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Research Article

# ANTIBACTERIAL ACTIVITY OF THREE STORED OLIVE OILS AND *MENTHA AQUATICA* L.

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## ABSTRACT

This study aimed to evaluate the antibacterial activity of three samples of stored olive oils (01 year, 12 years and 33 years) and essential oil of *Mentha aquatica* L. Two Gram positive bacteria strains: *Citrobacter freundii* ATCC 8090, *Staphylococcus aureus* ATCC 25923 and five Gram negative bacteria strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *klebsiella pneumoniae* ATCC 700603, *Bacillus subtilis* ATCC 6633 and *Schigella sonnei* were used in the present study. The results show that the olive oil stored for 33 years has evident antibacterial activity against bacteria strains used, especially against *Citrobacter freundii* ATCC 8090, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923, whereas all samples have no effect on *klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853.

**Keywords:** Olea europea L., Mentha aquatica L., stored olive oil, essential oil, antibacterial activity.

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## 1. INTRODUCTION

In traditional Algerian medicine; medicinal plants are used in the treatment of microbial diseases; among the most used plants are olives (the olive oil and stored olive oil) and mints.

The olive tree (*Olea europea* L.) is a familiar feature of the Mediterranean landscape. It may have originated in Syria, Asia Minor, Ethiopia, Egypt, or India. Since ancient times, it has contributed, in practical and symbolic terms, to the economy, health and haute cuisine of the inhabitants of the Mediterranean region [1].

The Olive tree is one of the first domesticated agricultural crops in Oleaceae family it is cultivated mainly for both edible oil and table olives [2], for fine wood, olive leaf, and as a renewable energy source [3]. Historically, olive leaves have been used as a remedy for fever and other diseases while olive oil was widely used in folk medicine and treatment, either as a treatment in itself, or as base for other preparations for internal or external use. Olive oil with poppy petals and sometimes spearmint balm was used as throat emollient and calmative for the joints. Threshed olive leaves and fruit were also used to soothe swollen glands [1].

Most of *Mentha* species are perennial herbs growing wildly in damp and wet places throughout temperate regions of Eurasia, North Africa, Australia and South Africa [4]. The Mints usually taken after a meal for their ability to reduce indigestion and colonic spasms, in addition to countering colds, coughs, constipation, stomach-ache or inflammation of the appendix and stomach trouble [5,6].

It is known in the North of Algeria, especially the Setif region and its environs that the olive oil (especially stored olive oil) and teas of *Mentha aquatica* L. are used to treat almost the same diseases (treating the respiratory and digestive system). The aim of this study is to evaluate the antibacterial activity of three samples of stored olive "*Olea europea* L." as well specie of mint "*Mentha aquatica* L.".

## 2. MATERIALS AND METHODS

### 2.1. Plant materiel

Three samples of stored olive oil were obtained from local farmers (older people), duration of its storage was (sample 1: stored for one year, sample 2: stored for 12 years and sample 3: stored for 33 years). These olive oils were extracted using traditional method (way of pressing) from fruits of *Olea europea* L.

The aerial parts of *Mentha aquatica* were collected in the flowering stage (September 2014) from the North of Algeria. The air-dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. The oil obtained was collected and dried over anhydrous sodium sulphate and stored at 4°C in sealed brown vials until use.

### 2.2 Antibacterial activity

The antibacterial activity of oil samples of *Olea europea* L. were evaluated by agar diffusion test “disc assay” [7].

two Gram positive bacteria and five Gram negative bacteria were used in the present study: *Staphylococcus aureus* ATCC25923, *Citrobacter freundii* ATCC 8090, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC700603 and *Schigella sonnei* (the non-reference strain used was isolated from human specimens at the laboratory of bacteriology of the university hospital center of Setif).

The bacterial inoculums were prepared (0.08-01 at 625 nm). Muller-Hinton agar (MH agar) was poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution and diluted essential oil (1:2, 1:5, and 1:10 w/w of Hexane for olive oil and DMSO for *M. aquatica* essential oil). Hexane and DMSO were used as negative controls, whereas Gentamicin (GM) was used as positive control. Petri dishes were incubated at 37°C during 18h to 24h. After incubation, inhibition zone diameters were measured and documented.

