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In vitro Assessment of Antimicrobial Activity of Honey Bees and *Nigella sativa* Against Selected Clinical Isolates from Shendi City

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Abstract

Background: An alarming rise in bacterial strains resistant to existing antimicrobial agents requires a renewed effort to seek agents effective against pathogenic bacteria resistant to antimicrobials. The new antimicrobial agents are will overcome this problem**.**

Objective: This study aimed to study the *in vitro* antimicrobial activity of different concentrations of Honey, petroleum ether extracts of medicinal plant *Nigella sativa* (seeds), and the mixture using the cup-plate agar diffusion method.

Materials and Methods: This cross-sectional observational study was carried out among One hundred samples were collected from urine, wound, ear, and eye swab, 95/100 (95%) showed bacterial growth, from which six types of pathogenic bacteria at Shendi city hospitals during March to June 2018.

Results: Of the total 95 clinical and standard specimens were confirmed as *Staphylococcus aureus* 20 (21%), *Escherichia coli* 20 (21%), *Pseudomonas aeruginosa* 15 (16%), and *Klebsiella pneumoneae* 30 (31%). In addition to *Candida albicans* 10 (11%). These results showed the activity of antimicrobial Petroleum ether extract of the *Nigella sativa,* and honey pronounced dose-dependent on standard strains and clinical isolates, while methanolic extract of *Nigella sativa* explained no activity.

Conclusion: The activity of *Nigella sativa,* honey, and the mixture exhibited high antimicrobial activity against all types of tested organisms both clinical and standard organisms. Therefore *Nigella sativa,* honey, and the mixture can be regarded as a broad-spectrum antimicrobial agent.

Keywords: *Antimicrobial Activity; Honey Bees; Nigella sativa; Petroleum Ether Extracts; Methanolic Extract*

Introduction

Microbial infection is a major public health problem in both developed and developing countries. Due to the misuse of antibiotics "used to treat these infections" the incidence of multiple antibiotic resistance among human pathogens is increasing. All this besides the undesirable side effects of antibiotics has forced scientists to search for new antimicrobial substances from natural sources [1].

Antimicrobial resistance is the ability of microorganisms to resist the effect of drugs leading to resistant infections, which may kill, can spread to other and imposes huge costs to individual and society. Misuse of antibiotics is the most important factor leading to antibiotic-resistant around the world [2]. In recent years, infective pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs ordinarily employed in the treatment of infectious diseases. Therefore, different antimicrobial methods are urgently required, and therefore this case has led to an evaluation of the therapeutic use of ancient remedies, like plants and plant-based products [3]. Herbal medicine is sometimes referred to as herbalism or traditional medicine. It is the application of herbs for their therapeutic or therapeutic value. A herb is a plant or a plant part evaluated for its medicinal, aromatic, or savory qualities. Herb plants produce and contain a difference of chemical substances that act upon the body [4]. Traditional medicine is defined as the health practices, approaches, knowledge, and beliefs incorporating plant, animal, and animal-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to prevent, diagnose and treat illnesses [1]. The seeds of *Nigella Sativa,* commonly known as black seed or black cumin have been used for medicinal purposes for centuries both as herb and pressed into oil in Asia, Middle East, and Africa [5].

Since old ancients, honey has been used traditionally for the treatment of many diseases including wound infections, respiratory tract infections, urogenital tract infections, and many others infections.

Many types of research to date have addressed honey and *Nigella sativa* antibacterial properties and their effects on many infections. The following Laboratory studies and clinical trials have shown that honey and *Nigella sativa* is an effective broad-spectrum antibacterial agent. the antibacterial activity of *Nigella sativa* against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) was studied and proved to have a strong inhibitory effect [6]. While in 2009 study under the title of the effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms showed that 100% of isolates were effectively inhibited by honey [7]. Local Sider and mountain Saudi honey were effective in inhibiting the *in vitro* growth of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii.* Sider honey was more potent than mountain honey in inhibiting these bacterial growths *in vitro* and both honey samples in the different concentrations were more effective against *E. coli* than other bacteria [8].

