



Synergistic effect of propolis and antibiotics on the *Salmonella Typhi* TY21

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Abstract:

The study was aimed at determining the synergistic effect of crude ethanolic extract of AL-Najaf propolis (EENP) with antibiotic (Ampicillin and Gentamycin) against *Salmonella typhi* by the method of disc diffusion. Result revealed that EEP a good antibacterial activity against bacteria at different concentrations 100,50 mg/ml and the results of Ampicillin–propolis combination on the bacterial isolates were; synergistic to words *Salmonella Typhi*. Statistical analysis showed significant differences between effect of Ampicillin and Ampicillin –propolis combination on bacterial isolates ,there was no significant differences between gentamycin and gentamycin–propolis combination at level ($P \leq 0.05$). but there are additive effect on *salmonella typhi*.

Key words: propolis extract, Gentamicin ,Ampicillin, synergistic effect.

التأثير التآزري للبروبوليس مع المضادات الحيوية ضد سالمونيلا تاي في

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الخلاصة:

هذه الدراسة تهدف الى تقييم التأثير التآزري للتركيزات المختلفة من المستخلص الايثانولي للبروبوليس العراقي الخام مع الامبيسلين والجنتاميسين ضد *salmonella typhi* وفقا لتقنية انتشار الاقراص الورقية وقد وضحت النتائج ان هناك تثبيط جيد ضد البكتريا في التركيزات 100,50 mg/ml. كما أظهرت نتائج خليط (الامبيسلين والبروبوليس) بأن لها تأثير تآزري على *salmonella typhi*. التحاليل الإحصائية أظهرت أن هناك اختلاف معنوي بين تأثير الامبيسلين و خليط (الامبيسلين والبروبوليس) , لكن لا يوجد اختلاف معنوي بين تأثير الجنتاميسين وخليط الجنتاميسين والبروبوليس عند مستوى ($P \leq 0.05$) , لكن هناك تأثير مضاف .

Aim of the study

- 1) the investigation of the antimicrobial activity of propolis collected from Iraq/Naja
- 2) the study of synergism between propolis and antibiotics.

Introduction :

Propolis is a resinous natural substance produced by honeybees from plant exudates, beeswax, and bee secretions. Propolis is composed of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances. However, the composition varies according to the geographical and plant sources, and the

collection season. The main function of propolis in honey bee hives is to control temperature, light, and humidity. Furthermore, it protects hives from pathogens and some colony invaders. Propolis has wide range of biological activities which include antimicrobial, antioxidant, anti-inflammatory, anesthetic and anticancer properties. (1,2).

Propolis was used specially in antiquity, in Egypt. There some thousand years BC, propolis was very well known to the priests who had monopolized medicine, chemistry and art of mummifying corpses. The fact that propolis was also known to the old Greeks is demonstrated by the very Greek name of it (3). Abu Ali bin Sina (Avicenna) distinguishes two kinds of wax in his well known work, the clean and the black wax. the black is the filth the hive. It is clear enough that the black wax is propolis that after Avicenna's testimony. Honey was mentioned in the Holy Quran 1400 years ago . As a natural honeybee hive product, propolis extracts have been used both internally and externally for thousands of years as a healing agent in traditional medicine. (4). Because of propolis diverse biological activities and increasing industrial interest, its chemical composition is of great importance. Furthermore, investigations have shown that individual compounds such as the flavonoids (6) are responsible for spasmolytic , anti-inflammatory , antiulcerative or antimicrobial activities(5,6). In laboratory tests, studies have shown broad spectrum antimicrobial activity of various propolis extracts. Synergism with certain antibiotics has been demonstrated. Depending upon its composition, propolis may show powerful local antibiotic and antifungal properties. (7).

Different researchers (8, 9, 10 ,11) have reported that propolis antibacterial activity is attributed to a number of phenolic compounds, mainly flavonoids, phenolic acids and their esters. Some prenylated

coumaric acids were isolated from propolis in several countries (12). *Salmonellae* are Gram-negative, facultative anaerobic, non-endospore forming, usually motile rods that cause gastroenteritis and enteric fever, constituting a significant ongoing threat to worldwide public health. Its virulence requires the expression of complex arrays of virulence factors that allow the bacterium to evade the host's immune system (13).