## 3. RESULTS AND DISCUSSION

The results of the antibacterial activity of oil samples of *Olea europea* L. are expressed by measuring the diameter of inhibition of the different concentrations of the samples in mm after 24 h of incubation at 37°C.

The strains *Pseudomonas aeruginosa* ATCC27853 and *Klebsiella pneumoniae* ATCC700603 were resistant to all samples of olive oils (Table 01) and (Fig. 01 and 02), while all the oil samples present important effect against *Citrobacter freundii* ATCC 8090 which exceeded those obtained by the antibiotic (Fig.03); in addition to this it seems clearly that the sample 03 has significant effect against *Staphylococcus aureus* ATCC 25923 with inhibition zone of 25mm for concentration 50% (Fig.04), this result is similar to those obtained by the antibiotic; also this olive oil exhibited weak inhibitory effect against *Schigella sonnei* and *Escherichia coli* ATCC 25922 (Fig.05 and 06); as well as with *Bacillus subtilis* ATCC 6633 with inhibition zone of 20mm, 35mm, and 37mm for concentrations 10%, 20% and 50% respectively while the sample 02 present important activity against this strain bacteria with inhibition zone of 18mm, 33mm, and 34mm for concentrations 10%, 20% and 50% respectively however the sample 01 has no effect against it.

Sample 03 has higher antibacterial activity as compared to other samples, where sometimes exceeded those obtained with antibiotic, in addition to this the samples 01 and 02 have similar antibacterial activity values unlike sample 03.

The effect of oil samples on positive bacteria is greater than negative bacteria. The largest effect of the olive oil samples obtained was on *Citrobacter freundi*i ATCC 8090, whereas *Klebsiella pneumoniae* ATCC700603 and *Pseudomonas aeruginosa* ATCC27853 where resistant.

In a previous study, different samples of olive oils (*Olea europaea* and *Olea ferruginea*) were effective (weak effect) against *Staphylococcus aureus* ATCC6538, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 but have no effect on the growth of *Bacillus subtlilis* ATCC 19659 [08].

Similar results were obtained by Agoramoorthy et *al*., (2007) [09] where they reported that the Gram-positive bacteria were more susceptible than the Gram-negative bacteria. In a previous study, the olive oil has important antibacterial activity against *Salmonella enteriditis* 13076 and *E. coli* ATCC 25922 [10].

The antibacterial activity of some fatty acids as linolenic acid and methyl 5,11,14,17-eicosatetraenoate against the Gram-negative as *Escherichia coli* were less compared to the Gram-positive bacteria such as *Staphylococcus aureus* [11].

The antimicrobial activity of unsaturated compounds such as oleic and linoleic acids is related to their degree of unsaturation, their chain length and the bacterial species tested [12], whereas some gastrointestinal bacteria were not affected during their growth by any of the long-chain of fatty acids "palmitic Acid and stearic Acid [13].

The storage of these olive oil increases the saturated fatty acids as palmitic acid and decreases the unsaturated fatty acids as oleic acid, as has been noted appearance of new compounds (17 new components were detected in sample 03 like nonanoïc acid, Chaulmoogric acid, Azelaic acid), or the disappearance of some components such as linoleic acid and linolenic acid. This change in the chemical composition may create a change in the therapeutic properties of olive oil [14]**.**

Michener et *al*. (1959) [15] reported in their study that the antibacterial effect of the straight-chain saturated fatty acids, those in which C = 2, 4, 6, 7, or 8 were inactive, C=10 were slightly active, whereas C=12, 14, 16, or 18 were active but oleic acid had little or no activity.

