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Original Article

CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF JORDANIAN PROPOLIS AND *NIGELLA SATIVA* SEED OIL AGAINST CLINICALLY ISOLATED MICROORGANISMS

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ABSTRACT

Objective: Increasing use of medicinal plants in the treatment of infectious diseases are due to the development of multi-antibiotics resistant microorganisms, and had alerted our interest in the examination of some natural products. This study was carried out to investigate the antimicrobial activity of Jordanian propolis, black seed oil (*Nigella sativa)* extract, alone or in combination against clinically isolated microorganisms (bacteria and fungi).

Methods: Jordanian propolis samples were collected. Aqueous and alcoholic extractions were done; black seed oil was extracted from *Nigella sativa* seeds. Seven clinical isolated microorganisms namely: *Micrococcus luteus, Bacillus pumilus, Bordetella bronchisptica*, *Enterococcus fecalis*, *Bacillus subtilis, and Staphylococcus aureus,* and one yeast strain namely *Candida albicans* were used. The antimicrobial activity was investigated by agar diffusion technique and microplate dilution to determine the MIC.

Results: The results indicated that the alcoholic propolis extract showed higher antimicrobial activity than the aqueous propolis extract. The antimicrobial activity of black seed oil was significantly higher than that of the propolis. Mixing propolis with black seed oil showed synergism effects against some microorganisms *as Enterococcus fecalis* (24±1.1), *Bordetella bronchisptica* (20±0.9) and *Candida albicans* (40±2.3), and additive with others as *Bacillus subtilis* (28±1.8).

Conclusion: Black seed oil and propolis might be used as a potential source of safe and effective natural antimicrobial in pharmaceutical and food industries.

Keywords: Propolis, Black seed (*Nigella sativa*) oil, Antimicrobial activity

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INTRODUCTION

Propolis is a gum produced by honey bees by assembling a gummy material from some trees, and processed in special ways by adding some bee's secretions. This material is used by bees in the construction of their hives, mainly to close the holes in the beehive, and is used as a protective barrier against bacteria and fungi, and has an antimicrobial effect against several human pathogens [1], against cariogenic organisms [2], against periodontal organisms [3], against respiratory infections [4], against gingival inflammation, against endodontic pathogens [5] and against oral ulcers [6]. Propolis presents numerous biological and pharmacological properties, such as immunomodulatory [7], antitumor [8], anti-inflammatory [9], antioxidant activity [10], neuroprotective activity [11], and hepatoprotective activity [12].

The chemical composition of propolis is dependent on the dominant vegetation cover. Propolis contains a variety of chemical compounds which mainly include polyphenols, flavonoids, amino acids, vitamins [13] and caffeic acid phenethyl ester [14]. Hundreds of compounds have been identified in different propolis samples of different botanical geographic origins, which may include fatty and phenolic acids and esters, substituted phenolic esters, flavonoids, terpenes, steroids, aromatic aldehydes, alcohols, sesquiterpenes, naphthalene, and stilbene derivatives [15]. Propolis generally contains 50 % resin, 30 % wax, 5 % pollen, 10 % aromatic oils and 5 % other organic residues [16]. However, the estimation of flavonoids remains crucial characterization of propolis.

The various biological activities of propolis have been attributed mainly to the presence of phenolic compounds, especially flavonoids and phenolic acids. Several of propolis constituents are present in food, which make it an attractive candidate as a natural preservative in new food applications [17]. Some of propolis components have antibacterial and antifungal action. Cinnamic acid and flavonoids contents were responsible for inhibiting bacterial motility [18].

