ISSN: 0976-7126
Mariri *et al.*, 16(9):27-40, 2025

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Antibacterial Effects of Some Antibiotics and Essential Oils Against *Brucella abortus* Inside Goat's Macrophages and New Promising Treatments

Ayman Al-Mariri*, Basel Alobeid, Nizar Dayuob and Laila Al-Hallab

Department of Molecular Biology and Biotechnology, Atomic Energy Commission, P.O. Box 6091, Damascus Syria.

Article info

Received: 11/04/2025

Revised: 12/07/2025

Accepted: 18/09/2025

© IJPLS

www.ijplsjournal.com

Abstract

Brucellosis is an infectious disease caused by bacteria. Animals that are most commonly infected include sheep, cattle, goats, pigs, and dogs. Nowadays, the resistance of microbial infections to antibiotics has threatened the health of societies. Thus, this article introduces the antibacterial effects of some antibiotics and plant-derived essential oils so that they can be used as promising choices to develop a new anti-*Brucella* therapy. *B. abortus* isolate was obtained from milk samples collected in previous work from different Syrian provinces. Essential oils extraction was acquired using water steam distillation device. Macrophages were isolated from blood and infected with *B. abortus* at a ratio of 1:100 bacteria/macrophage.

Brucella strains have been shown resistant to most antibiotic groups used in this study, whereas it was detected an excellent synergistic activity between Levofloxacin- Cefprozil, and Levofloxacin – Tetracycline with a $\log_{10}$ decreasing from 3.367, 3.303 to a value of 0 after 96h of infection respectively.

Cinnamon 1% was revealed the best antibacterial activity against *B. abortus* strains with a decreasing value of $\log_{10}$ reached 0.95 after 96h of infection. A strong significant inhibitory effect was observed for *Cinnamon* 0.1% in combination with a 1% concentration of the other plant's oil extracts.

Keywords: Antibiotics, *Brucella*, Essential oils, Macrophage, Synergistic effect, Treatment

Introduction

Brucellosis is an old, infectious and common zoonosis that's caused by Gram-negative bacteria from the genus *Brucella*. It is transmitted through direct contact with infected animals or by using unpasteurized dairy products of goats, pigs, camels, sheep, buffalo and cows, or inhalation of aerosols. Veterinarians and people working in slaughterhouses are at high risk of developing this disease; also, it is more common in children than in adults [1]. Despite humans are incidental hosts, brucellosis continues to be a major public health concern worldwide because more than 500000 cases each year are diagnosed with this disease, most of them live in the developing countries, furthermore, the number of brucellosis cases are over 10000000 people in some countries which making this disease is endemic.

***Corresponding Author**
E.mail: ascientific@aec.org.sy

Four different *Brucella* species, *B. suis*, *B. melitensis*, *B. abortus* and *B. canis* can be pathogenic to humans [2] Through these species, *B. melitensis* is the most infectious species, followed by *B. suis* and *B. abortus*. *Brucella* species are intracellular, obligate and Gram-negative coccobacilli facultative intracellular bacterium and are not able to produce spores and nonmotile [3]. *Brucella* species have no classic virulence genes encoding plasmids, pili, capsules or exotoxins, and have resistance to phagocytes and proliferation inside phagocytic cells.

The Damascus goat, also known as Shami, is a breed of goat with unique mouth and head shape raised in Syria, Cyprus and Lebanon and it is considered one of the oldest breeds around the world [4]. The predominant color is dark honey and there is a black and white color in addition to a mixture between the three colors [5].

Shami's goats are considered one of the best goat veins for milk production, with an average daily quantity of (2.5) kg, the total milk is (265) kg in the first season and (500) kg in the second season [6].

It has large number of distinctive characteristics; it can withstand high temperatures up to 41°C and colds up to 6°C [4].

Nowadays, the resistance of microbial infections to antibiotics has threatened the health of societies. It is responsible for millions of deaths every year worldwide. In 2013, 9.2 million deaths have been reported because of infection. The evolution of resistance has caused antibacterial drugs to becoming less effective or even ineffective.

In recent years, various strategies have been suggested to overcome the resistance to antibiotics. One of these strategies is to achieve the combination of non-antibiotic drugs with antibiotics, which may increase the desirable antibacterial activity [2].

According to this case, phytochemicals have exhibited a potent activity. They can work alone or in combination with antibiotics to enhance the antibacterial activity against a wide range of bacteria.

B. melitensis and *B. abortus* are the most important cause of brucellosis in sheep and goats. It can cause abortion during the fourth month of pregnancy in goats [7].

Thus, this article introduces the antibacterial effects of some antibiotics and plant-derived essential oils (EOs) or extracts so that they can be used as promising choices to develop a new anti-*Brucella* therapy, as suitable alternatives to conventional antibiotics for brucellosis, as much as possible.

1. Materials and Methods

Sampling

B. abortus isolate was obtained from milk samples collected in previous work from different Syrian provinces at the Immunology and Microbiology Laboratory, AECS; and it was identified using molecular method [8].

Microorganisms and growth conditions

Brucella was grown under an optimal condition in 2YT agar supplemented with the following antibiotics to inhibit the growth of organisms other than *Brucella*: cycloheximide (100 mg), bacitracin (25,000 units), polymyxin B sulphate (5,000 units), vancomycin (20 mg), nalidixic acid (5 mg) and nystatin (100,000 units) [9].