Salmonella are responsible for human and animal diseases that range from mild gastroenteritis to host-disseminated enteric fever (13). Bacterial drug resistance is an important world problem (14,15). Poppe *et al.* (16) verified that *Salmonella* serovars, isolated from food or infected animals, were resistant to several antibiotics. (17) and Stoner *et al.* (18) also reported an increased resistance of *Salmonella* serovars to several antimicrobial drugs.

The antimicrobial activities of propolis toward various pathogens have been widely investigated. Propolis poses bacteriostatic activity against different bacteria, and in high concentration it has a bactericidal activity(14,2). Synergism between propolis and antibacterial agents has been observed (19,20). In this regard, it was found that there is synergism between propolis and antimicrobial drugs against *S. aureus* especially those agents that interfere on bacterial protein synthesis (20).

Material and Methods:

Propolis samples

Propolis samples were collected from hives of honey bees of Najaf / Iraq in (2012-2013). Propolis samples were cleaned, free of wax, paint, wood, cut into small pieces, and placed in clean container.

Ethanolic extract of propolis:

Ten gm of propolis were mixed with 100 ml of ethanol in dark brown bottle and left for 7 days at room temperature and in dark place. the container was shaken 2 or 3 times per day and returned to warm dark

place. The liquid was filtered through Whatman No.1 and the water was evaporated by oven at 45 °C, then the extract was weighed and stored in dark clean container for further using . Ethanolic extract was dissolved by Dimethyl Sulfoxide (DMSO), sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were Prepared.

Bacterial strains

Standard bacterial strains and local isolates used in this study is *Salmonella typhi* TY21. The Standard bacterial strains were activated and cloned three successive times in Nutrient agar and stored on nutrient agar slants at 4 °C. The identification of the local bacterial isolates was confirmed using conventional biochemical tests (21). Antimicrobial susceptibility was tested using paper disc agar diffusion method (22). Paper discs (5 mm) were sterilized by autoclave and soaked in a propolis extracts (ethanolic solution) with different concentrations 50mg/ml,100mg/ml).

Preparation of antibiotics discs and measurement of inhibition zone

Antibiotics susceptibility was tested by using the paper disc agar diffusion method (25) and the zones of inhibition were subsequently measured in mm .Gentamycin 0.1mg,Ampicillin 0.03.

Antibiotics and propolis susceptibility test bacteria

The solutions (antibiotic with propolis solution) were applied by using disc , The plates were incubated at 37 C° for overnight. The size of zone of inhibition was measured .

Statistical analysis

Snedecor and Cochran for Statistical analysis ($P \leq 0.05$) to show if there is any significant differences between result of disc diffusion method of propolis ethanolic extract (32).

Result and Discussion:

We could verify that *Salmonella Typhi* is susceptible to propolis from Al-Najaf at concentration (100mg/ml, 50mg/ml). (Figure 1,2,3,4). As a general rule extract is considered active against both bacteria and fungi when the inhibition zone is greater than 6 mm (23). the effect of EEP was elevated when the concentration increased to 100mg/ml The zones of inhibition of *Salmonella Typhi* were 45 mm whereas the zones of inhibition of 50 mg/ml were 30mm (table1).

EEP a good antibacterial activity against bacteria at different concentrations 100,50 mg/ml . Statistical analysis showed significant differences after treating the microorganisms with propolis ethanolic extract at different concentrations of disc diffusion ($P \leq 0.05$).(table,1,2,3)

This result indicated that the active components of propolis were concentrated in the sample. This was in agreement with reports of several papers which indicated that each propolis sample contained 80–100 chemical compounds with different concentrations (7, 9,11,12 , 24 and 25)

A possible explanation for propolis action mechanism may be the fact that one or some of its constituents caused a significant inhibition of bacterial mobility, besides ion permeability alteration on the inner bacterial membrane (26).This effect of ethanolic extract of propolis reflects its antibiotic action on *Salmonella*, suggesting its possible use as an alternative control of *Salmonella* infection.