Several extraction methods were used to prepare the propolis extraction, such as hydro-distillation, an organic solvent extraction method [19]. The ethanol extraction method is suitable for obtaining low-wax propolis extracts rich in biologically active compounds [16]. Biologically active substances of propolis have low solubility in water, and the amount of phenolic compounds in water extracts is lower than the amount in alcoholic extracts [20]. Aqueous propolis extracts and their major compounds possess higher pharmacological activity, as compared to alcohol extracts [21]. This variation in effectiveness may be due to the fact that the aqueous extract inhibits the generation of free radicals more effectively than the alcoholic extract [22]. Ethanolic extracts of propolis have been found to be effective against a broad range of bacteria, especially Gram-positive bacteria species [23]. Water soluble derivatives of propolis and its polyphenolic compounds significantly reduce the growth and proliferation of tumor cells [24]. It was also reported that phenolic compounds present in oil extracts of Brazilian propolis have effective antimicrobial and antitumor activity [25].

The black seeds of *Nigella sativa* contain a fixed oil of unsaturated fatty acids that represent 32-40% of the seed components, and volatile oil of saturated fatty acid in 0.4-0.5% [26]. Fixed and volatile oils have various therapeutic properties like antitumor activity, antioxidant activity, anti-inflammatory activity, antibacterial activity, and a stimulatory effect on the immune system, and they are effective against multi-antibiotic resistance bacteria [27]. The essential oil of the seeds has also dosed dependent anti-bacterial

activity on Gram positive and Gram-negative bacteria [28]. The fixed oil had more potent anti-bacterial effect against gram positive than gram-negative bacteria [29]. Many active principles have been isolated from black seed oil including thymoquinone, which is the main component.

Antimicrobial agents are one of the most significant arms in fighting infectious diseases, the emergence of drug resistant microorganisms' leads to investigate for newer drugs with lesser resistance. The objective of this research was to investigate the antimicrobial activity of Propolis and black seed oil, against clinical isolates bacterial and fungal strain when they are used alone or together.

MATERIALS AND METHODS

Chemicals

The following chemicals and materials were used: Ethanol 99.9 % (Super Chem Inc, Sarasota), distilled water, All chemicals in the biochemical analysis were purchased from Sigma/Aldrich (St. Louis, MO, USA).

Instrumentation

Analytical balance with a precision 0.01 mg (Phoenix Instrument, USA), autoclave machine (Rypa, Spain), incubator (EuroStar, EU), vortex mixer (Labinco, India), hot plate magnetic stirrer (Dragon, China), sterile tubes and sterile swab (MWe, UK), micropipette (Oxford, USA), Ultrasonic liquid processor (Qsonica LLC, USA).

Culture media

Muller Hinton broth, Muller Hinton agar, Sabouraud's dextrose broth, Sabouraud's agar was obtained from Oxoid Laboratories, Hampshire, UK.

Extraction of propolis

Jordanian propolis samples were collected by honey bees *(Apis mellifera*) during March and April 2015 from the Al Balqa area, located about 25 km west of Amman/Jordan. In this region the majority of the common pine trees is *Pinus helplines*. The collected propolis was washed thoroughly with water, dried in the air and was stored at-20 °C, then were cut into small pieces and extracted with water or with ethanol as the following:

Aqueous extraction

Propolis was macerated with deionized water for 2 h at 80 °C. The sample was mixed using magnetically stirred for 24 h and treated with liquid ultrasonic processor for 30 min at 60% amplitude, 20 kHz, and 500 watt. The extract was mixed again for 1 h. Then centrifuged for 15 min at 5000 RPM. The aqueous extracts were filtered using 15 µm filter paper.

Alcoholic extraction

Propolis was macerated with 99.9% ethanol with mixing (using magnetical stirrer) for 24 h. The sample was treated with liquid ultrasonic processor for 2 h at 60% amplitude, 20 kHz, and 500 watt. The extract was mixed with magnetically stirrer again for 1 h, centrifuged for 15 min at 5000 RPM. The alcoholic extracts were filtered using 15 µm filter paper. The pure extracts were stored at 4 °C in amber vials in the dark to prevent photo isomerization [30].

Black seed oil extraction

Black seed oil was extracted from the seeds of Nigella sativa with cold press at 4 °C in dark [31], the method extracted 100% pure organic oil. The extracted oil was preserved for the antimicrobial activity testing.

