Further studies on American foulbrood disease of honey bees, *Apis mellifera*

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VII. ENGLISH SUMMARY.

A. Effects of crude natural antibacterial substances on *Paenibacillus larvae larvae*:

1. Effects of essential oils on the bacterial growth of *P. l. larvae*:

1.1. Essential oils extracted from plant Family Rutaceae:

The essential oil of *Citrus Limon* was extracted from leaves and peels also, used fruit juice. The essential oil of *Citrus reticulata* and *Citrus sinensis* were extracted from leaves and peels. The above mentioned essential oils compared with antibiotics. Tylosine which already used by beekeepers was used as a reference antibiotic to identify the most effective essential oil by calculate the relative efficiency percentage (R.E.). The other two antibiotics Doxycycline and Tylo-Doxine were tested in our previous work. The inhibition zones were measured.

1.1.1. The essential oils extracted from *Citrus limon*:

The essential oil of leaves of *C. Limon* was higher antibacterial activity. Such essential oil exhibited 164.97% R.E. with inhibition zone of 27.50 mm. followed by the peels of *C. Limon* exhibited the least effective with 86.98% R.E and inhibition zone of 14.50 mm .When leaves mixed with peels the relative efficiency was 98.98% with inhibition zone of 16.50 mm and them the *C. Limon* juice recorded of 61.15% R.E with 10.50 mm inhibition zone.

1.1.2. The essential oils extracted from *Citrus reticulata*:

Data indicated that the essential oil of leaves of *C. reticulate* recorded 102.37% R.E. with 14.67 mm inhibition zone. In case of *C. reticulate* peels recorded 68.60% R.E. less than Tylosine but the essential oil of peels of *C. reticulate* when mixed with Leaves recorded 100.00% R.E.

1.1.3. The essential oils extracted from *Citrus sinensis*:

The essential oils of leaves and peels of *C.sinensis* recorded 80.34% and 18.77% R.E, respectively and the essential oil of mixture of leaves and peels recorded 50.88% which means that there is an agonistic effect for peels when mixed with leaves essential oil.

The essential oil of leaves *C. Limon* showed significant difference compared with Tylosine. But all of the peels and mixture of leaves and peels of *C. Limon* leaves and mixture of leaves and peels of *C. reticulata* showed high effect with no significant difference. However, such essential oils can be used to control the *P. l. larvae* rather than antibiotics as a natural alternative compounds.While *C. Limon* juice, leaves, mixture of leaves and peels and peels of *C. reticulate* and peels of *C. sinensis* recorded lower effect with significant different. Such essential oils can be mixed with antibiotics to reduce the antibiotics doses used to control the AFB diseases.

1.2. Essential oils extracted from plant Family Cupressaceae:

1.2.1. The essential oils extracted from *Thuja oreintalis*:

The tested extracts varied in their efficiency in inhibiting the growth of the pathogen. The essential oil obtained from leaves of *T. oreintalis* by hydro-distillation method was more effective than that extracted using ethanol in inhibiting the growth of *P. l. larvae*. The hydro-distilled extract caused inhibition zone of 10.67 mm and had R.E. of 62.14%, while ethanol extract caused inhibition zone of 7.17 mm and had R.E. of 43.01%. Generally, essential oils from *T. oreintalis* leaves exhibited weak antibacterial activity compared to the three tested antibiotics. Statistical analysis revealed that crude essential oil extracted from *T. oreintalis* leaves either by hydro-distillation or ethanol showed significantly lower antibacterial activity against *P. l. larvae* than that exhibited by any of tested antibiotics. .

1.2.2. The essential oil extracted from *Cupressus sempervirens*:

The essential oil extracted from leaves of *Cupressus sempervirens* recorded light effect with 65.99% R.E and 11.33 mm inhibition zone.

1.3. Essential oils extracted from plant Family Moringaceae:

1.3.1. The essential oil extracted from *Moringa oliefera:*

The essential oil of leaves of *Moringa olifera* did not have any effect on this type of bacteria despite of its effectiveness against of many other bacteria.

1.4. Essential oils extracted from plant Family Myrtaceae:

1.4.1. The essential oil extracted from *Syzygium aromaticum*:

The essential oils extracted from buds *Syzgium aromaticum* recorded 91.71% R.E with 14.83 mm inhibition zone with a significant difference.