The bio-typing of the bacteria was performed with the use of following tests: CO_2 requirement, H_2S production, urease, catalase and oxidase positivity, growth in the presence of dyes (thionine and basic fuchsine), and reaction with monospecific anti-A and anti-M sera (Thermo Fisher Scientific, UK) [10]. Isolates were stored in 2YT medium supplemented with a final glycerol concentration of 15% at –20°C.

DNA isolation and amplification by PCR

Isolation of DNA was carried out according to the method of cetyl trimethyl ammonium bromide (CTAB) modified by Ausubel et al., 2003 [11]. DNA pellet was resuspended in 100 µl of TE buffer and the concentration and purity were determined by spectrophotometer. A dilution of 100 ng/µl of purified DNA was made and stored at −20°C until required for further use.

For the identification of *Brucella* isolates specific oligonucleotide primers were used for a multiplex PCR assay. The primers sequences are shown in table 1.

PCR amplifications were carried out using Thermocycler (Techne Inc, TC-512, UK) with a 25-µl reaction mixture containing: 3 mM $MgCl_2$, 200 µM dNTPs, 10 pM of each primer, 1X reaction buffer, 1 U Taq DNA polymerase

(Fermentas, Germany) and 2 µl of template DNA (100 ng).

After initial denaturation of template DNA at 95°C for 5 min, the PCR profile was as follows: 35 cycles of 45s of template denaturation at 95°C; 30s of primer annealing at 55°C and 1 min of primer extension at 72°C; with a final extension at 72°C for 10 min. The presence of PCR products was determined by electrophoresis of 10 µl of reaction product in 1% agarose gel staining with ethidium bromide (0.5 µg/ml) in TAE 1X electrophoresis buffer for 1 h at a voltage of 70 and were visualized under UV light with the use of a 100 bp molecular weight DNA ladder (GeneRuler DNA Ladder Mix), (Thermo Fisher Scientific, UK) for the validation of length of the amplified products. Another bacterial genus was used as a negative control and sterile water was used to monitor any contamination with *Brucella* DNA.

Essential oils (EOs) extraction

Bark of *Cinnamomum verum* (*Cinnamon*) and rhizome of *Zingiber officinale* (Ginger) were purchased from local market in Damascus, Syria, then grounded and powdered using electrical blender prior to steam distillation.

Aerial parts, leaves of *Thymus syriacus* (*Thyme*) and *Mentha piperita* (*Mentha*), peels of *Citrus aurantium dulcis* (*Orange* peel) were collected during the flowering season from their natural habitat in Syria, these parts were cleaned and dried; then grounded and powdered using electrical blender prior to steam distillation.

Isolation of EOs was acquired using water steam distillation device (Clevenger-type apparatus) according to the European Pharmacopoeia method [12].

Briefly, 100g of each powdered dried plant was used for extraction EOs were released from plant material and evaporated into the hot steam.

Steam was applied for 3 h and the supernatant EOs were dried through anhydrous sodium sulfate (Na_2SO_4), filtered and stored in tighten brown colored bottle vials at (4°C). The oil was diluted in dimethyl sulfoxide (DMSO) and used for the antimicrobial efficacy test.

The yields of essential oil expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation:

Yield% = (amount of extracted oil (g)/amount of dry vegetal matter mass (g)) x100.

It was about (21, 4.6, 3.4, and 3%) with regard to *Mentha*, *Ginger*, *Cinnamon* and *Orange* respectively.

Antibiotics used in the study:

Antibiotics that were used: Cefprozil (CEF) a second-generation cephalosporin antibiotic, Cefixime$3H_2O$ (CFM), Cefotaxime sodium (CTX) and Ceftazidime (CAZ) a third-generation cephalosporin antibiotics, Levofloxacin (LEV) a third-generation fluoroquinolones and Tetracycline (TET). These antibiotics were purchased as powder (Sigma-Aldrich, Germany) and were added at a concentration of 50 ,100, 25, 50, 3.12, and 6.25 µg/µl for CEF, CFM, CTX, CAZ, LEV, and TET respectively according to the MIC values of a previous study [13].

Macrophages isolation from goat

Macrophages were isolated from blood. Briefly; 15 ml of whole blood from healthy goats were collected and peripheral blood cells were collected from whole blood by Ficoll gradient centrifugation. Collected cells were plated in tissue culture dishes (TPP, Switzerland) at a density of 3×10^6 cells/ml and macrophages were obtained by adhesion for 24h in RPMI-1640 (EuroClone, Italy) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (EuroClone, Italy), 2 mM L-glutamine (EuroClone, Italy), 1% non-essential amino acids (Gibco™, Germany), 0.1 mM sodium pyruvate (EuroClone, Italy), 50 µM 2-mercaptoethanol (EuroClone, Italy), and 10% fetal bovine serum (FBS) (EuroClone, Italy). After 24h cells were washed twice with RPMI-1640 to remove a majority of the non-adherent cells and cultured for an additional five days. The macrophage cell purity is >90% and cell viability is determined by trypan blue dye exclusion.