Black seed oil and propolis mixture

The mixture of black seed oil with propolis was prepared as follows: 1 g of propolis incubated at 80 °C for 2 h with 10 ml of Black seed oil, mixed and stirred using magnetically stirred for 1.30 h at 60 °C in an orbital shaker at 200 RPM. The extract was filtered using 15 µm filter paper.

Antibiotic containing disks

The disks were purchased from the local market (from company Abtek biological Ltd, Liverpool, UK).

Tested microorganisms

Six clinically isolated bacterial strains, namely: *Micrococcus luteus, Bacillus pumilus, Bordetella bronchisptica*, *Enterococcus fecalis*, *Bacillus subtilis, and Staphylococcus areas* and one yeast strain, namely: *Candida albicans* were used in this study, and were obtained from patients attending Prince Faisal hospital in Al-Zarqa/Jordan. All bacterial strains used throughout the present investigation were maintained on nutrient agar slants, while fungal isolates were maintained on Sabouraud dextrose agar. The cultures were stored at 4 °C, with regular transfer at monthly intervals, and their morphological characteristics confirmed by macroscopic and microscopic examination.

Screening for antimicrobial activity

The method of Rios 1998 [32] was used, to determine the antimicrobial activity of propolis extracts and black seed oil. Müller-Hinton agar plates seeded with an inoculum of 1.5x 10^8CFU/ml (equivalent to 0.5 McFarland) of freshly prepared microorganisms were used for this test. A well was cut in the agar medium with a sterilized (0.8 mm) cork borer. The oil or propolis extract was introduced into the wells (100 µl), and the plates were then incubated at 37 °C for 24 h. All plates were examined for the presence of zones of inhibition. The diameters of the zones were measured in millimeters in accordance with performance Standards for Antimicrobial Disk Susceptibility Tests (NCCL, 2002) [33].

Determination of minimal inhibitory concentration (MIC)

The most promising extract identified from the screening test was used for MIC determination by dilution method. This test was performed in sterile 96-well micro-titer plates [34]. The microorganism cultures were diluted in Müller-Hinton broth at a density adjusted to 0.5 McFarland turbidity. The final inoculum concentration was 1.5 x10^8CFU/ml of bacterial cultures. The wells were filled with 80 µl of sterile broth, 20 µl sterile tween 80 to the first row of the wells and 100 µl of the extracts were added to the first well in the row, and then serial two fold dilution was done. Each well was inoculated with 100 µl of 0.5 McFarland standard bacterial or fungal suspensions so that each well got 1.5 x10^8 CFU/ml. The 96-well micro-titer plates were covered, placed in plastic bags and incubated at 37 °C for 24 h. The MIC was the lowest concentration of the extract that inhibit microorganism growth (clear well).

Antibiotic susceptibility testing for the used microorganisms

Nine different antibiotics were selected, and the test was done using disc-diffusion method [35]. The concentration of antibiotics and interpretation of the size of inhibition zone was in accordance with performance standards for antimicrobial disk susceptibility tests, NCCL, 2002 [33]. All the antibiotic disks were purchased from Abtek biological Ltd, Liverpool, UK.

All antimicrobial testing was done in triplicate and the mean was calculated. The results of antimicrobial testing are reported and compared with those of standard drugs.

Propolis chemical characterization

Total protein estimation method were from Lowry *et al.* 1951 [36].

Total carbohydrate measurement method was from Simoni *et al.* 2002 [37].

Total lipid measurement method was from Righ *et al.* 1972 [38].

Flavone/Flavonols (FF) content: Samples were prepared according to the method of Marghitas *et al.* 2007, the absorbance was measured against a blank at 425 nm, Galangin was chosen as internal standard [39]

Flavonones/Dihydro Flavonols (FD): Samples were prepared according to Marghitas *et al.* 2007 method. The absorbance was read immediately at 486 nm against a blank, using pinocembrin as internal standard [39].

Statistical analysis

Statistical analysis was carried out using Student's t test by statistical packages for social science software (SPSS). Values are expressed as mean±SD and values of $p<0.05$ were considered

statistically significant. The relationships between variables were calculated using Pearson Correlation Coefficients.