1.4.2. The essential oils extracted from *Eucalyptus camaldulensis*:

Data indicated that the essential oil extracted from the leaves of *Eucalyptus camaldulensis* showed 65.36% R.E with 11.00 mm inhibition zone. The essential oil showed less relative efficiency than antibiotics with no significant difference while seeds extraction showed the highest for inhibitory of bacteria with 75.28% R.E and 12.67 mm inhibition zone. The percentage of essential oil from seeds of *Eucalyptus camaldulensis* is very high and when mixed with the essential oil of leaves showed 63.40% R.E and 10.67 mm inhibition zone.

1.5. Essential oils extracted from plant Family Lamiaceae:

1.5.1. The essential oils extracted from *Ocimum bacilicum*:

The results showed that the essential oil of leaves of *Ocimum bacilicum* recorded 87.80% R.E with 13.17 mm inhibition zone. While seeds of same plant showed 84.47% R.E with 12.67 mm inhibition zone. So, the seeds were less effective than leaves but with

no significant difference and when leaves and seeds mixed together the R.E recorded 90.0% with 13.50 mm inhibition zone

1.5.2. The essential oil extracted from *Thymus vulgaris*:

The essential oil of *T. vulgaris* extracted from whole plant was the most effective essential oil with 183.49% R.E. and 29.67 mm inhibition zone than any all of tested essential oils and antibiotics. Such essential oil recorded higher antibacterial activity about 1.83-fold than that of the reference antibiotic with significant difference.

1.5.3. The essential oil extracted from *Mentha piperita*:

The present results showed that the essential oil of *M. piperita* leaves plus stems recorded 118.18% R.E and 19.5 mm inhibition zone. The essential oil showed the higher effect by about 1.18-fold than that of the reference antibiotic with not significant difference.

1.5.4. The essential oil extracted from *Mentha pulegium*:

The essential oil of leaves plus stems of *M. pulegium* recorded lower antibacterial activity with the R.E value 85.88% and 14.17 mm inhibition zones. The antibacterial activity of the reference antibiotic was higher than tested essential oil with about 14% with no significant difference compared with any of the tested antibiotics.

1.6. Essential oils extracted from plant Family Anacardiaceae:

1.6.1. The essential oils extracted from *Schinus terebinthifolius*:

The essential oil of leaves of *Schinus terebinthifolius* recorded 65.71% R.E and 11.17 mm inhibition zone compared with seeds 54.94% R.E and 9.17 mm inhibition zone . In case of mixture of them recorded 65.71% R.E.

2. Effects of extracts of propolis on the bacterial growth of *P. l. larvae*:

The antibacterial activity of the aqueous extract of propolis showed low effect with 49.01% R.E and 8.17 mm inhibition zone. While the ethanol extract of propolis recorded 65.97% R.E with 11.00 mm inhibition zone, them both extracts are mixed together the R.E recorded 52.10% and 8.67 mm inhibition zone. It means that the two extracts of propolis showed low effect on the bacteria, *P. l. larvae.*

B. Effects of mixing natural antibacterial substances with antibiotics on *Paenibacillus larvae larvae:*

1. Inhibiting effects of mixing essential oils with antibiotics on the bacterial growth of *P. l. larvae*:

The essential oils of twenty plant parts extracts from different plants mixing with the three tested antibiotics were used in these experiments to evaluate their inhibition activity against *P. l. larvae* alone or when mixed in equal proportions with each of the tested antibiotics: Tylosin, Doxycycline and Tylo-Doxine (Controls).

1.1. Mixing essential oils of plant Family Rutaceae with antibiotics:

1.1.1. Mixing of essential oils of *Citrus limon* with antibiotics:

Data observed that the essential oil of leaves of *C. Limon* recorded 162.45% R.E with 30.33 mm inhibition zones as single but it was less effective when mixed with the antibiotic, Tylosin, recorded 95.50% R.E with 17.83 mm inhibition zone. Also, when such essential oil mixed with antibiotic Doxycycline the R.E value recorded 102.68% with 19.17 mm inhibition zones. While mixing with compound antibiotic Tylo-Doxcine recorded 104.45% R.E with 19.50 mm inhibition zone.