Materials and Methods

Design of study

This was a prospective cross-sectional and hospital base study.

Duration of study

From March 2018 to June 2018.

Study population

Non - Probability Sampling in Shendi hospitals - Sudan.

Sample size

A total of one hundred samples (n = 100) were collected.

Ethical approval

Permission was issued by the College of Ethical Committee, Shendi University, and the ethical committee of the hospital. Volunteers were informed and had got all the information about the research study.

Data collection

Data were collected from the patients using a structured questionnaire.

Study setting

Different hospitals and clinical centers are located in Shendi locality, River Nile State, Sudan. Shendi is a town in northern Sudan on the east bank of the River Nile 150 km northeast of Khartoum (16°41'N 33°25'E).

Specimen collection

Under the aseptic condition, wound swabs were collected using sterile cotton swabs moistened with sterile normal saline, urine and stool were collected in sterile screw-capped universal containers.

Culture of urine specimen

Different types of culture media (CLED agar, Blood agar, Macconckey Agar, and Chocolate blood Agar) were used for the identification and isolation of clinical isolates.

Interpretation of culture growth

The plates were observed for any bacterial colonies to grow significantly. The bacteria were well isolated and then identified by colonial morphology, Gram stain, and biochemical tests.

Preparation of plant extract

Extraction was carried out according to the method described by Sukhdev and his colleagues (2008). A Hundred grams of the plant sample was coarsely powdered using mortar and pestle and successively extracted with petroleum ether and methanol using the soxhlet extractor apparatus. Extraction was applied for four hours with petroleum ether and eight hours for methanol until the color of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally, the extracts were allowed to dry in Petri dishes till complete dryness.

Preparation of bacterial suspension

Clinical isolates were isolated from different samples in the sterile slope of nutrient and standard bacteria were brought from the microbiology department of the National Institute for Research. Ten ml of normal saline were distributed in test tubes and sterilized in an autoclave at 121oC for 15 mins. A loopful of the purified bacterium was inoculated in sterile normal saline. Inoculum density was compared with McFarland standard solution.

Procedure of inoculation in Mueller Hinton agar plates and applying *Nigella sativa* oil, Honey, and the mixture

The cup plate agar well diffusion methodology was adopted with some minor alterations to evaluate the antimicrobial activity of the prepared extract. 0.2 ml of bacterial suspension (standard and clinical isolates) were taken with automatic pipettes using the sterile tips and added to twenty ml of molten Mueller Hinton media and mixed and poured in a sterile plate. The media were allowed to set and solidify for minutes, make wells using a sterile Cork borer of 10 mm diameter. Alternated cups were filled with 0.1 ml of different concentrations of *Nigella sativa* oil, Honey, and the mixture (100%, 50%, 25%, and 12.5%), using automatic pipettes and Allowed to diffuse at room temperature for 30 min then the plates were incubated in an incubator in the upright position at 37oC for 18 hours.

Interpreting the sensitivity of *Nigella sativa* oil, Honey, and the mixture

The diameters of the resultant growth inhibition zones were measured in mm and the result was recorded. The inhibition zones with a diameter less than 12mm were considered as having no antibacterial activity [9].

Data analysis

Data were entered, check, and analyzed using Microsoft Excel 2007and SPSS (Statistical Package of Social Science) soft program version 11.5. Proportional data were presented as frequencies and percentages.