**Table 1.** Antibacterial activity of stored olive oil samples measured as diameter of inhibition

| Bacteria strains | Sample 01 (concentration w/w) | | | Sample 02 (concentration w/w) | | | Sample 03 (concentration w/w) | | | Antibiotic |
|---|---|---|---|---|---|---|---|---|---|---|
| | **50%** | **20%** | **10%** | **50%** | **20%** | **10%** | **50%** | **20%** | **10%** | **Gentamicin** |
| *Staphylococcus aureus* ATCC 25923 | - | - | - | - | - | - | 25.67±0.49 | 10.10±0.65 | 7.96±0.95 | **25.83±0.76** |
| *Escherichia coli* ATCC 25922 | - | - | - | - | - | - | 10.29±0.70 | 08.15±1.06 | - | **25.16±0.70** |
| *Pseudomonas aeruginosa* ATCC27853 | - | - | - | - | - | - | - | - | - | **22.50±0.50** |
| *Citrobacter freundii* ATCC 8090 | 20.05±0.42 | 18.85±0.54 | - | 36.59±1.96 | 20.12±0.79 | 17.55±0.63 | 38.76±1.08 | 27.60±2.09 | 22.05±0.68 | **20.33±0.57** |
| *Schigella sonnei* | - | - | - | - | - | - | 9.73±0.80 | 10.22±0.31 | 8.97±0.72 | **12.16±0.28** |
| *Klebsiella pneumoniae* ATCC700603 | - | - | - | - | - | - | - | - | - | **22.66±0.28** |
| *Bacillus subtilis*ATCC 6633 | - | - | - | 34.33 | 33.12 | 18.03 | 37.76 | 35.01 | 20.28 | **33.25** |