So, the essential oil of *C. Limon* peels recorded 88.90% R.E and 14.67 inhibition zones when tested alone. While mixed this essential oil with each of the antibiotics, Tylosin and Doxycycline separately showed 98.97% R.E with 16.33 inhibition zone which means that found an increase in its effect with no significant difference about the effect of essential oil single but when it was mixed with the compound antibiotic, The antibiotic Tylo-Doxcine recorded the effect 82.85% R.E and 13.67 inhibition zone with no significant difference.

1.1.2. Mixing of essential oils of *Citrus reticulata* with antibiotics:

Data indicated that the R.E. of the essential oil for leaves of *C. reticulata* alone was 118.11% and 20.67 inhibition zone. While essential oil and antibiotic Tylosin mixed together as a compound gave the R.E. value 93.31% and inhibition zone also decreased to be 16.33 mm. It means that the bactericidal activity of the essential oil for *C. reticulata* leaves was decreased by mixing with different significant.

Also, such decreased occurred when the antibiotic Doxycycline was mixed with the essential oil of leaves of *C. reticulata* which recorded 101.89% R.E and 17.83 mm inhibition zone without significant difference. The same trend was followed with the antibiotic Tylo-Doxcine.

Data show the essential oil of Peels of *C. reticulata* recorded 67.91% R.E and 12.00 mm inhibition zone. When the essential oil mixed with the antibiotic Tylosin the effect increased and recorded 104.70% R.E with 18.50 mm inhibition zone. In case of mixing with antibiotic Doxycycline the R.E value recorded 100.91% and 17.83 mm inhibition zone. While when mixed with compound antibiotic Tylo-Doxcine the R.E recorded 99.04% and 17.50 mm inhibition zone.

1.1.3. Mixing of essential oils of *Citrus sinensis* with antibiotics:

The essential oil of leaves of *C. sinensis* recorded 107.38% R.E and 21.83 mm inhibition zone when used alone. Such essential oil exhibited 92.62% R.E and 18.83 mm inhibition zone when mixed with antibiotic Tylosin. While mixing with antibiotic Doxycycline showed 104.92% R.E and 21.33 mm inhibition zone. In case of mixing with compound antibiotic Tylo-Doxcine the R.E value recorded 106.59% and 21.67 mm inhibition zone .

The essential oil of Peels of *C. sinensis* recorded the lowest effects with 49.97% R.E and 8.33 mm inhibition zone. While when such essential oil mixed with Tylosin the R.E value recorded 109.96% and 18.33 mm inhibition zone. In the same time, when mixed with Doxycycline the R.E recorded 106.96% and 17.83 mm inhibition zone. When the compound antibiotic Tylo-Doxcine mixed with such essential oil it recorded 101.98% R.E and 17.00 mm inhibition zone.

1.2. Mixing of essential oils extracted from plant Family Cupressaceae with antibiotics:

1.2.1. Mixing of essential oils of *Thuja oreintalis* with antibiotics:

There was few effect of essential oil extracted by hydro distillation from leaves of *Thuja oreintalis*. While mixed such essential oil with antibiotics Tylosin, Doxycycline and Tylo-Doxcine the effects increased with no significant difference. Also, the essential oil of leaves of *Thuja oreintalis* extracted using ethanol recorded low effect when tested alone but when mixed with antibiotics Tylosin, Doxycycline and Tylo-Doxcine it recorded 19.33, 18.83 and 19.83 mm inhibition zone, respectively with no significant difference.

1.2.2. Mixing of essential oils of *Cupressus sempervirens* with antibiotics:

The essential oil of leaves of *Cupressus sempervirens* show fewer effects against *P. l. larvae*. While the effect increased gradually when it mixed with antibiotics Tylosin and Tylo-Doxcine recorded 109.89% R.E and 111.73% R.E, respectively, with no significant difference. On the other hand, in case of mixed with antibiotic Doxycycline it recorded 99.08% R.E and 18.33 mm inhibition zone with significant difference compared with the antibiotic alone.

1.3. Mixing of essential oil extracted from plant Family Moringaceae with antibiotics:

1.3.1. Mixing of essential oil of *Moringa oliefera* with antibiotics:

The essential oil obtained from *M. oliefera* had no effect in inhibiting *P. l. larvae* growth, when evaluated alone. While these mixed with any tested antibiotic, its efficiency increased to be approximately equal to that of any control antibiotic. Based on R.E. values, they were 96.30, 99.10 and 95.34% when mixing with Tylosin, Doxycycline and Tylo-Doxine, in respect. Statistical analysis revealed that these mixtures of antibiotics with crude hydro-distilled extract of *M. oliefera* seeds did not significantly differ either from each other or from control antibiotic in inhibiting the growth of pathogen. Moreover, all of them achieved significant higher antibacterial activity than that exhibited by crude extract alone.