Results

A total of one hundred patients with symptoms of urinary tract infection, wound infection, Ear and Eye problems were enrolled in this study during the period from March to June 2018. Out of one hundred specimens, 31 samples were taken from patients with mean age Less than 20 age group, 42 from 21 - 40 age group, 15 from 41 - 60 age group, 9 from 61 - 80 age group. and 3 from More than 80 age groups (Table1). Out of 100 specimens, 53 (53%) were from males and 47 (47%) were from females (Table 2). In this study, six types of bacteria were isolated. The isolated bacteria were *K.pneumoniae* 30/95 (31%), *E.coli* 20/95 (21%), *S.aureus* 21/95 (21%), *Ps.aeruginosa* 15/95(16%), In addition to *Candida albicans*10 (11%), (Table 3). Out of twenty strains tested. the oil extract of *N. sativa* exhibited showed (90%) activity, Honey which showed low activity when compared with *Nigella sativa* oil (83%), In contrast with *N. sativa* oil and honey the mixture exhibited the highest activity, it showed (100%) against *E. coli* (Table 4). Out of twenty strains tested. the oil extract of *N. sativa* exhibited showed activity (100%), Honey which showed low activity when compared with *Nigella sativa* oil (25%), In contrast with *N. sativa* oil and honey the mixture exhibited the highest activity, it showed (100%) against *S. aureus* (Table 5). Out of thirty strains tested, the oil extract of *N. sativa* exhibited showed activity (66%), Honey which showed low activity when compared with *Nigella sativa* oil (25%), In contrast with *N. sativa* oil and honey the mixture exhibited the highest activity, it showed (72%) against *K. pneumoniae* (Table 6). Out of fifteen strains tested. the oil extract of *N. sativa* exhibited showed (88%) activity, Honey showed no activity when compared with *Nigella sativa* oil, In contrast, with *N. sativa* oil and honey the mixture exhibited the highest activity, it showed (87%) against *P. aeruginosa* (Table 7). Out of ten strains tested. the oil extract of *N. sativa* exhibited showed (100%) activity, Honey which showed low activity when compared with *Nigella sativa* oil (27%), In contrast with *N. sativa* oil and honey the mixture exhibited the highest activity, it showed (100%) against *C. albicans* (Table 8). The oil extract of *N. sativa*, honey, and the mixture presented variable activity against standard strains *S. aureus* ATCC 25923, followed by *K. pneumoniae* ATCC 53657, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 7596, as shown in (Table 9). The highest mean diameter of the growth inhibition zone in mm (MDIZ) of *N. sativa* oil for *S. aureus* was 47.9 mm at conccentration100%, while the lowest MDIZ was 39.8mm at concentration 12.5%. The highest MDIZ was 45.6mm for *C. albicans* at 100%, the lowest MDIZ was 21.5 mm at 25%. The highest MDIZ for *P. aeruginosa* was 19.9 mm at 100% the lowest MDIZ was 9.4 mm at 12.5%. For *E. coli* the highest MDIZ was 17.2 mm at 100%, while the lowest was 7 mm at 12.5%. The highest MDIZ was 14.2 mm at 100% for *K. pneumoniae*, while at concentration 12.5% showed no activity. The highest MDIZ of honey for *E. coli* was 26.6 mm at 100% while the lowest MDIZ was 12.1 mm at 12.5%. The highest MDIZ was 19.7 mm at 100% for *S. aureus* and the lowest MDIZ showed no activity at 12.5%. For *K. pneumonia*e the highest MDIZ was 16.4mm at 100%, the lowest MDIZ showed no activity at 12.5%. The highest MDZI was 12.9 mm at 100% for *C. albicans*, it showed no activity at 12.5%, the highest MDIZ was 4mm at 100% for *P. aeruginosa*, and it showed no activity at 12.5%. The mixture of *N. sativa* and honey showed the highest MDIZ 46.5 mm at 100% for *S. aureus*, the lowest MDIZ was 40.2 mm at 25%. For C*. albicans* the highest MDIZ was 41bmm at 100%, the lowest MDIZ was 25.3 mm at 12.5%. The highest MDIZ for *E. coli* was 28nmm at 100%, the lowest MDIZ was 13.7 mm at 12.5%. For *P. aeruginosa* the highest MDIZ was 15.1n mm at 100%, and the lowest MDIZ was 4.1n mm at 12.5%, while *K. pneumoniae* showed MDIZ 11.7n mm at 100% and the lowest MDIZ 3.6 mm at 12.5% (Table 10).