Macrophage infection

Isolated cells were cultured in RPMI 1640 with 10% FBS. A total of $1x10^5$ cells were cultured in each well of the tissue culture test plate. For macrophage growth assays, 96-well microtiter plates were seeded with 2×10^5 macrophages/well and infected with *B. abortus* at a ratio of 1:100 bacteria/macrophage. Cells were incubated for one h at 37°C in 5% CO_2. Extracellular bacteria were removed by three washes with PBS, followed by treatment with 25 µg/ml of

gentamicin (Sigma–Aldrich, Germany) for 30min. Then, the cells were maintained by adding a medium containing 5 µg/ml of gentamicin. To evaluate the effect of antibiotics and plant EO extracts on the ability of *Brucella* to invade goat's macrophages, each antibiotic and EO at 1% concentration, were added alone or in synergism, after 2, 4, 24, 48, 72, and 96h of infection, the cells were washed three times with PBS and lysed with 0.1% Triton.

After the incubation at room temperature for 5min, the lysates were plated on 2YT agar and incubated for 48h at 37°C; in order to determine the intracellular bacterial count. All experiments were performed in triplicate. Macrophages infected with *B. abortus* without adding any antibiotics or EO were considered as positive control and the same conditions but without adding the bacteria as a negative control.

In brief; the treatment was included five groups: (i) the control group without any additives or without bacteria as positive and negative control respectively, (ii) antibiotics only, (iii) antibiotics in synergism, (iv) EO only, and (v) EO in synergism.

Statistical study

Antibacterial properties of antibiotics and EO were analyzed with one-way repeated measures analysis of variance (ANOVA) to compare the difference of three replicates between each pair of means. Data were transformed into $\log_{10}$ CFU. All analyses were done by using SPSS Statistical Software V16. Differences were deemed statistically significant at $p<0.05$.

Results and Discussion

Identification of bacteria

All the isolates were Gram-negative coccobacilli, with biochemical tests positive for oxidase, catalase, urease, and hydrogen sulfide. They grew on media containing basic fuchsin but failed to grow on thionin media and required carbon dioxide for growth. No agglutination was observed with antisera M and R for all of the isolates, while all of them were showed agglutination with *Brucella*-monospecific antiserum A, and according to these tests, the isolates were apparently classified to be *B. abortus* [8].

PCR result

Genomic DNA of *B. abortus* was isolated and amplified by using multiplex PCR. The expected amplicons sizes were approximately 794bp for *B. melitensis*, 498bp for *B. abortus*, and 302bp for *Brucella* sp. (Fig 1).

Macrophage infection

Brucellae are considered to be a selected agent by the CDC because of the weakened nature of brucellosis, the lack of a safe and effective human vaccine [3], the easy entry of the microorganism by aerosolized and the low infectious dose (10–100 organisms), which increase the potential of the *Brucella* species to be used as agents of biological warfare and bioterrorism [2]. For these reasons, it was necessary to evaluate the antibacterial activity of some antibiotics and EOs against *Brucella's* isolates.

It was noticed that the $\log_{10}$ for *Brucella's* counts inside Shami's goat macrophages was increased from 3.84 after 2h of infection to reach a value of 8.83 after 96h whereas the ability of these macrophages to kill these bacteria without any antibiotics was the lowest and this result was in agreement with many kinds of research like Sathiyaseelan et al., 2000 [14] which indicated an increase in CFU of *Brucella* occurred between 24h and 48h after infection of macrophage populations ranging from $\log_{10}$ 0.5 to 1.5.

The activity of some antibiotics in elimination *B. abortus* was low such TET where the $\log_{10}$ of *Brucella's* population was 4.24 after 2h of infection and increased up to 5.51 after 96h. In addition, there wasn't any protection effect observed for Cephalosporins used in this study including Cefixime$3H_2O$, Cefotaxime sodium, and Ceftazidime, where the $\log_{10}$ for CFU of *B. abortus* population was increased from 3.65, 3.85, and 4.34 for the previous antibiotics respectively (after 2h of infection) to reach a value of 5.33, 5.21, and 5.30 after 96h of infection (Fig 2). This result was agreed with Motamedi et al., 2010 [15] which indicated that *Brucella's* isolates were resistant to TET that preventing bacteria from reproducing through the inhibition of protein synthesis, whereas the study of Wendell, 1990 [16] indicated that TET was the most active antibiotic against *Brucella* and one of the least toxic chemotherapies for human brucellosis. Also, Valderas et al. 2008 [17] demonstrated an

intracellular bacteriostatic activity for TET and Doxycycline in *B. abortus*, and it was indicated that all tested isolates were susceptible to TET by Baykam et al., 2004 [18].

Safi and Al-Mariri, 2012 [13] were assured that no activity was observed in all isolates when Cefixime3H_2O, Cefotaxime sodium, Ceftazidime, and other antibiotics were used, and this finding was in accordance with this study. According to Xu x-L et al., 2013 [7] the MIC values of LEV and CAZ were at medium level, but the bacteriostatic rate of CAZ was better. Also, there was a report on the successful treatment of brucellosis by LEV and CAZ by Hashemi et al., 2012 [19].

These results were may be due to the reason of the harmful effect of acidic pH on the efficiency of antibiotics against *Brucella* sp. It may be thought that most antibiotics lose their bactericidal activity against intracellular *Brucella* sp. because it was multiplied in acidic cell compartments within macrophages and this explanation was mentioned by Akova et al., 1999 [20].

In this study LEV was demonstrated a good activity against *B. abortus* with a $\log_{10}$ decreasing from 3.82 after 2h down to a value of 1.12 after 96h of infection and this result was due to its ability to readily penetrate macrophages (Fig 2).

