determine the combined effects of TQ and THQ and antibiotics. Sterile filter paper discs (mm, Whatman No. 1) containing µg of thymoquinone and thymohydroquinone were used along with commercial antibiotic discs of ampicillin (Ap), chefalexin (Cx), gentamicin (Gn), tetracycline (Tc), chloramphenicol (Cm), sulfamethoxazole (Sx), ciprofloxacin (Cp) (Oxoid, UK). The selected bacterial strains were grown in liquid media for 18 h. Each culture was diluted to 10^7 CFU ml^{-1} and was used to flood the surface of Muller-Hinton agar plates (Oxoid), in triplicate and the plates were dried. At first, the individual inhibitory effects of thymoquinone and thymohydroquinone and individual antibiotics were determined. The drug discs were placed in such a manner that the inhibitory circles would just touch each other tangentially. The plates were incubated at 37°C for 18 h. The zones of inhibition produced by each drug were measured in three different directions around the disc and mean diameter was recorded. The diameter on inhibition zone produced due to the individual and mutual effects of the antibiotic and thymoquinone or thymohydroquinone were recorded. Data were analyzed as follows: (i) indifference, when both the zones of inhibition remaine unaffected; (ii) antagonism, when the zones of inhibition receded and assumed kidney shape; (iii) synergism, when there was enlargement of zones at the site of meeting the inhibition zones of antibiotic and TQ or THQ.

Determination of minimum inhibitory concentration [MIC) and minimum bactericidal concentration (MBC) of TQ and THQ: The minimum inhibitory concentration (MIC) was determined by a broth dilution method in test tubes as follows: various dilutions of the oils in Muller-Hinton broth was added to 5 ml of broth tubes containing 10 cfu/ml of bacterial cells. The tubes were then incubated on an incubator shaker. The highest dilution (lowest concentration), showing no visible growth, was regarded as MIC. 0.1 ml of the cell suspensions from the tubes showing no growth were sub-cultured on nutrient agar plates for bacteria to determine if the inhibition was reversible or permanent. Each experiment was performed in triplicate. MBC was determined as the highest dilution (lowest concentration) at which no growth occurred on the plates.

RESULTS

The bacteriostatic activity of TQ and THQ was determined by broth dilution method. *S. aureus* a gram-positive bacteria was highly susceptible to TQ having MIC as low as 3 µg/ml. On the other hand gram-negative bacteria were less susceptible to both TQ and THQ. The MIC of TQ to gram-negative bacteria ranged from 200 t0 1600 µg/ml. *S. flxeneri* was more susceptible to both TQ and THQ followed by *Sal. enteritidis* and *Ps. aeruginosa* (Table 2). Unlike TQ, THQ was less effective against

Table 1: Bacteria used in the study and their source

Bacteria	Characteristics	Source
Escherichia coli	K12 standard strain, nalidixic acid resistant	Upsala University, Sweden
Pseudomonas aeruginosa	PAO1 standard strain	Ohio University, USA
Shigella flexneri	Ap, Tc, Cm, Sx	Clinical isolate, Taif University, KSA
Salmonella Typhimurium	Ap, Sm, Tc, Sx	Clinincal isolate, Taif University, KSA
Salmonella Enteritidis	Ap, Sm, Sx	Clinical isolate, Taif University, KSA
Staphylococcus aureus	ATCC25923 Standard strain	Ohio State University, USA
Staphylococcus aureus	Ap, Cf, Sm, Gn	

Table 2: Minimum inhibitory concentration and minimum bactericidal concentration [µg/ml) of thymoquinone and hydrothymoquinone against some pathogenic bacteria

	Thymoquinone		Thymohydroquinone	
Bacteria	MIC	MBC	MIC	MBC
Escherichia coli	800.0	800.0	1600.0	1600.0
Salmonella Typhimurium	800.0	800.0	800.0	800.0
Salmonella Enteritidis	400.0	800.0	800.0	800.0
Shigella flexneri	200.0	200.0	800.0	800.0
Pseudomonas aeruginosa	400.0	1600.0	400.0	1600.0
Staphylococcus aureus [standard)	3.0	6.0	400.0	800.0
Staphylococcus aureus [clinical str)	3.0	6.0	400.0	800.0

Table 3: Combined effect of thymoquinone and hydrothymoquinone with antibiotics against different bacteria

		Bacteria			
Volatile oil ingredient	Antibiotic	*E. coli*	*Ps. aeruginosa*	*Sal. typhimurium*	*S. aureus*
Thymoquinone	Ap	I	-	A	S
	Cx	S	I	A	S
	Cm	S	I	S	S
	Tc	S	A	S	S
	Gn	I	I	A	S
	Sx	I	-	A	S
	Cp	A	I	I	S
Thymohydroquinone	Ap	S	-	A	S
	Cx	I	A	S	S
	Cm	I	S	I	S
	Tc	I	I	I	S
	Gn	S	S	A	S
	Cp	I	I	I	S
	Sx	S	-	I	S