Ages groups	Number	%
Less than 20	31	31%
21-40	42	42%
41-60	15	15%
16-80	9	9%
More than 80	3	3%
Total	**100**	**100%**

Table 1: *Distribution of clinical samples according to age group.*

Gender	Frequency	Percentage
Male	57	57%
Female	43	43%
Total	**100**	**100%**

Table 2: *Distribution of clinical specimens according to the gender.*

Isolate	Frequency	Percentage
1. K.pneumoniae	30	31%
2. E.coli	20	21%
3. S.aureus	20	21%
4. P. aeruginosa	15	16%
5. Candida albicans	10	11%
Total	**95**	**100%**

Table 3: *Frequency and percentage of isolated organisms.*

Concentration	*Nigella Sativa*		Honey		Mixture	
	S	**R**	**S**	**R**	**S**	R
100%	19	1	20	0	20	0
50%	19	1	20	0	20	0
25%	19	1	19	1	20	0
12.5%	15	5	8	12	20	0
Percentage	**90%**	**10%**	**83%**	**16%**	**100%**	

Table 4: *Antimicrobial activity of Nigella sativa, honey and the mixture against twenty E. coli.*

Concentration	*Nigella Sativa*		Honey		Mixture	
	S	R	S	R	S	R
100%	20	0	20	0	20	0
50%	20	0	0	20	20	0
25%	20	0	0	20	20	0
12.5%	20	0	0	20	20	0
Percentage	**100%**		**25%**	**75%**	**100%**	

Table 5: *Antimicrobial activity of Nigella sativa, honey and the mixture against S. aureus.*

Concentration	*Nigella Sativa*		Honey		Mixture	
	S	R	S	R	S	R
100%	30	0	30	0	30	0
50%	29	1	0	30	27	3
25%	20	10	0	30	20	10
12.5%	0	30	0	30	10	20
Percentage	**66%**	**34%**	**25%**	**75%**	**72%**	**28%**

Table 6: *Antimicrobial activity of Nigella sativa, honey and mixture against thirty K. pneumoniae.*

Concentration	*Nigella Sativa*		Honey		Mixture	
	S	R	S	R	S	R
100%	15	0	0	15	15	0
50%	14	1	0	15	14	1
25%	12	3	0	15	12	3
12.5%	12	3	0	15	11	4
Percentage	**88%**	**12%**	**100%**		**87%**	**13%**

Table 7: *Antimicrobial activity of Nigella sativa, honey and the mixture against fifteen P. aeruginosa.*

Concentration	*Nigella Sativa*		Honey		Mixture	
	S	R	S	R	S	R
100%	10	0	10	0	10	0
50%	10	0	1	9	10	0
25%	10	0	0	10	10	0
12.5%	10	0	0	10	10	0
Percentage	**100%**		**27%**	**73%**	**100%**	

Table 8: *Antimicrobial activity of Nigella sativa, honey and the mixture against ten C. albicans.*

STD organisms	*Nigella Sativa*				Honey				Mixture			
	100%	*50%*	*25%*	*12.5%*	*100%*	*50%*	*25%*	*12.5%*	*100 %*	*50%*	*25%*	*12.5%*
S. aureus	S	S	S	S	S	S	-	-	S	S	S	S
E. coli	S	S	S	S	S	-	-	-	S	S	S	S
K.peu[1]	S	S	S	R	S	-	-	-	S	S	S	-
P. aeru[2]	S	S	S	S	S	-	-	-	S	S	-	-
C.albi[3]	S	S	S	S	S	-	-	-	S	S	-	-

Table 9: *Antimicrobial activity of N. sativa, honey and the mixture against standard organisms.*

[1]K. peumoniae,

[2]P. aeruginosa,

[3]C. albicans

(-) No antimicrobial activity.