This result was supported by the study of Hooper, 2000 [21], which indicated an antibacterial activity combined with better pharmacological properties such as fewer adverse effects and easier dosing that made this drug an attractive alternative in the treatment of brucellosis. Also, Lang and Rubinstein, 1992 [22] indicated that Fluoroquinolones and newer macrolides have a good anti-*Brucella* activity *in vitro*.

CEF was exhibited a good activity against *B. abortus* with a $\log_{10}$ decreasing from 3.68 after 2h down to a value of 1.45 after 96h of infection, but despite of CEF can be used to treat *Brucella's* infections, *B. abortus* has developed resistance towards it in varying degrees and this was given by the CEF Susceptibility and Resistance Datasheet 2013 [23], and this was noticed by previous research done by Safi and Al-Mariri which indicated that no activity was observed at all Syrian regions when CEF and other antibiotics were used [13].

The treatment of brucellosis is still problematic, because of high rates of treatment failure or relapses, and due to the intracellular nature of this organism, treatment requires combined regimens by replacing monotherapy with more potent synergistic that may increase efficacy and reduce treatment duration [24], for this purpose we studied the synergistic effect between some selected antibiotics to evaluate the antibacterial activity against our isolates.

In some cases, antibacterial combinations restore potency to ineffective drugs or enhance an antibiotic's potency targets and destroys mechanisms of bacterial resistance thereby allowing the antibiotic to function properly, interacting with the host to trigger defensive mechanisms [24].

Although our study found that TET was the least effective agent, the combination of TET-LEV showed the best synergistic effect inside goat's macrophages. It was detected an excellent synergistic activity between LEV-CEF, and LEV-TET with a $\log_{10}$ decreasing from 3.36, 3.30 after 2h down to a value of 2.30 and 2.33 after 96h of infection respectively according to the mentioned synergism (Fig 3).

Though several antibiotic combinations have been used in the treatment of brucellosis, there was very limited data about *in vitro* synergistic activity of *Brucella* strains in the literature.

There wasn't any synergistic activity studied between LEV-CEF or LEV–TET, whereas there were many studies about the synergistic activity of antibiotic combinations against *Brucella* species, in a study by Rubinstein et al. 1991 [25], various antibiotic combinations were tested against *Brucella* in combination studies, but none of them exhibited actual synergy. Also, Qadri et al., 1989 [26] in an earlier study reported the absence of synergy between quinolones and other antibiotics against *Brucella*. Moreover, in a study by Rubinstein et al., 1991 [25] it was shown that the combination of Ciprofloxacin with Minocycline exhibited the slowest bacterial killing, whereas combinations of Streptomycin with other antibiotics achieved the fastest killing. In a study by Akova et al., 1999 [20], the combination of Ofloxacin with Rifampin was tested against 20 isolates and there was indifference between the two antibiotics and the

combination exhibited antagonism, indifference, additive effect, and synergy. In the study of Matthew and Ioannis 2005 [27], the combination of Rifampin and Doxycycline was found to be the most synergistic. On the other hand, in a retrospective study by Tekkok et al., 1993 [28], Ofloxacin monotherapy led to a higher probability of brucellosis relapse than the combination of Ofloxacin and Rifampin in a small number of patients.

Despite the fact that quinolones are not recommended in first-line therapy for brucellosis, Kilic et al., 2008 [29] detected 43.7% and 25% synergistic activity and 56.2% and 43.7% additive activity in CIP-SXT (Trimoxazole) and TET-MXF (Moxifloxocin) combinations, respectively. These results may lead to consider to use of quinolones as an alternative choice if toxicity occurs in the classical combinations or as part of a second-line regimen in patients who don't improve or respond to disease relapse after therapy [27].

Finally, there are promising reports regarding the use of quinolone as the third agent in therapeutic combinations for complicated and difficult-to-treat cases of brucellosis such as the study of Manosuthi et al., 2004 [30].

There is a need to find alternative strategies to deal with infections resulting from drug-resistant bacteria, due to an increase in antibiotic-resistant bacteria and the lack of new antibiotics being brought onto the market. The development of alternatives to antibiotics and the discovery or development of adjuvants are among the potential strategies proposed [19]. EOs and their components form a part of phytochemicals that are seemed to have such effects, according to *in vitro* studies [31].

On the other hand, brucellosis was remained a major public health concern, especially in developing countries, and because of the antimicrobial resistance, multiple drug-resistant strains of *Brucella* have developed. For this reason, natural plant sources were evaluated for their antibacterial effects as an alternative and complementary medicine against *Brucella* isolates which were found to be highly pathogenic to human beings.

Brucella $\log_{10}$ counts inside Shami's goat macrophages were significantly studied by some EOs treatments compared with untreated control. It was noticed that there were significant activities shown when using *Thymus* 1% against the tested *Brucella's* strains with a $\log_{10}$ of counts decreased from 4.3 after 2h down to 1.5, after 96h of infection comparing to the control with $\log_{10}$ 8.64 after 24h, while a moderate activities were noticed when using each of *Orange* peel 1%, or *Ginger* 1% with a $\log_{10}$ increasing from 4.25 and 3.72 after 2h up to 4.54, and 4.21 after 96h of infection (Fig 4).