RESULTS

The results show

Propolis chemical characterization. The propolis gave Flavone/ Flavonols of 0.1593% w/w and Flavonones/Dihydro Flavonols (FD) of 7.5180% w/w.

Black seeds oil characterization

Chemical analysis of black seed oil indicated it was 20% protein, 37% carbohydrate, and 37 % fats and oils (unsaturated fatty acid and volatile oil) in addition to minerals, this is in agreement with others [40], most of the pharmacological effects are due to quinine constituents of which Thymoquinone and melanin are the major components, this is in accordance with other workers [41, 42].

Thymoquinone and other component of black seed oil were qualitatively estimated according to Aljabre *et al.* 2005 [41] and Houghton *et al.* 1995 [42]. Good level was detected compared to others.

Antimicrobial activity measurement by disk diffusion method

The antimicrobial activity of the aqueous and alcoholic propolis extraction, black seed oil, and both of them together are presented in table 1.

Table 1: The zone of inhibition diameter in millimeter (mm) of the aqueous and alcoholic propolis extracts, black seed oil and both together, against clinically isolated microorganisms

Microorganisms	Aqueous propolis	Alcoholic propolis	Black seed	Black seed oil+propolis
	mm	mm	mm	mm
	Mean	Mean	Mean	Mean
Micrococcus luteus	15.8±0.8	19.4±1.2	28±0.9	28±2.1
Bacillus pumilus	13.6±0.6	17.4±1.0	28±1.0	28±2.4
Bordetella bronchisptica	6.6±0.3	8±0.3	0	20±0.9
Enterococcus fecalis	8.8±0.4	7.8±0.4	0	24±1.1
Bacillus subtilis	14.4±0.7	15.4±0.9	26±0.9	28±1.8
Staphylococcus aureus	13.8±0.2	13.4±0.7	28±0.8	28±2.0
Candida albicans	15.2±0.8	14±0.5	14±0.06	40±2.3

*The results were the mean±standard deviation (SD) of triplicate results and were the diameter of the test–The diameter of the control (solvent).

It can be seen that the aqueous propolis extract has similar antimicrobial activity against *M. luteus, B. pumilus, B. subtilis, S. aureus and C. albicans.* The zone of inhibition diameter was 15.8±0.8, 13.6±0.6, 14.4±0.7, 13.8±0.2 and 15.2±0.8 mm respectively and has less antimicrobial activity against *B. bronchisptica* (6.6±0.3 mm) and *E. fecalis* (8.8±0.4 mm) compared to other bacteria. Alcoholic propolis has higher antimicrobial activity than the aqueous extract, but the differences are not significant. The zone of inhibition diameter for the alcoholic propolis extract against *M. luteus, B. pumilus, B. subtilis, C. albicans, S. aureus, B. bronchisptica, E. fecalis,,* and were 19.4±1.2, 17.4±1.0, 15.4±0.9, 14±0.5, 13.4±0.7, 8±0.3, 7.8±0.4 mm respectively.

The antimicrobial activity of black seed oil against bacteria and fungi were significantly higher than that of propolis extracts. The inhibition zone diameter against *M. luteus, B. pumilus, B. subtilis S. aureus and C.* albicans were: 28±0.9, 28±1.0, 26±0.9, 28±0.8, and 14±0.06 mm respectively. Although, it has no antimicrobial activity against *B. bronchisptica* and *E. fecalis.*

When propolis extract was mixed with black seed oil, the antimicrobial activity was significantly increased against all of the tested microorganisms when compared with the antimicrobial activity of propolis alone; it was increased to 28±2.1, 28±2.4, 20±0.9, 24±1.1, 28±1.8, 28±2.0 and 40±2.3 mm against *M. luteus, B. pumilus*, *B. bronchisptica*, *E. fecalis, B. subtilis, S. aureus, and C. albicans* respectively. While the addition of the black seed oil to propolis significantly increased the antimicrobial activity against *B. bronchisptica*, *E. fecalis, and C. albicans* when compared with the activity of the black seed oil alone, while the antimicrobial activity against *M. luteus, B. pumilus*, *B. subtilis, and S. aureus* were similar as the activity alone.