1.4. Mixing essential oils of extracted from plant Family Myrtaceae with antibiotics:

1.4.1. Mixing of essential oil of *Syzygium aromaticum* with antibiotics:

The essential oil of *Syzygium aromaticum* buds when tested alone it recorded 96.31% R.E and 17.50 mm inhibition zone. But when mixed with antibiotics Tylosin and

Doxycycline the effect decreased and also the effect decreased when such essential oil mixed with compound antibiotic Tylo-Doxcine with R.E value 77.99% and 14.17 mm inhibition zone.

1.4.2. Mixing of essential oils of *Eucalyptus camaldulensis* with antibiotics:

The present results indicated that the essential oil extracted from leaves of *Eucalyptus camaldulensis* recorded 55.49% R.E and 10.33 mm inhibition zone. Such essential oil when mixed with the antibiotics Tylosin and Doxycycline the effect increased to 14.83 mm inhibition zone in both cases. But in case of mixed with Tylo-Doxcine strong effect was observed with significant difference. The essential oil extracted from seeds of such plant recorded 47.95% R.E and 7.83 mm inhibition zone when tested alone. But when mixed with antibiotics Tylosin, Doxycycline and Tylo-Doxcine recorded 13.50, 15.33 and 14.33, respectively.

1.5. Mixing of essential oils extracted from plant Family Lamiaceae with antibiotics:

1.5.1. Mixing of essential oils of *Ocimum bacilicum* with antibiotics:

The effect of the essential oils of *Ocimum bacilicum* leaves recorded 111.76% R.E and 19.00 mm inhibition zone when tasted alone against *P. l. larvae* and not found significant difference. The effect decreased when such essential oil mixed with antibiotic Tylosin and recorded 101.94% R.E and 17.33 mm inhibition zone. When the essential oil mixed with Doxycycline the effect decreased to 105.88% R.E and 18.00 mm inhibition zone and in case of mixing with compound antibiotic Tylo-Doxcine the R.E recorded 104.88% and 17.83 mm inhibition zone.

The essential oil of seeds of *Ocimum bacilicum* recorded 96.63% R.E and 14.33 mm inhibition zone with no significant difference. While mixing with antibiotic Tylosin the R.E value increased to be 107.89% and 16.00 mm inhibition zone, while mixing with Doxycycline showed 105.66% R.E and 15.67 mm inhibition zone. On the other hand, the mixing with antibiotic Tylo-Doxcine recorded 97.77% R.E and 14.50 mm inhibition zone with no significant difference.

1.5.2. Mixing of essential oil of *Thymus vulgaris* with antibiotics:

The results showed that the essential oil of *Thymus vulgaris* was more effective than all of the other extracted essential oils. It recorded 190.80% R.E and 34.67 mm inhibition zone with significant difference between the essential oil and antibiotic when each compound tested alone. While mixed such essential oil with all antibiotics the effects decreased with a significant difference.

1.5.3. Mixing of essential oil of *Mentha piperita* with antibiotics:

It is observed that the essential oil of *Mentha piperita* recorded 132.99% R.E and 22.17 mm inhibition zone. This means that such essential oil was more effective than the antibiotics on growth of the strain of the tested bacteria when each of them was tested alone.

1.5.4. Mixing of essential oil of *Mentha pulegium* with antibiotics:

Data showed that the essential oil of *Mentha pulegium* recorded 110.76% R.E and 18.83 mm inhibition zone. On the other hand, when mixed with antibiotics no significant difference for these mixtures.

1.6. Mixing of essential oils extracted from plant Family Anacardiaceae with antibiotics:

1.6.1. Mixing of essential oils of *Schinus terebinthifolius* with antibiotics:

Present results indicated that the essential oil of leaves of *Schinus terebinthifolius* showed fewer effects. When the essential oil tested alone it recorded 64.80% R.E and 9.83 mm inhibition zone.Such the essential oil when mixed with antibiotic Tylosine recorded 112.06% R.E and 17.00 mm inhibition zone. While when mixed with antibiotics Doxycycline and Tylo-Doxcine showed 110.94 and 16.83 mm inhibition zone with no significant difference between antibiotics effect alone and essential oil effect mixed with such antibiotics.