These findings were in agreement with many studies such as those of Viuda et al., 2008 [32] which proved that *Orange* and mandarin EOs had the moderate inhibition effect upon tested bacteria. Whereas Arshad et al., 2014 [31] reported that the activity of *Orange* oil for gram-negative organisms was significantly lower as compared to gram-positive isolates, however for *Thymus* EO Nostro et al., 2007 [33] reported it was had very strong inhibitory effects against tested bacteria, even in diluted forms. Lambert et al., 2001 [34] demonstrated that Carvacrol and Thymol, the principal components of *Oregano* and *Thyme* EOs, killed bacteria pathogens mostly by damaging their cytoplasmic membrane integrity and this finding was in agreement with this study.

Some researchers showed that the inhibitory effect of *Thyme* EO was more than extracts of this plant and with increasing of concentration; the antimicrobial properties were enhanced [35].

It wasn't noticed antibacterial activity when using *Mentha* EO and the $\log_{10}$ for CFU increased from 3.41 after 2h of infection up to 7.64 after 96h, whereas it was active up to 72 h with CFU $\log_{10}$ about 3.27 (Fig 4). These findings were in concert with the study of Al-Mariri et al., 2012 [36] which revealed that *Mentha* volatile EO had significant activities against *B. abortus* 544. Whereas, no antibacterial activity was demonstrated by the EOs of *Mentha* against *B. melitensis* in the study of Al-Mariri and Safi 2013 [37]. Also, Al-Bayati, 2009 [38] reported that *M. longifolia* L. volatile EO had antimicrobial activity against some gram-positive pathogenic, but did not have any activity against gram-negative ones. Mkaddem et al., 2009 [39] found that the *Mentha* volatile EO were very active against *K. pneumoniae* and *L. monocytogenes*; whereas they were less effective against *E. coli*.

Concerning *Cinnamon* 1%, it was revealed the best antibacterial activity in Shami's goat macrophages against *Brucella* strains with a $\log_{10}$ value of 3.41 after 2h of infection and a decreasing value reached 0.86 after 96h of infection which could be made it a potential source of new antibacterial agents (Fig 5).

In a previous work performed in our laboratory, Safi and Al-Mariri, 2014 [40] indicated the activity of *Cinnamon* EO, it was showed the highest antibacterial activities against *B. melitensis* with MIC_{50} values of 3.125 and also revealed a good activity against 90% of isolates (MIC_{90}=6.25 µl/ml).

Our finding is in accordance with a report by Al-Mariri et al., 2012 [36] which indicated that *C. verum* volatile oil at a concentration of 1% exhibited a strong inhibitory effect against *B. abortus* 544 inside the human macrophages, and the $\log_{10}$ CFU increased from 3.11 to 4.9 after 24 and 96h respectively after the infection [41] reported that the *Cinnamon* bark volatile EO revealed excellent antimicrobial activity against tested gram negative and gram-positive bacteria at concentrations ranging from 0.31% to 10% (v/v). Also, Ooi et al., 2006 [42] studied the antimicrobial effect of *Cinnamon* against gram-negative bacteria and reported that it was effective against a broad spectrum of bacteria, and its efficacy was related directly to the presence of active components, such as cinnamaldehyde cinnamyl acetate and cinnamyl alcohol, plus a wide range of other volatile substances.

In contrast *Cinnamon* EO, when applied at a concentration of 0.1% wasn't showed any significant inhibitory effect against *B. abortus* isolates with a value of $\log_{10}$ for *Brucella's* counts increasing from 4.22 up to 8.10 after 2 and 96h of infection respectively, compared to using *Cinnamon* at a percentage of 1% and this result was incompatible with the research of Al-Mariri et al., 2012 [36] which assured that *Cinnamon* EO, when applied at a concentration of 0.1%, did not show any considerable inhibitory effect against *B. abortus* 544 in comparison to the control group. Whereas Senhaji et al., 2007 [43] reported that viable bacterial counts decreased from $1x10^7$ to $1x10^4$ CFU/ml when bacterial cells were incubated at 37°C for 2h in the presence of 0.025% concentration of *Cinnamon* EO. However, it was almost completely eliminated after 30min of incubation in the presence of 0.05% concentration of *Cinnamon* EO, and these findings weren't compatible with our study. In contrast, a strong significant inhibitory effect was observed when a 0.1% concentration of *Cinnamon* EO was applied in combination with a 1% concentration of the other plant's oil extracts in comparison with the control without oils. The $\log_{10}$ CFU was reached a value of 0.99 for *Cinnamon* and *Mentha*, 0.93 for *Cinnamon* and *Ginger*, 1.10 for *Cinnamon* and *Orange* peel and 0.96 for *Cinnamon* and *Thymus* after 96h of infection (Fig 6). This was mean that *Cinnamon* oil at a concentration of 0.1% was associated with increased antibacterial activity and had good synergism with different EOs as antibacterial agents against *B. abortus*, which was assured with a previous study by Al-Mariri et al., 2012 [36] that showed the mixture of 1% concentrations of individual EOs and a small amount of *Cinnamon* oil 0.1% was associated with enhanced antibacterial activity. Also, Probst et al., 2011 [44] findings showed that a combination of *Cinnamon* with *Peppermint*, *Ginger*, and *Clove* EOs produced synergistic antibacterial effects against gram-positive and gram-negative microorganisms. Moreover, it was in agreement with Nanasombat and Wimuttigosol, 2011 [45] results, which revealed that *Cinnamon* oil in combination with Nutmeg or Makaen (Zanthoxylum limonella Alston) EO showed a synergistic effect against some negative and positive gram bacteria that were resistant to antibiotics.

These synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or several substances of an extract [46].