Antibiotic susceptibility testing

The antibiotic susceptibility testing was done using (Tetracycline 300 µg, Gentamicin 10 µg, Cefazolin 30 µg, Neomycin 30 µg, Ampicillin 10 µg, Nitrofurantoin 300 µg, Vancomycin 30 µg and Penicillin G 10 IU), against *B. subtilis, B. pumilus, S. aureus, E. fecalis, M. luteus, and C. albicans,* the result is shown in table 2. The results showed variation in the susceptibility of microorganisms, in general, most of the tested microorganisms showed no susceptibility or low to the commonly used antibiotics (table 2) as *E. fecalis* and *B. bronchisptica.*

The minimum inhibition concentration determination, MIC

The minimum inhibitory concentration (MIC) of propolis extracted with different solvent (water, alcohol), black seed oil, and propolis with black seed oil, against all tested microorganisms is shown in table 3.

Table 2: Antibiotic susceptibility testing (zone of inhibition diameter in mm) of clinically isolated microorganisms using commonly available antibiotics

Microorganism	TE	GN	CZ	N	AM	F	VA	P 10
Bacillus subtilis	12±0.5 mm	8±0.6 mm	4±0.1 mm	7±0.3 mm	7±0.5 mm	7±0.5 mm	9±0.8 mm	8±0.8 mm
Bacillus pumilus	17±1 mm	15±0.5 mm	9±0.3 mm	9±0.8 mm	11±1 mm	7±0.4 mm	11±0.4 mm	13±1.2 mm
Staphylococcus aureus	14±0.8 mm	6±0.4 mm	10±0.5 mm	5±0.4 mm	12±0.8 mm	8±0.2 mm	6±0.2 mm	9±0.6 mm
Enterococcus fecalis	3±0.1 mm	0	0	0	5±0.2 mm	6±0.5 mm	5±0.4 mm	0
Micrococcus luteus	13±1 mm	10±0.8 mm	5±0.2 mm	7±0.4 mm	9±0.4 mm	0	6±0.5 mm	16±1.2 mm
Bordetella bronchisptica	10±0.8 mm	6±0.4 mm	0	5±0.3 mm	0	0	0	0

*The results were the mean±standard deviation (SD) of the zone of inhibition diameter of triplicate results, TE tetracycline 300 µg, GN gentamicin 10 µg, CZ cefazolin 30 µg, N neomycin 30 µg, AM ampicillin 10 µg, F nitofurantoin 300 µg, Va vancomycin 30 µg, P 10 penicillin G 10 IU, mm millimeter.

Table 3: The minimum inhibition concentration (MIC) of aqueous propolis, alcoholic propolis, black seed oil, and for propolis mixed with black seed oil against strains of *B. subtilis, B. pumilus, S. aureus, E. fecalis, M. luteus,* and *C. albicans*

Microorganism	black seed oil	Alcoholic propolis extract	Aqueous propolis extract	Propolis extract+black seed oil
Bacillus subtilis	8±0.5µg/ml	8±0.3 µg/ml	31±0.8 µg/ml	8±1 µg/ml
Bacillus pumilus	125±2.2 µg/ml	4±0.5 µg/ml	4±0.3 µg/ml	31±1.4µg/ml
Staphylococcus aureus	31±0.6 µg/ml	8±0.6 µg/ml	8±0.8 µg/ml	125±3 µg/ml
Enterococcus fecalis	8±0.4 µg/ml	16±1 µg/ml	4±0.5 µg/ml	31±0.8 µg/ml
Micrococcus luteus	8±0.5 µg/ml	1±0.3 µg/ml	31±1.5 µg/ml	8±0.5 µg/ml
Candida albicans	63±4 µg/ml	8±1.2 µg/ml	16±1.2 µg/ml	125±4 µg/ml
Bordetella bronchisptica	8±0.2 µg/ml	63±1.2 µg/ml	8±0.6 µg/ml	125±3.0 µg/ml