The activities of propolis combination with antibiotics on the bacterial isolates were studies.(table,1,2,3.),figure (1,2,5,6). The results of Apmicillin–propolis combination on the bacterial isolates were; synergistic to words *Salmonella Typhi* Statistical analysis showed significant differences between effect of Apmicillin and Apmicillin –propolis combination on bacterial isolates there was no significant differences between gentamycin and gentamycin–propolis combination at level

(P0.05) ,(table,1).but there are additive effect on salmonella typhi A synergistic effect of different propolis samples with commercial antibiotics was reported worldwide(27). Beta-lactamic antibiotics (Amoxicillin, Ampicillin and Cefalexin) act on a group of proteins called "Penicillin-binding-proteins" (PBP), found in the periplasmic space of Gram-negative bacteria. These PBP are involved in the peptideoglican synthesis - an important structure of bacteria wall (28). Although little is known about the mechanisms of propolis antibacterial action, Takaisi-Kikuni and Schilder (29) verified that ethanolic extract of propolis acted on *S. agalactie* growth, inhibiting the protein synthesis. Mirzoeva *et al.* (26) reported that propolis and some of its cinnamic and flavonoid components were found to uncouple the energy transducing

cytoplasmic membrane and to inhibit bacterial motility. Koo *et al.* (30) suggested that propolis could act on the enzymatic activity of *S. mutans* and *S. sanguis*. Krol *et al.* (31) related that propolis had a marked synergistic effect on the antibacterial activity of streptomycin and cloxacillin towards *Staphylococcus aureus*. Scheller *et al.* (27) observed a synergistic effect between an ethanolic extract of propolis and antibiotics used against mycobacteria.

In our work, propolis seemed to aid beta-lactamic antibiotics in PBP inhibition, what could explain the synergistic effects. Besides, propolis could also diminish the resistance of the bacteria wall to antibiotics, as suggested by Mirzoeva *et al.* (26), promoting beta-lactamics action on PBP.

Table (1) . Effect of different concentration of Al-Najaf propolis and antibiotics (Ampicillin and Gentamicin) on the Salmonella Typhi

Treatment	Zone of inhibition Mean \pm SE
Ampicillin 0.03mg	29 \pm 0.1 c
Gentamicin 0.1mg	27 \pm 0.15 c
100mg/ml Propolis + Ampicillin	40 \pm 0.11 b
100mg/ml Propolis + Gentamicin	27 \pm 0.24 c
50 mg/ml Propolis	30 \pm 0.16 c
100 mg/ml Propolis	45 \pm 0.15 a

Means with the same letter are not significantly different. T tests (LSD) ($P \leq 0.05$).