Conclusion

The objective of this study was to develop an effective and cheap therapy against *B. abortus* inside goat's macrophages. *Brucella* strains have been shown resistant to most antibiotic groups used in this study, whereas it was detected an excellent synergistic activity between LEV-CEF, and LEV–TET. Also, *Cinnamon* EO at a concentration of 1% is used separately, or at a concentration of 0.1% in combination with other oils and represented an

alternative source of natural antimicrobial materials, and might replace conventional chemical antimicrobials.

The high specific activity of *Cinnamon* at low and non-toxic concentrations suggests that it could be used in clinical practice for the treatment of Brucellosis in animals and humans, whereas more specific studies are recommended to examine this alternative therapy.

Table 1: Oligonucleotide primers used for multiplex PCR.

primer	DNA sequence (5'–3')	Gene target	Reference
Bru-Ab	GACGAACGGAATTTTTCCAATCCC	alpha-ketoglutaratedependent dioxygenase	[10]
Bru-Me	AAATCGCGTCCTTGCTGGTCTGA	hypothetical protein	
Bru-IS711	TGCCGATCACTTAAGGGCCTTCAT	*IS711*	
Bru-F	CCTTTTCGAGCACTTCGG	*ugpB*	
Bru-R	AGCTATCGCGCTCACCAT		

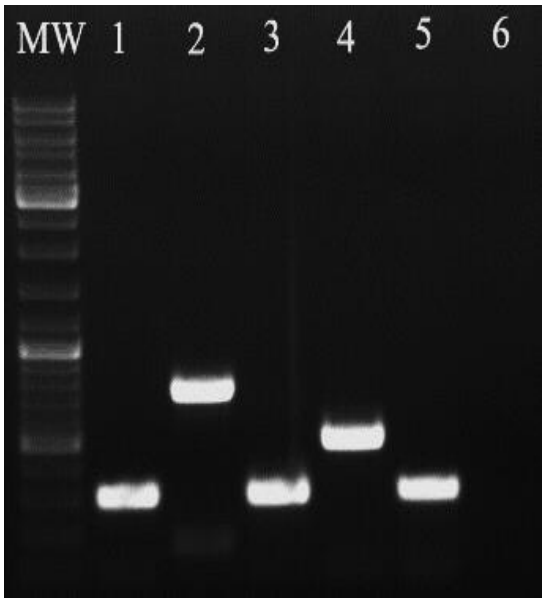


Fig 1: PCR Electrophoresis on agarose gel 1%: MW: 100bp molecular DNA ladder, 1: *B.* melitensis, 2: *B. melitensis*, 3: *B. abortus*, 4: *B. abortus*, 5: *Brucella* sp., 6: Negative control.

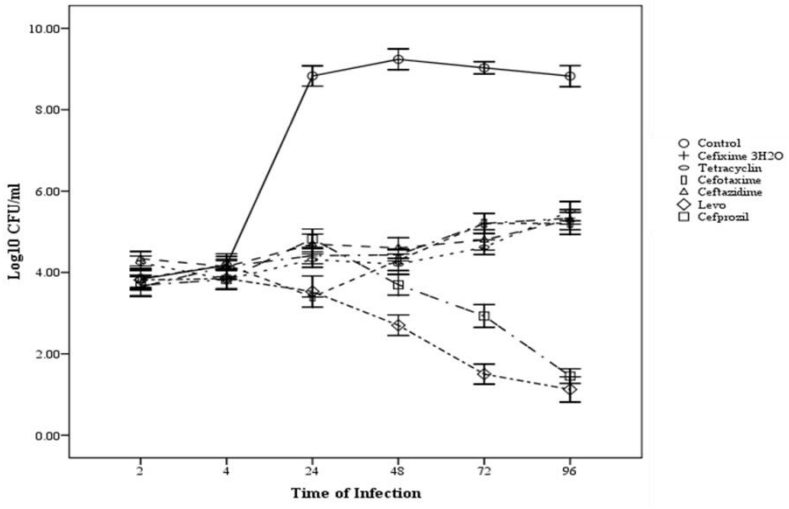
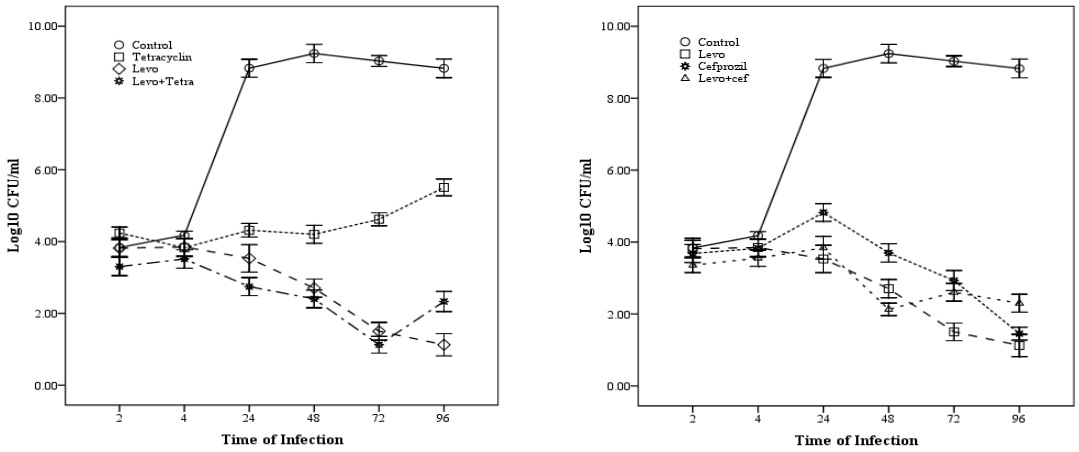


Fig 2: The antimicrobial effects of the studied antibiotics as log_{10} CFU/ml after 2 to 96h from macrophages infection with *B. abortus*. It was noticed that there were statistically significant differences with (p<0.05) between the antibiotics and control, and between (lev and antibiotics except for cef) for the time above 24h. No significant difference was shown between lev and cef at all times of the study.