(S) Susceptible.

organisms	*Nigella sativa*				Honey				Mixture			
	100%	*50 %*	*25 %*	*12.5%*	*100%*	*50%*	*25%*	*12.5 %*	*100 %*	*50 %*	*25 %*	*12.5 %*
E. coli	17.2	13.4	13.3	7.0	26.6	19.7	16.4	12.1	28	21.9	16.8	13,7
S. aureus	47.9	44.4	42.8	39.8	19.7	0	0	0	46.5	48.2	40.2	44.7
***K. pneu*[1]**	14.2	11.6	7.6	0	16.4	0	0	0	11.7	9.7	4.7	3.6
***P. aeru*[2]**	19.9	15.7	13.5	9.4	0	0	0	0	15.1	12.3	9.1	4.1
***C. albic*[3]**	45.6	43.6	21.5	29	12.9	1.3	0	0	41	41	33.2	25.3

Table 10: *The mean diameter of growth inhibition zone (MDIZ) in millimeter (mm)of Nigella sativa oil, honey and mixture against clinical isolates.*

[1]*K. peumoniae.*

[2]*P. aeruginosa.*

[3]*C. albicans*

Interpretation

Mean diameter of growth inhibition zone (MDIZ) in (mm)

MDIZ≥ 12 highly susceptible.

MDIZ< 12 resistance.

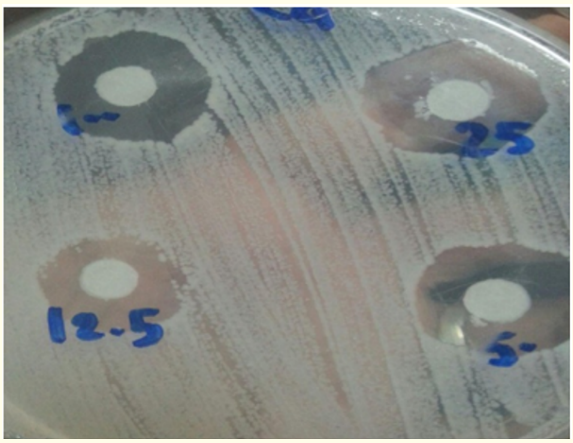

Figure 1: *Susceptibility of C. albicans ATCC 7596 to Nigella sativa at different concentrations.*

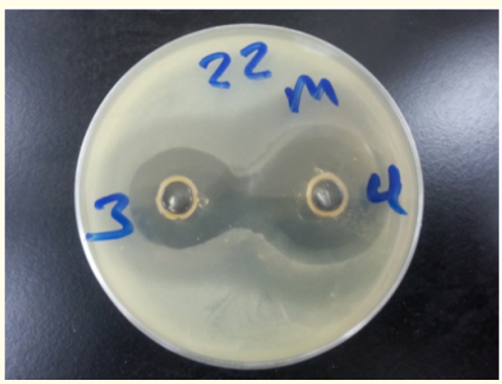

Figure 2: *Susceptibility of C. albicans to Mixture at different concentrations.*

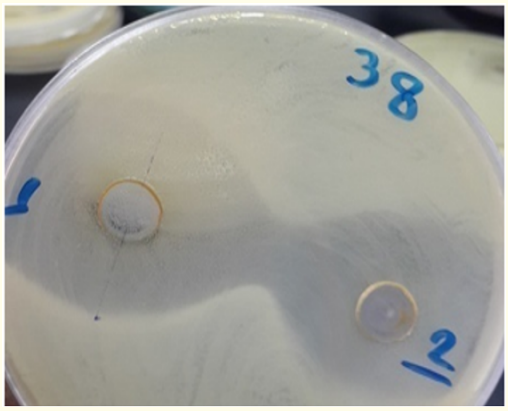

Figure 3: *Susceptibility of E. coli to Honey at different concentrations.*

Discussion

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains and this became a major cause of failure of the treatment of communicable disease [10]. Plants' fundamental oils and extracts are used for thousands of years, in food preservation, pharmaceutical, alternative medication, and natural treatments. Therefore, it is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care. Essential oils are potentials sources of novel antimicrobial compounds, especially against bacterial pathogens. *In vitro* studies during this work showed that the essential oils inhibited microorganism growth however in varying degrees of the effect. In the present study, *N. sativa* oil, honey, and the mixture exhibited activity against some of the selected bacterial and fungal strains. The oil extract of *N. sativa* produced a wide diameter of inhibition zone because it contains the major active substance (thymohydroquinone) required for antimicrobial activity, and the honey contains an active component (methylglyoxal) which is required for antimicrobial activity.