*The results were the mean±standard deviation (SD) of triplicate results

The MIC of black seed oil, and alcoholic propolis, against *B. subtilis* was 8±0.5, 8±0.3 µg/ml compared to that of 31±0.8 µg/ml for aqueous propolis, no decrease in the MIC was detected when the results of them are compared to the result of mixing them together (8±1 µg/ml). The MIC of propolis against *B. pumilus* was significantly lower than that of black seed oil (4±0.5µg/ml and 125±2.2 µg/ml) respectively, and was the same for alcoholic and aqueous extracts of propolis (4±0.5, 4±0.3 µg/ml). The same results were detected when comparing the MIC of propolis and black seed oil against *S. aureus* (8±0.6 µg/ml, 31±0.6 µg/ml respectively), and when they mixed together the MIC was increased to 125±3 µg/ml. The same results were detected when comparing the MIC of alcoholic propolis extract and black seed oil against *M. luteus,* the alcoholic extract of propolis gave better MIC than black seed oil (1±0.3 µg/ml, 8±0.5 µg/ml respectively), and no better MIC when they mixed together. The MIC of the alcoholic propolis extract was better than black seed oil and when they mixed together. These results indicate that propilis extracts and black seed oil possessed considerable antimicrobial activity, and this is in agreement with other researchers [1-6, 9, 43], with the alcoholic propolis extract being the most potent, which may indicate that the potent activity of propolis is lipophilic compound, and mixing them together did not improve their antimicrobial activity, which indicate that they have components which are antagonized. Surprisingly the aqueous propolis extract gave better MIC than the black seed oil and the alcoholic propolis extract (4±0.5 µg/ml, 8±0.4 µg/ml, and 16±1 µg/ml respectively) against *E. fecalis.*

Black seed oil and the mixture of black seed oil with propolis have the highest antibacterial activity against *S. aureus* with zones of inhibition of 28±0.8, 28±2.0 mm compared with propolis extract alone with a zone of inhibition of 13.8±0.2, 13.4±0.7 mm. The MIC for propolis, black seed oil and propolis with black seed oil were 8±0.6, 31±0.6 and 125±3 µg/ml respectively. The effect of propolis, black seed oil and propolis with black seed oil against *S. aureus* was better than the effect of all tested antibiotics, the highest zone of inhibition diameter was 14±0.8 mm when tetracycline 300 µg antibiotic was used.

Propolis extracts possess less antimicrobial activity against *B. subtilis* and *B. pumilus* compared with the antimicrobial activity of black seed oil; also, there is a little interaction effect of propolis when mixed with black seed oil. The inhibition zones of propolis extracted with water, propolis extracted with Alcohol, black seed oil and propolis mixed with black seed oil against *B. subtilis* were 14.4±0.7, 15.4±0.9, 26±0.9 and 28±1.8 mm respectively, and against *B. pumilus* were 13.6±0.6, 17.4±1.0, 28±1.0, and 28±2.4 mm. All antibiotics had lower activity against *B. subtilis,* the highest effect was tetracycline 300 µg with inhibition zone of 12±0.5 mm.

The highest effect against *M. luteus* was for black seed oil (28±0.9 mm), and for the mixture of propolis with black seed oil (28±2.1 mm) compared with propolis extracted with water (15.8±0.8 mm), and propolis extracted with alcohol (19.4±1.2 mm). No interaction effect of propolis and black seed oil was obtained in our study against *M. luteus.*

A maximum zone of inhibition was obtained against *B. bronchisptica* when propolis mixed with black seed oil (20±0.9 mm), compared with black seed oil (no effect) and propolis extracted with water (6.6±0.3 mm) or extracted with alcohol (8±0.3 mm).