(A) (B)

Fig 3: Synergistic inhibitory effect of antibiotics after 2-96h of infection; (A): synergism between LEV and TET (✱) on *B. abortus* inside goat macrophages in comparison with each of them alone (◊) and (□); (B): synergism between LEV and CEF (△) on *B. abortus* inside goat macrophages in comparison with each of them alone (□) and (✱). There were statistically significant differences with (p<0.05) between TET and each of LEV and LEV-TET (A). It was noticed that there was no statistically significant difference between LEV, CEF, and LEV-CEF (B).

ISSN: 0976-7126
Mariri *et al.*, 16(9):27-40, 2025

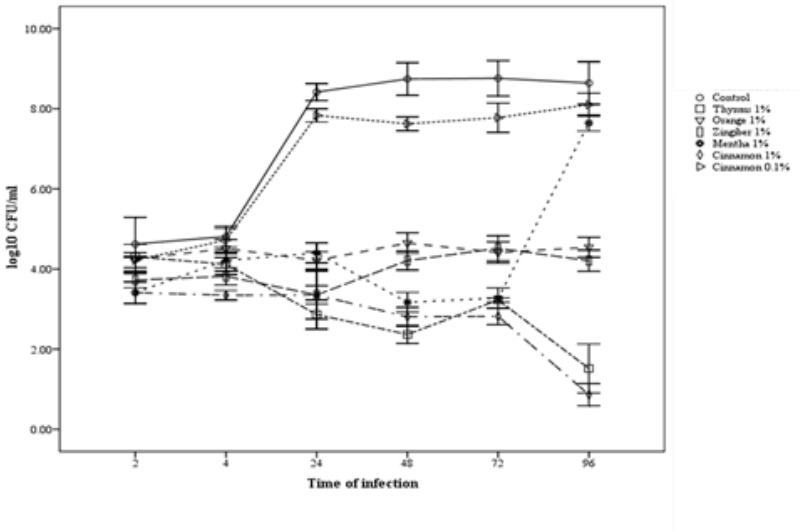


Fig 4: The antimicrobial effects of the studied oil extracts as log_{10} CFU/ml after 2 to 96h from macrophages infection with *B. abortus*; (○): Without oil, (□) *Thyme* 1%, (▽) *Orange* 1%, (▯) *Zingiber* 1%, (□) *Mentha* 1%, (◊) *Cinnamon* 1%, (▷) *Cinnamon* 0.1%. It was noticed that there was a statistically significant difference with ($p<0.05$) between each of *Zingiber, Orange, Mentha,* and *Cinnamon* 1% against *Thyme* 1%, and *Cinnamon* 0.1%. There were no significant differences shown between *Zingiber, Orange, Mentha,* and *Cinnamon* 1%.

Fig 4: The antimicrobial effects of the studied oil extracts as log_{10} CFU/ml after 2 to 96h from macrophages infection with *B. abortus*; (○): Without oil, (□) *Thyme* 1%, (▽) *Orange* 1%, (▯) *Zingiber* 1%, (□) *Mentha* 1%, (◊) *Cinnamon* 1%, (▷) *Cinnamon* 0.1%. It was noticed that there was a statistically significant difference with ($p<0.05$) between each of *Zingiber, Orange, Mentha,* and *Cinnamon* 1% against *Thyme* 1%, and *Cinnamon* 0.1%. There were no significant differences shown between *Zingiber, Orange, Mentha,* and *Cinnamon* 1%.

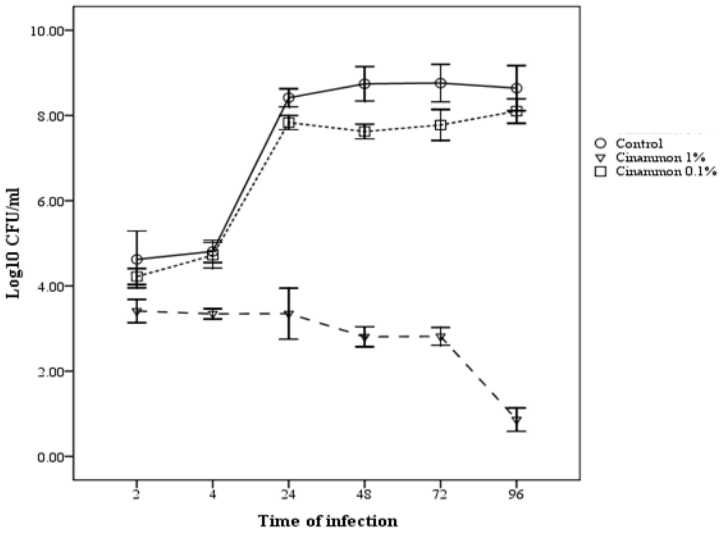


Fig 5: Antibacterial effect of *Cinnamon* oil extracts at a concentration of 1% (▽) and 0.1% (□) after 2-96h of infection on *B. abortus* inside goat macrophages in the comparison with the control (○). It

was noticed that there was a statistically significant difference with (p<0.05) between *Cinnamon* 1%, and *Cinnamon* 0.1%.