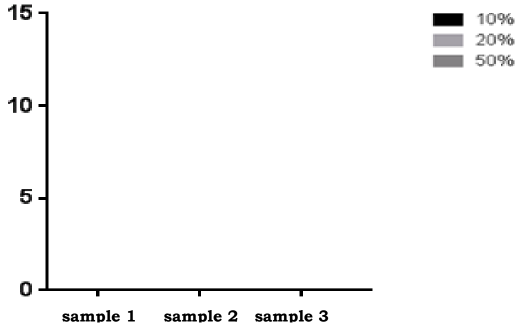


**Fig.1.** Antibacterial activity of the samples 1, 2, 3 against *Ps.*

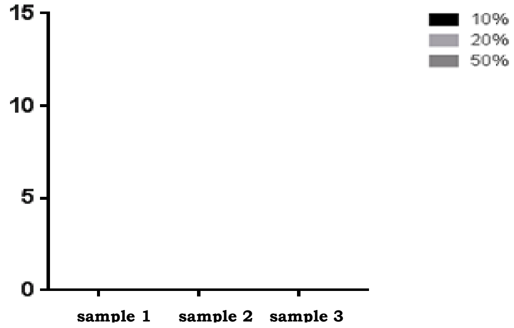


**Fig.2.** Antibacterial activity of the samples 1, 2, 3 against *K. pneumoniae*

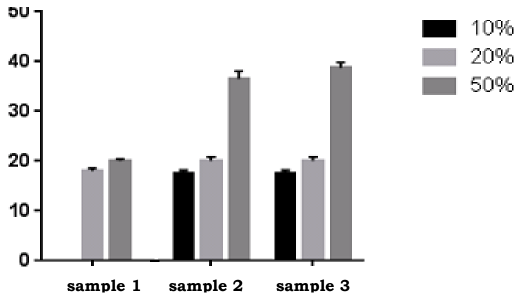


**Fig.3.** Antibacterial activity of the samples 1, 2, 3 against *C. freundii* ATCC 8090

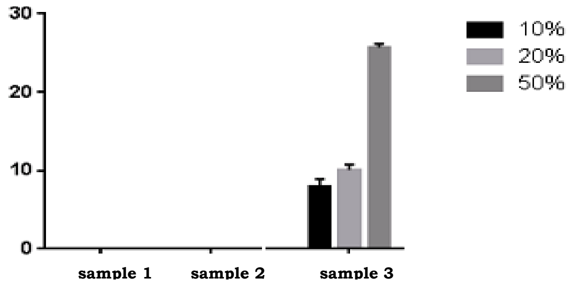


**Fig.4.** Antibacterial activity of the samples 1, 2, 3 against *S. aureus* ATCC

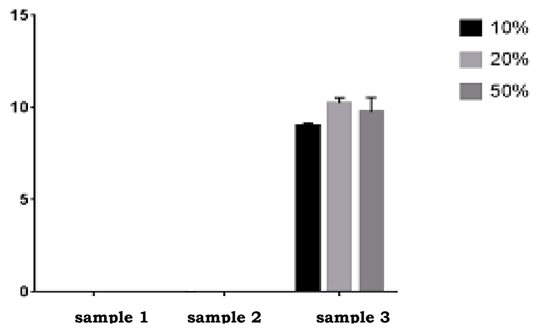


**Fig.5.** Antibacterial activity of the samples 1, 2, 3 against *Schigella sonnei*

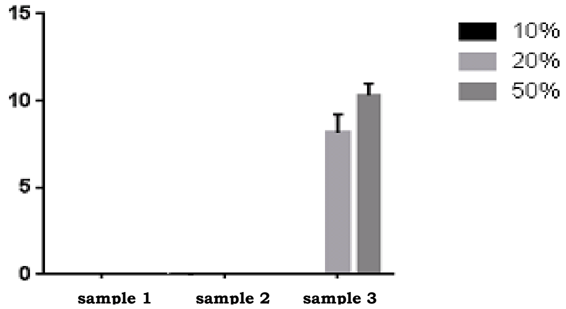


**Fig.6.** Antibacterial activity of the samples 1, 2, 3 against *E. coli* ATCC

The results of antibacterial activity of *M. aquatica* essential oil show significant activity against all bacteria strains tested except *Pseudomonas aeruginosa* ATCC 27853 which was resistant while the inhibition zone of the antibiotic was 22.5±0.5mm.

The biggest result was against *Staphylococcus aureus* ATCC 25923, *Schigella sonnei*, *Escherichia coli* ATCC 25922, *Citrobacter freundii* ATCC 8090 and *Klebsiella pneumoniae* ATCC 700603 respectively. Some bacteria strains were more sensible to the essential oil than the antibiotic (GM) such as *S. aureus* ATCC 25923, *Schigella sonnei*, *E. coli* ATCC 25922 (table 02 and Fig. 07), whereas this oil present weak activity against *K. pneumoniae* ATCC 700603. It seems clear that the essential oil has a dose dependent activity against susceptible species. The antibacterial activity of the essential oil was evaluated using different concentrations which showed a difference in results according to the concentration used.

**Table 2.** Antibacterial activity of *M. aquatica* essential oil measured as diameter of inhibition (mm)

| Bacteria strains | *M. aquatica* essential oil concentration (w/w) | | | Antibiotic |
|---|---|---|---|---|
| | **100%** | **50%** | **20%** | **(GM)** |
| *Staph ylococcus aureus*ATCC 25923 | 52.35±2.71 | 26.24±0.53 | 18.68±1.29 | 25.83±0.76 |
| *Escherichia coli* ATCC 25922 | 34.01±0.95 | 12.23±0.32 | 8.96±0.45 | 25.16±0.76 |
| *Pseudomonas aeruginosa* ATCC 27853 | 00 | 00 | 00 | 22.5±0.5 |
| *Citrobacterfreundii* ATCC 8090 | 18.34±0.62 | 12.97±0.45 | 11.44±0.43 | 20.33±0.57 |
| *Schigella sonnei* | 39.41±0.78 | 19.11±0.42 | 11.01±0.47 | 12.16±0.28 |
| *Klebsiella pneumoniae* ATCC 700603 | 12.67±1.13 | 9.67±0.4 | 00 | 22.66±0.29 |