In this study, significantly maximize in a zone of inhibition against *C. albicans* was observed for the mixture of propolis with black seed oil (40±2.3 mm) compared with single effect propolis extracts (15.2±0.8, 14±0.5 mm) or black seed oil (14±0.06 mm).

DISCUSSION

The use of plant extracts and phytochemicals, both for their antimicrobial properties were known for ages [44]. The results of the work showed that black seed oil, propolis extracts and both together possessed considerable antimicrobial activity against some Gram positive bacteria (*B. subtilis, B. pumilus, S. aureus, E. fecalis, M. luteus),* Gram negative bacteria *(B. bronchisptica*) and yeast (*C. albicans)*, this is in agreement with other researchers [1-6, 9, 43, 45], although the tested microorganisms used in this study showed high resistance against the most available antimicrobial agents (table 2). The alcoholic propolis extract being the most potent; although black seed oil showed excellent antimicrobial activity against the majority of the tested microorganisms (28-26 mm diameter), black seed oil shows no activity against *B. bronchisptica* and *E. fecalis,* with the aqueous propolis extract was the least potent among them (table 1). This could be explained by the fact that the active component is present in the alcoholic propolis extract in a higher amount than the aqueous propolis extract, this result is in agreement with other [29].

When propolis was added to the black seed oil, the antimicrobial activity was significantly increased against all tested microorganisms, the zone of inhibition diameter was increased to 20±0.9, 24±1.1, and 40±2.3 mm against *B. bronchisptica*, *E. fecalis* and *C. albicans* respectively (table 1), while black seed oil alone showed no activity against *B. bronchisptica* and *E. fecalis*. Enhancement of the antimicrobial activity of black seeds oil and propolis extract was achieved by combing them together, suggesting the presence of a potent synergistic activity against some microorganisms and addition to others.

Alcoholic extract of propolis had the lowest MIC as 1±0.3, 4±0.5, 8±0.6, 8±1.2 µg/ml against *M. luteus, B. pumilus, S. aureus and C. albicans* respectively (table 3). The fact that the alcoholic propolis extract possessed higher antimicrobial activity than the aqueous extract may prove that the potent activity of propolis is due to lipophilic components in addition to hydrophilic components.

Using the MIC determination to describe the antimicrobial activity of black seed oil gave better results as shown in table 3, black seed oil showed good MIC against *B. bronchisptica*, *E. fecalis* (8±0.2, 8±0.4µg/ml), while evaluating their antimicrobial activity using agar diffusion test (table 1) revealed that black seed oil showed no antimicrobial activity against *B. bronchisptica*, *E. fecalis,* which may indicate that the diffusion of the active components were retarded (molecular size or permeability barrier) in the agar medium and contributed to such variation [46].

The good shelf-life of propolis and black seed oil as well as other desirable characteristic features which had been reported by others, the good antimicrobial activity of the two makes them suitable ingredients in pharmaceutical, nutraceutical and cosmetic products.

CONCLUSION

According to the present study, it can be concluded that black seeds oil and propolis had proven to show significant antimicrobial

activity, against various microbial types through different inhibitory mechanisms. Our results revealed the possibility of using them together in treating various underlying causes of different infection as a replacement of antimicrobial agents to lower the development of antimicrobial resistant microorganisms.

However, additional studies are required to evaluate and explore the specific cellular, and molecular mechanisms of the antimicrobial activity of black seed oil and propolis.

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CONTRIBUTION OF EACH AUTHOR

Dr. Sabah Al-muhtaseb is the chief author, supervised the work from the beginning to the end, responsible for writing the biochemical part.

Dr. Najah Al-muhtaseb and Dr. Sabah Al-Muhtaseb were responsible for the biochemical investigations.

Mahmoud Al-Masri (MSc) was responsible for the laboratory work

Prof. Elham Al-kaissi was responsible for writing and supervised the microbiology part.

Dr. Ibrahim Al-Adham was responsible for the flavone/flavonls and Flavonones/Dihydro Flavonols determination and the Microbiology part

Dr. Amjad Abu Sirhan was responsible for the statistic part

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest

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