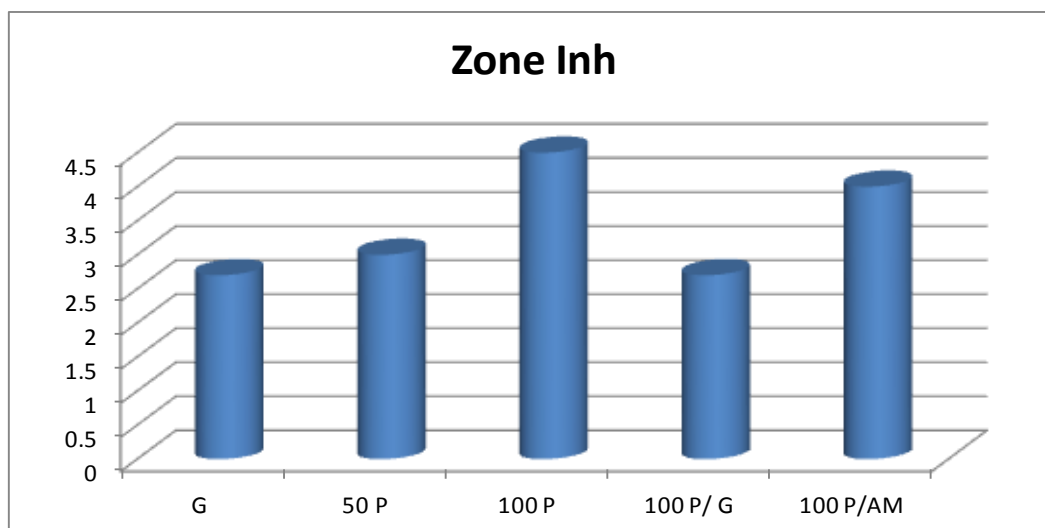


Figure (1) . Effect of different concentration of Al-Najaf propolis and antibiotics (Ampicillin and Gentamicin) on the Salmonella Typhi by disc diffusion method as compares with Gentamicin

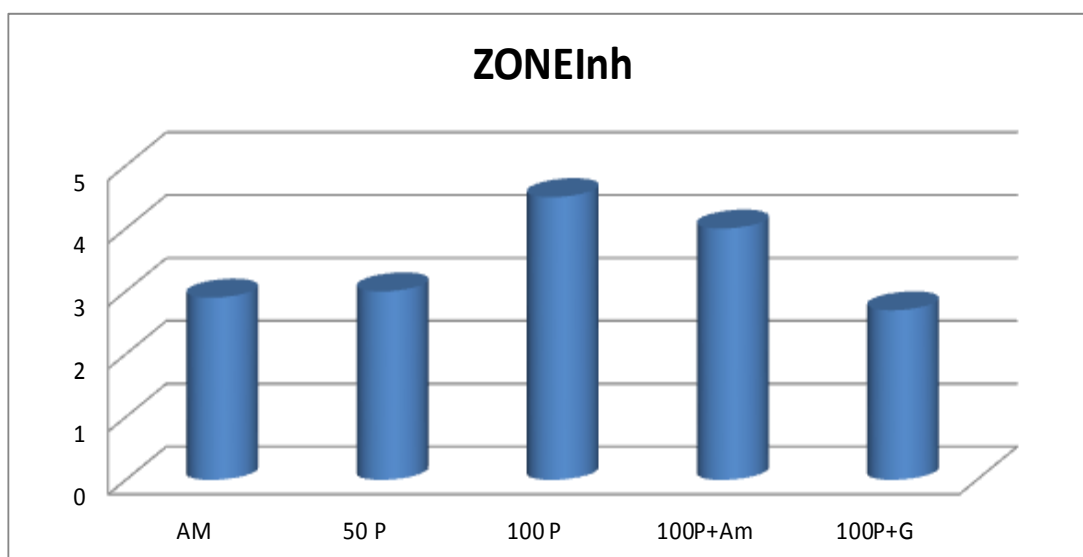


Figure (2). Effect of different concentration of Al-Najaf propolis and antibiotics (Ampicillin and Gentamicin) on the Salmonella Typhi by disc diffusion method as compares with Ampicillin.

Table 2. Effect of different concentration of Alnajaf propolis and antibiotics (ampicillin and gentamicin) on the Salmonella Typhi as compares with Ampicillin

Treatment	Zone of inhibition Mean \pm SE
Ampicillin 0.03mg	29 \pm 0.1 c
100mg/ml Propolis + Ampicillin	40 \pm 0.11 b
100mg/ml Propolis + Gentamicin	27\pm0.24 c
50 mg/ml Propolis	30 \pm 0.16 c
100 mg/ml Propolis	45\pm 0.15 a

Means with the same letter are not significantly different. T tests (LSD) ($P \leq 0.05$).

Table (3) . Effect of different concentration of Al-Najaf propolis and antibiotics (Ampicillin and Gentamicin) on the Salmonella Typhi as compares with Gentamicin

Treatment	Zone of inhibition Mean \pm SE
Gentamicin 0.1mg	27 \pm 0.15 c
100mg/ml Propolis + Ampicillin	40 \pm 0.11 b
100mg/ml Propolis + Gentamicin	27\pm0.24 c
50 mg/ml Propolis	30 \pm 0.16 c
100 mg/ml Propolis	45\pm 0.15 a

Means with the same letter are not significantly different. T tests (LSD) ($P \leq 0.05$).