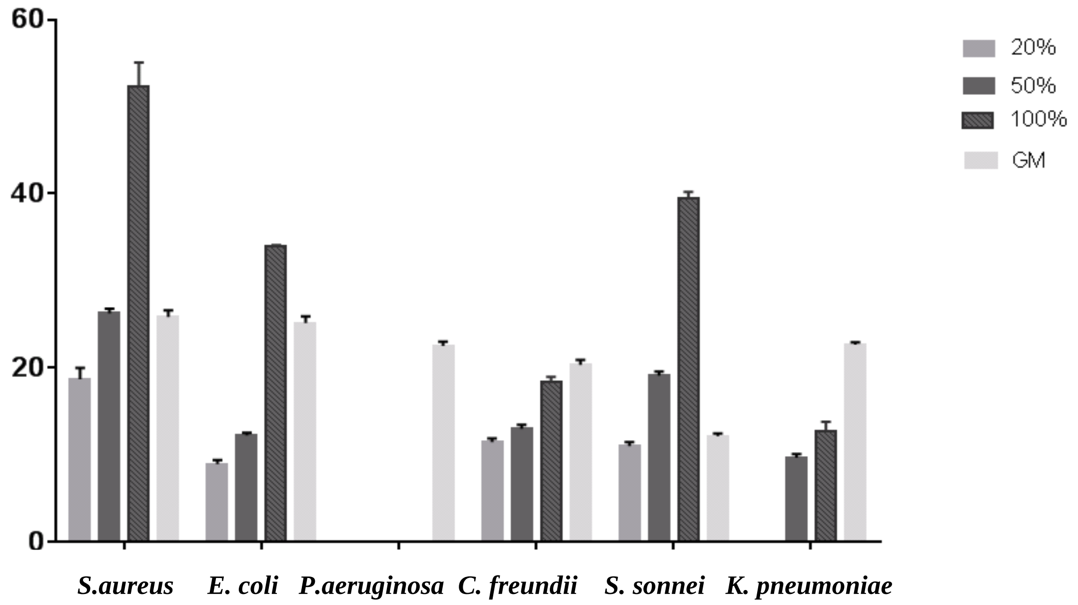


**Fig.7.** Antibacterial activity of the *M. aquatica* essential oil against *S. aureus* ATCC 25923, *S. sonnei*, *E. coli* ATCC 25922, *C. freundii* ATCC 8090 and *K. pneumoniae* ATCC 700603 and *P.aeruginosa* ATCC 27853 at different concentrations, measured as diameter of inhibition (mm).

Getahun et *al*. (2008) [16] reported that the oil of *M. aquatica* showed good activity against a series of Gram positive and Gram-negative bacterial strains.

Our results are in good agreement with the findings of essential oil of *M. piperita* from Madagascar [17], but they differ with those obtained in study of peppermint (*Mentha piperita*) from Pakistan [18] and *Mentha pulegium* L. from Iran [19].

On the other hand, the results of antibacterial activity of olive oil samples: (01, 02, 03) and the essential oil of *M. aquatica* differ in the inhibition diameters of different bacterial strains, *C. freundii* ATCC 8090 was more susceptible to the three olive oil samples than the essential oil of *M. aquatica*, with inhibition zone of 20.05±0.42, 36.59±1.96, 38.76±1.08 12.97±0.45 at concentration of (50%), as shown in Fig. 08;

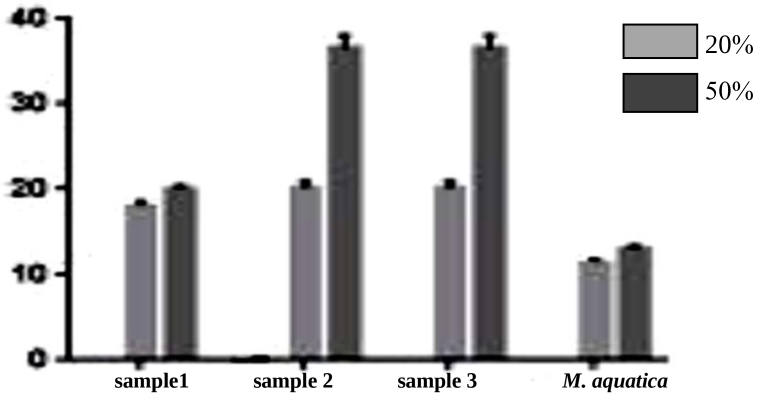


**Fig.8.** Antibacterial activity of the three olive oil samples and *M. aquatica* essential oil against *C. freundii* ATCC 8090, measured as diameter of inhibition (mm).