The effect of essential oil extracted of *Schinus terebinthifolius* seeds was higher than that of the essential oil of leaves. It recorded 66.95% R.E and 11.83 mm inhibition zone with a significant difference. On the other hand, when mixed with antibiotics Tylosine and Doxycycline the R.E value recorded 90.55, 87.72, respectively, with a significant difference. The mixing of essential oil with compound antibiotic Tylo-Doxcine recorded 84.89% R.E and 15.00 mm inhibition zone with no significant difference.

2. Effects of mixing of extracts of propolis with antibiotics on the bacterial growth of *P. l. larvae*:

The aqueous extract exhibited weak inhibitory effect on the growth of *P. l. larvae*: inhibition zone of 7.67 mm with 41.64% R.E. But, the mixture with Tylosin was affected as Tylosin, while mixing with either Doxycycline or Tylo-Doxine had lower efficiency than involved control antibiotic, without significant difference. Thus, the inhibitory effect of propolis aqueous extract on the growth of *P. l. larvae* was significantly lower than that of any mixture or tested antibiotic. So, there is a benefit effect by mixing such extract with antibiotics especially Tylosin for achieving the same effect of antibiotics.

The ethanol extract of propolis, also exhibited weak inhibitory effect on the growth of *P. l. larvae*: inhibition zone of 4.97 mm with 32.06% R.E. But, mixing it with Tylosin or Doxycycline had a synergistic effect, while it was additive in the case of mixing with Tylo-Doxine. Furthermore, the inhibitory effect of propolis ethanol extract on the growth of *P. l. larvae* was significantly lower than that of any mix or tested antibiotic. Thus, this extract was useful as the other extracts when mixed with antibiotics. Moreover, ethanol extract of propolis had R.E. value lower (32.06%), compared with aqueous extract (41.64%), on contrary of obtained findings in previous experiments.

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C. Effects of different concentrations of potential essential oils on *Paenibacillus larvae larvae*:

The tested essential oil varied in inhibiting the growth of the tested bacterium compared with the commonly used antibiotic Tylosine which was used as a reference to identify the most effective essential oil by calculate the relative efficiency percentage.

1. The effects of diluting essential oils from plant Family Rutaceae:

1.1. *Citrus limon* essential oils:

The essential oil of leaves of *Citrus limon* showed higher antibacterial effect than antibiotic reference and recorded 213.56% R.E with significant difference between the essential oil and the antibiotics. The essential oil showed the same effect at concentrations of 50% and 12.5% recorded 125.24% and 100.95% R.E, respectively, but with no significant difference.

The present results showed that the antibacterial activity of the essential oil of peels of *Citrus limon* when tested alone at a concentration of 100% recorded 101.06% R.E with no significant difference compared with the antibiotic reference Tylosine. But when dilution such essential oil at concentrations (50, 25, 12.5and 6.25%) found decreased on the effect essential oil with (71.06, 56.09, 52.42 and 41.18%) R.E, respectively.

On the other, hand the amount of essential oil was higher in the peels *Citrus limon* than leaves. So it can be used mixed with the essential oil of leaves (50% percentage for each in mixture). The essential oil of mixture leaves and peels of *Citrus limon* recorded 166.69% R.E at a concentration of 100%. It means that the effect of such essential oil was higher than those of the antibiotics with a significant deference. Also, dilution of such essential oil with 50% concentrates recorded higher effect on *P. l. larvae* than antibiotics and no significant difference are found. Concentrations of 25, 12.5 and 6.25% showed low effect and recorded 69.15, 58.24 and 49.00% R.E with significant deference.

1.2. *Citrus reticulata* essential oils:

Data showed that the essential oil of leaves of *Citrus reticulata* recorded 121.00% R.E at a concentration of 100%. While other concentrations recorded low effect on perventation growth of the bacteria *P. l. larvae.*

Also, the present results indicated that the essential oil of *Citrus reticulata* peels recorded low effect at all tested concentrations. When the essential oil of leaves and peels of *Citrus reticulata* were mixed together the mixture was effective with significant deference only at a concentration of 100% and recorded 76.00% R.E. While the concentrations of 50, 25, 12.5 and 6.25% showed low effects.