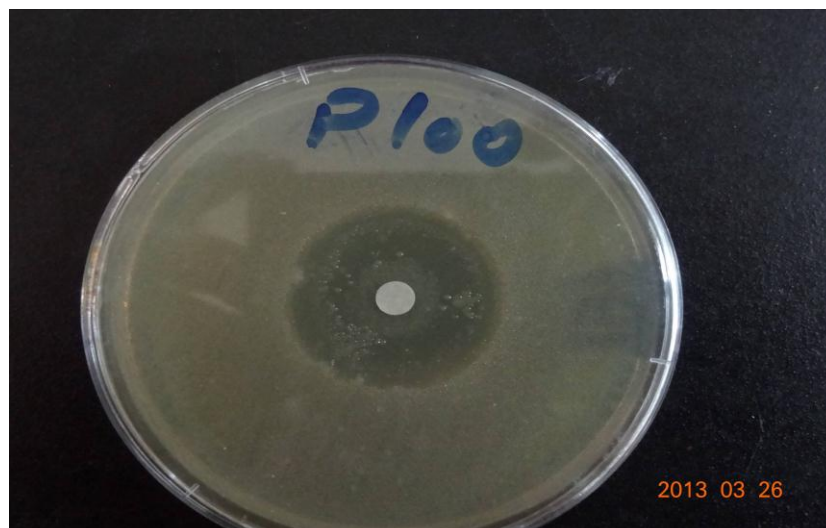


Figure 3: Zone inhibition of *salmonella* growth on MHA produced by ethanol extracts of propolis 100mg/ml.



Figure 4: Zone inhibition of *salmonella* growth on MHA produced by ethanol extracts of propolis 50mg/ml.



Figure.5 Zone inhibition of *salmonella* growth on MHA produced by ethanol extracts of propolis +Ampicillin.



Figure 6: Zone inhibition of *salmonella* growth on MHA produced by ethanol extracts of propolis +Gentamycin.

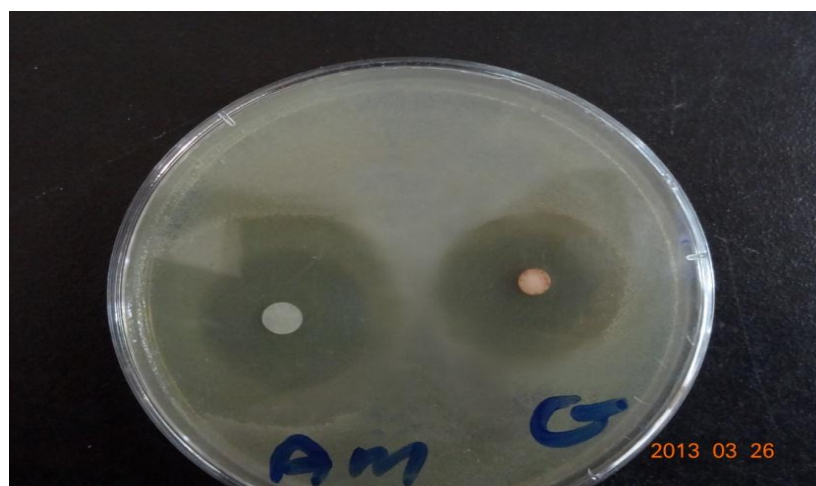


Figure 7: Zone inhibition of *salmonella* growth on MHA produced by Gentamycin and Ampicillin.

Conclusion:

We conclude that Al-Najaf propolis had an antibacterial action on *Salmonella* Typhi, Al-Najaf propolis shows synergistic effect with Ampicillin antibiotics acting on the cell wall, and additive effects with gentamicin. This result is important to reduce the antibiotic clinical doses and their marked side effects.

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