but the result is different with *E. coli* ATCC 25922 which present a low sensitivity to the essential oil of *M. aquatica*, inhibition diameters were estimated at 08.96 ± 0.45 and 12.23 ± 0.32 mm in concentrations 20% and 50%, respectively, while their sensitivity was weak towards the sample 03 of olive oil with inhibition diameters of 08.15± 1.06 mm and 10.29 ± 0.70 mm at concentrations 20% and 50% respectively, while the oils of samples 01 and 02 have no effects on this bacteria strain, (Figure 09).

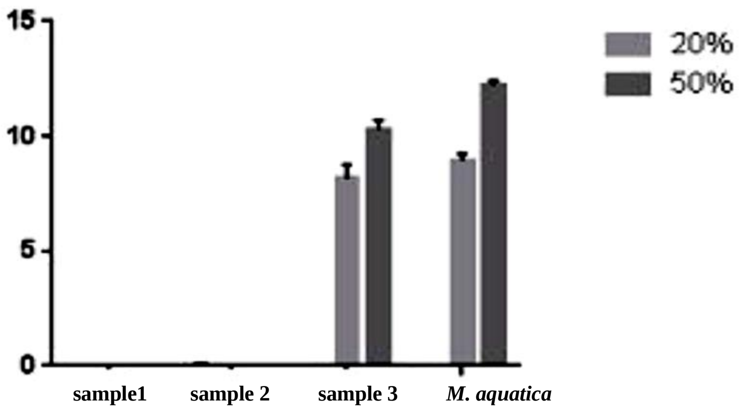

**Fig.9.** Antibacterial activity of the three olive oil samples and *M. aquatica* essential oil against *E. coli* ATCC 25922, measured as diameter of inhibition (mm)

In turn, *Schigella sonnei* show a less sensitivity to sample 03 with inhibition zone of 10.22±0.31 mm and 09.73 ± 0.80 mm in concentrations 20% and 50% respectively, but it was no susceptible to the samples 01 and 02, whereas this bacteria strain was sensitive to the essential oil with inhibition zone of 11 and 19 mm in concentrations 20% and 50% respectively, as shown in Figure 10.

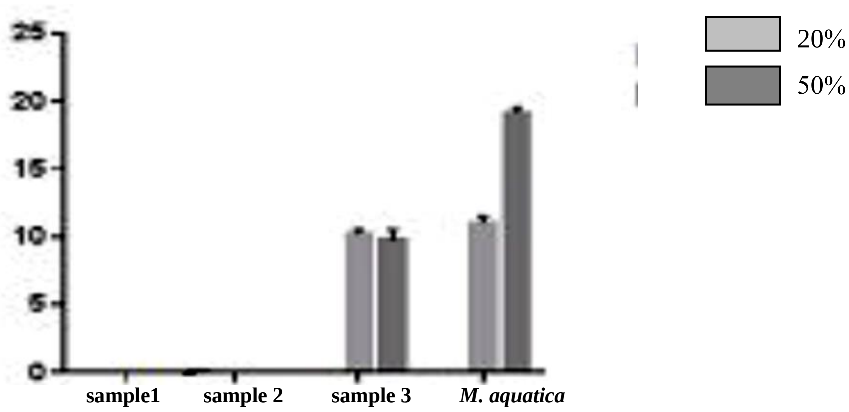


**Fig.10. A**ntibacterial activity of the three olive oil samples and *M. aquatica* essential oil against *Schigella sonnei,* measured as diameter of inhibition (mm)

*Staphylococcus aureus* ATCC 25923 was resistant to sample oil 01 and 02 while this bacteria strain was sensitive to sample 03 and essential oil of *M. aquatica* with inhibition diameters of 10.10 ± 0.65 mm and 18.68 ± 1.29 mm respectively in concentrations 20%, 25.67 ± 0.49 mm and 26.24 ± 0.53 mm. In order of 50% concentration, the results are shown in Figure (37); these last two results are close to each other and to the result of the positive witness, as shown in Figure 11.