The results showed that *N. sativa* oil has the highest activity against *S. aureus*, the lowest activity against *K. pneumoniae*. Honey showed the highest activity against *E. coli*, the lowest activity against *P. aeruginosa*. While the mixture showed the highest activity against the selected clinical isolates when compared with *N. sativa* and honey, but the highest activity was against *S. aureus*, the lowest activity against *K. pneumoniae.* In this study petroleum ether extract of *Nigella sativa* showed remarkable antimicrobial activity against the standard and clinical isolates of *S. aureus, E. coli, Ps. aeruginosa,* and *K.pneumoniae.* While methanolic extract showed no activity, these results agreed with that obtained by [11]. However, negative results do not indicate the absence of bioactive constituents, since active compound (s) may be present in insufficient quantities in the methanolic extract to show activity with the dose levels employed. These findings are in agreement with several studies cited below. Toama found that *N. sativa* was active against Gram-positive bacteria *(S. aureus)* and yeast cells [12]. In 1989 Islam Sk, found that *N. sativa* oil has an antifungal effect [13]. In 1996 Bilal and his colleagues found a fairly good activity against most Gram-positive bacteria and some of the Gram-negative ones [14]. In 2005 Nazma Ara and his colleagues reported that the volatile oil showed strong activity against Gram-negative, Gram-positive bacteria, and *Candida albicans* [15]. In 2008 Zuridah. H found that *N. sativa* oil was active against Gram-positive cocci *(S. aureus),* Gram-negative bacteria (*E. coli*, *K. pneumonia,* and multidrug-resistant clinical strains of *P. aeruginosa*), and Gram-positive bacilli (*Bacillus cereus*) [16]. In 2009 Salman MT, Khan R.A., and shuku found that *Nigella sativa* oil has activity against multidrug-resistant clinical strains of *P. aeruginosa* [17]. The best inhibition zone obtained by petroleum ether extract of *Nigella sativa* was 48 mm in diameter against *S.aureus* ATCC 25923 and 63 mm against clinical isolates of *S.aureus* at a

concentration of 100 mg\ml. Followed by 14 mm against *K. pneumoniae* ATCC 53657 and 18 mm against clinical isolates of *K. pneumoniae* at a concentration of 100 mg\ml, then 17 mm against *E. coli* ATCC 25922 and 23 mm against clinical isolates of *E.coli* at a concentration of 100 mg\ml. Out of three methicillin-resistant *Staph. aureus* tested a pair of were sensitive to oil ether extract of *Nigella sativa*, these results agreed with that description by [6]. The founding of our study as regards the antimicrobial activity of honey agrees with several studies cited below. Hyungjaerm reported on the antimicrobial activity of different floral sources of honey against bacterial isolates, the results showed that 92.5% of bacteria was inhibited by honey [18]. Alandejani and his colleagues, study under the title of the effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms showed that 100% of isolates were effectively inhibited by honey [7]. NurAzida evidenced the antibacterial properties of honey and its result in wound management was created leading to that both Gram-negative bacteria isolated were completely inhibited by the honey tested [19]. Algurashi found that local Sider and mountain Saudi honey were effective in inhibiting the *in vitro* growth of *E. coli, K. pneumoniae, P. aeruginosa*. Both honey samples at different concentrations were more effective against *E. coli* than other bacteria (Alqurashi *et al.*, 2013). Modified diffusion technique was selected to conduct our research; because some trials used the disc diffusion technique and proved that discs were the source of contamination.

Conclusion

It was concluded that both *Nigella sativa* and honey; possess antimicrobial activity, but with varying degrees of effectiveness. The mixture was the most potent antimicrobial agent followed by *Nigella sativa* oil and honey. We believe this investigation together with previous studies provided support to the antimicrobial properties of honey and *Nigella sativa*. The herbal medicinal practice could provide a source for new drugs and therefore efforts should be directed to evaluate traditional medicinal practice based on scientific methodologies available. Resort a new source of antimicrobial agents to treat antibiotic-resistant microbes to avoid the high cost and side effects of medications. These results support the use of some plants as folk medicine.

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