1.3. *Citrus sinensis* essential oils:

From the present data observed that the essential oil of leaves of *Citrus sinensis* recorded 115.71% and 90.63% R.E at a concentration of 100% and 50%, respectively, with no significant deference between such essential oil and antibiotic reference. Concentrations of 25, 12.5 and 6.25% showed low effect with R.E values of 57.70, 53.09 and 49.01%, respectively.

2. The effects of diluting essential oils from plant Family Myrtaceae:

2.1. *Syzygium aromaticum* essential oil:

The present data observed that the essential oil of *Syzygium aromaticum* buds recorded 108.56 and 95.47% R.E at concentrations of 100% and 50%, respectively, with no a significant deference. But at concentrations of 25, 12.5 and 6.25% were 79.66, 65.81 and 55.48% R.E, respectively, with a significant deference.

2.2. *Eucalyptus camaldulensis* essential oils:

Obtained data showed that the essential oil extracted from the leaves and seeds of *Eucalyptus camaldulensis* and mixture recorded 68.94, 72.26, and 62.18% R.E at a concentration of 100%. While such essential oils at the other concentrations recorded a very low antibacterial activity against *P. l. larvae*. Dilution of such essential oil to concentrations of 50, 25, 12.5 and 6.25% were also performed because the *Eucalyptus camaldulensis* has an effective role in the control many diseases in the honey bee, it was necessary to prove a role it in control of American foulbrood disease.

3. The effects of diluting essential oils from plant Family Lamiaceae:

3.1. *Ocimum bacilicum* essential oils:

The essential oil of leaves of *Ocimum bacilicum* was effective at a concentration of 100% with 111.76% R.E. Dilution of such essential oil to concentrations of 50, 25, 12.5 and 6.25% the effect decreased to 70.81, 52.57, 50.03 and 28.01% R.E, respectively. The essential oil of *Ocimum bacilicum* seeds was effective only at a concentration of 100% with 104.88% R.E. While at the other concentrations was low effect.

3.4. *Thymus vulgaris* essential oil:

Obtained data emphasized the high efficiency of the crude essential oil from *T. vulgaris* of whole plant, beside its tested diluents, in inhibiting the growth of *P. l. larvae*. However, the crude oil (100%) was the most effective treatment achieving inhibitory activity with 245.28% R.E. almost 2.5 fold more than that exhibited by any control antibiotics.

The *T. vulgaris* essential oil diluted to half concentration significantly decreased its activity to be with 198.20% R.E. approximately double that of any tested antibiotic. Moreover, the same trend was observed when the concentration decreased to be 25 or 12.5% which still exhibiting high activities with 177.91 and 163.36% R.E., respectively. But, the oil, at all of these concentrations, was more effective against *P. l. larvae* than any control antibiotics. While the lowest concentration of 6.25% had antibacterial activity with 92.74% R.E. as effective as any of tested control antibiotics, based on statistical analysis. The same trend was followed in case of the tested species of Family Lamiaceae, the antibacterial activity of *T. vulgaris* essential oil was concentration-dependent.

3.2. *Mentha piperita* essential oil:

The present results showed that the essential oil of *Mentha piperita* recorded 122.28% R.E at a concentration of 100%. Dilution of such essential oil to 50, 25, 12.5 and 6.25% recorded R.E value 83.33, 69.15, 54.73 and 50.00%, respectively, with a significant deference.

3.3. *Mentha pulegium* essential oil:

The essential oil of *Mentha pulegium* when tested against the bacteria with a concentration of 100% recorded 97.32% R.E with no significant deference between effects such essential oil and antibiotics. While at the other tested concentrations low effect was observed with a significant deference compared with the tested antibiotics.

4. The effects of diluting essential oils from plant Family Anacardiaceae:

4.1. *Schinus terebinthifolius* essential oils:

The essential oil of leaves, seeds and mixture of *Schinus terebinthifolius* showed 70.39, 62.06 and 64.83% R.E, respectively, at a concentration of 100%. At the other tested concentration of 50, 25 and 12.5% there were a very low antibacterial activity while at a concentration of 6.25% was not effective against the bacteria. However, in all cases of decreasing efficiency in the activity was noticed as the dilution increase till 12.5% concentration, then sudden drop happened by the last dilution. On the other hand, each of efficient treatment using leaves oil was more effective, with no significance, than corresponding treatment using seeds oil, and the oils mixture followed the same trend.