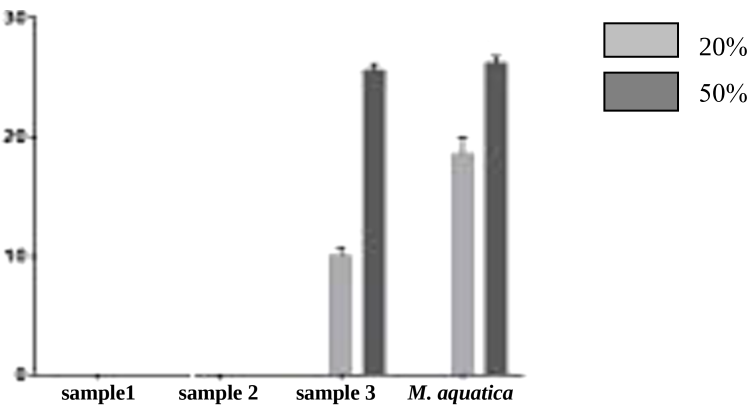


**Fig. 11.** Antibacterial activity of the three olive oil samples and *M. aquatica* essential oil against *Staphylococcus aureus* ATCC 25923, measured as diameter of inhibition (mm)

The essential oil of *M. aquatica* present a weak effect against the *Klebsiella pneumoniae* ATCC700603 with an inhibition diameter equal to 09.67 ± 0.4 mm at 50% concentration and no effect at 20%, unlike oil samples 01.02 and 03 which have no effect against this bacterial strain, as shown in Figure 12.

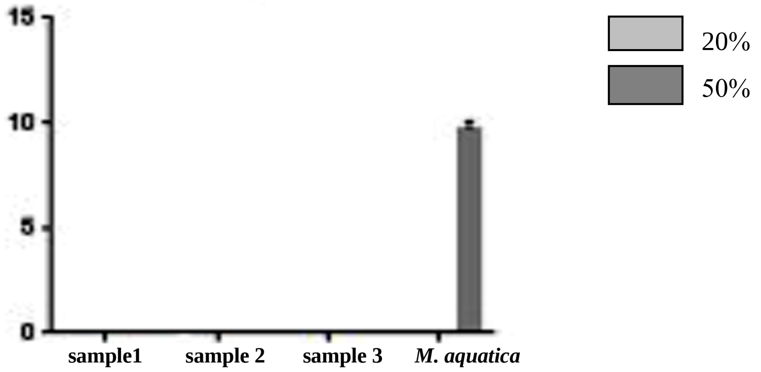


**Fig. 12.** Antibacterial activity of the three olive oil samples and *M. aquatica* essential oil against *K. pneumoniae* ATCC700603, measured as diameter of inhibition (mm)

## 4. CONCLUSION

This study was conducted in light of what is known in local folk medicine and the selection of both plants was intentional and not random. The study has shown that olive oil samples and *Mentha aquatica* essential oil have a significant antibacterial activity.

The results show that the oils have different antibacterial activity depending on the nature of the extract and the concentrations used. It is noted that *Mentha aquatica* essential oil and olive oil sample 03 have important antibacterial activity against pathogenic bacteria strains tested; against the samples 01 and 2 of olive oil that have a very low activity.

The results indicate that the essential oil of *Mentha aquatica* used in this study inhibited development of most bacteria tested, while the olive oils samples have low effect against these bacteria strains. In general, tested Gram negative bacteria appear more resistant than Gram positive ones especially in the case of olive oil samples.

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