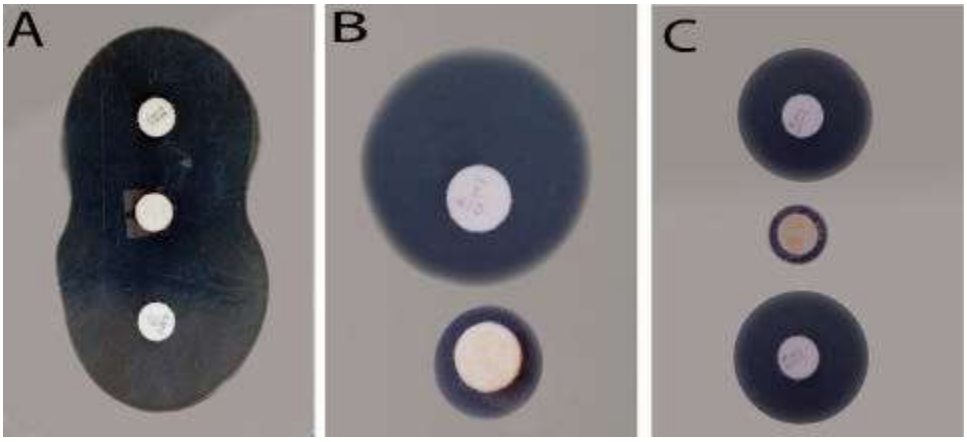

Fig. 1: Representative examples of the combine effect of TQ and antibiotics which demonstrate synergism [A], anatgonism [B] and indifference[C].

S. aureus and its MIC was close to that of gram-negative bacteria. The MIC of THQ for both gram-positive and gram-negative bacteria ranged between 400 and 1600 µg/ml (Table 2). The MBC of Both TQ and THQ was usually equal to or double that MIC, except in the case of TQ against *P. aeruginosa* where the MBC was four times more than the MIC (Table 2).

Both TQ and THQ showed synergism when combined with all tested antibiotics against the gram-positive *S. aureus* (Fig. 1 and Table 3). In the case of gram-negative bacteria was more common in *E. coli*. In the later bacteria synergism was detected in TQ with Cx, Cm and Tc and was detected in THQ with Ap, Gn and SX (Table 3). In *Sal.* Typhimurium, TQ was synergistic with Cm and Tc and THQ was synergistic with Cx (Table 3). Synergism was detected in *Ps. aeruginosa* in only THQ in its combination with Cm and Gn (Table 3).

Antagonism was detected in 9 combinations of the tested antibiotics with TQ and THQ out of the 56 tested (16.1%), as shown in Table 3. It was detected in 6, 2 and 1 in combinations in *Sal.* Typhimurium, *Ps. aeruginosa* and *E. coli* respectively (Table 3). No inhibition zone of inhibition was detected for Ap, and Sx in *Ps. aeruginosa*, therefore, no combined effect was recorded (Table 3).

DISCUSSION

The antibacterial and antifungal activity of black seed and its crude extracts have been demonstrated by several research groups [5,25,29,30]. This activity was demonstrated against both pathogenic and environmental bacteria causing food spoilage [10]. The major pharmacologically active principles in the volatile

oil of back seed were identified as TQ, THQ, dithymoquinone and thymol [25, 27,31].

The objective of this research was to examine the antibacterial activity of the two main components of black seed oil which are TQ and THQ and to investigate their interaction with some commonly used antibiotics against gram-positive and gram-negative bacteria.

Both TQ and THQ exerted antibacterial activity against both gram-positive and gram-negative bacteria. *S. aureus, Sal.* Typhimurium, *Sal.* Enteritidis and *Sh. flexeneri* clinical isolates were multi-drug resistant and were equally as sensitive as standard sensitive strains to both TQ and THQ. The susceptibility of resistant bacterial isolates to black seed oil and its crude extract was reported by other investigators [27,30]. In this study both antibiotic sensitive and resistant *S. aureus* were highly susceptible to TQ. Three and 6 µg/ml were enough to inhibit and kill the bacteria respectively. This is very interesting because *S. aureus* clinical isolates are frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones [32].

On the other hand the concentration of THQ required to inhibit and kill *S. aureus* was 400 and 800 µg/ml which is 100 times more than that of TQ.

TQ and THQ to inhibited and killed gram-negative bacteria at concentrations ranged between 200 and 1600 µg/ml. The MIC and MBC concentrations, of THQ found in this study were relatively higher than the concentrations reported by Toama, *et al.* [33]. *P. aeruginosa* was as susceptible to TQ and THQ as other gram-negative bacteria, though, this bacteria is well known for its intrinsic resistance to antimicrobials [34,35].

Synergism was reported recently by disc diffusion method between diethyl ether extract of black seed and some antibiotics against *S. aureus, E. coli, P. aeruginosa and Sal*. Typhimurium[25]. In this study we investigated the combined activity of TQ and THQ with several antibiotics against the same bacteria. Both TQ and THQ exerted synergism in *S. aureus* with all investigated antibiotics (Ap, Cx, Cm, Tc, Gn, Cp and Sx). In gram-negative bacteria, synergism, antagonism and indifferent effects were detected. Synergism and antagonism were detected respectively in 11 and 9 of the 38 combinations of gram-negative bacteria.

This study demonstrated that TQ and THQ have inhibitory and lethal effects against both gram-negative and gram-positive bacteria and when combined with antibiotics they may exert synergistic activity. Therefore, both TQ and THQ could be used as antibacterial drugs. It was suggested that black seed oil might be used as a preservative for food [10], and the combination of garlic with black seed has been used traditionally for treatment of urinary tract infection [36]. Data obtained from this study suggest that TQ and THQ may be used for treatment of infections alone or in combination with some antibiotics, especially in case of the higly susceptible gram-positive bacteria *S. aureus*.

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