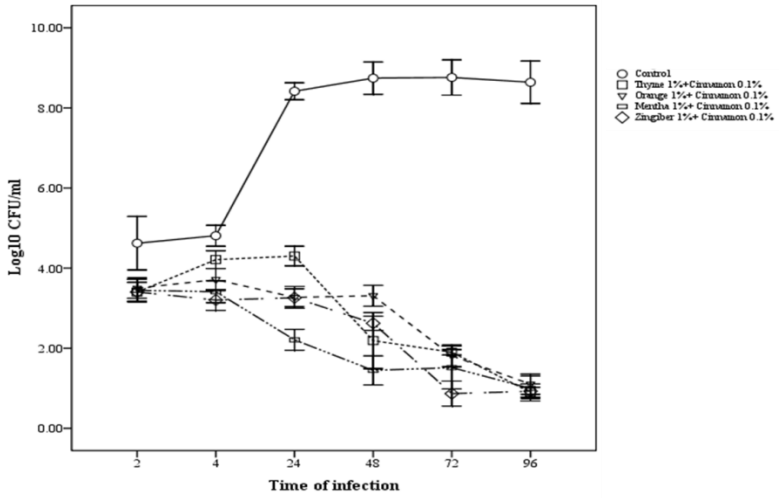


Fig 6: Synergistic inhibitory effect of oil extracts after 2-96h of infection; (○): Bacteria without oils, (□) synergism between *Cinnamon* 0.1% and *Thyme* 1%, (▽): Synergism between *Cinnamon* 0.1% and *Orange* 1%, (▭): Synergism between *Cinnamon* 0.1% and *Mentha* 1%, (◊):Synergism between *Cinnamon* 0.1% and *Zingiber* 1%; on *B. abortus* inside goat macrophages in comparison with the effects of *Cinnamon* 0.1% alone. There were no significant differences shown between the following synergism (*Orange* 1%- *Cinnamon* 0.1%), (*Mentha* 1%- *Cinnamon* 0.1%), (*Zingiber* 1%- *Cinnamon* 0.1%). a statistically significant difference with (p<0.05) was shown for the synergism (*Thyme* 1%- *Cinnamon* 0.1%) against the other synergism after 96h of infection.

Acknowledgments
The authors would like to thank the Director General of the AECS and the Head of the Molecular Biology and Biotechnology Department for their support.

References
1. Lai S.H, Zhou H., Xiong W., Gilbert M., Huang Z.H, Yu J. (2017). Changing epidemiology of human brucellosis, China, 1955–2014, *Emerg Infect Dis*, *23(2): 184–194*.
2. WHO. (2017). The World is Running Out of Antibiotics, WHO Report Confirms. September 2017. Available online: https://www.who.int/news/item/20-09 2017-the-world-is-running-out-of-antibiotics-who-report-confirms (accessed on 22 May 2022).
3. Al-Mariri A., Al-Hallab L., Alabras R., Kherbik H., Khawajkiah M. (2022). Protection against virulent *Brucella* spp. by gamma-irradiated *B. ovis* in BALB/c mice model, *Clin Exp Vaccine Res*, *11:53-62*.
4. Mavrogenis A.P., Antoniades N.Y. and Hooper R.W. (2006). The Damascus (Shami) goat of Cyprus, *Anim. Genet. Resour. Inf., 38: 57-65*.
5. Ganskopp D., Cruz R., Fajemisin B. (1997). Relationships among variables indexing selective grazing behaviour of goats, *Appl. Anim. Behav*. Sci., *51: 75-85*.
6. Alsheikh S.M. (2013). Prediction of daily milk yield using different body measurements of Shami goats in Sinai. Egypt Am-Eurasian, *J. Agric Environ Sci*, *13(2):218–221*.
7. Xu X.L, Chen X, Yang P.H, Liu J.Y, Hao X.K. (2013). *In vitro* drug resistance of clinical isolated *Brucella* against antimicrobial agents, *Asian Pac J Trop Med*, *6:921-924*.

8. Al-Mariri A., Haj-Mahmoud N. (2010). Detection of *Brucella abortus* in Bovine Milk by Polymerase Chain Reaction, *ACTA VET. BRNO, 79: 55-61*.
9. Al-Mariri A. (2015). Isolation of *Brucella Melitensis* strains from Syrian bovine milk Samples, Bulgarian *J. Vet. Med., 18 (1): 40-48*.
10. Bricker B.J, Halling S.M. (1994). Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella sui*s bv. 1 by PCR, *J Clin Microbiol, 32:2660-2666*.
11. Ausubel F.M., Brent R., Kimgston R.E., Moore D.D., Seidman J.D., Smith J.A., Struhl K., Eds. (2003). *Curr. Protoc. Mol. Biol.*, p*p. 241–245. New York, John Wiley and Sons, inc; pp. 208-221*.
12. European Pharmacopoeia. (2011). *7thEd. Council of Europe: Strasbourg, France. 99*.
13. Safi M., & Al-Mariri A. (2012). Efficacy evaluation of some antibiotics against syrian *Brucella* spp isolates, in vitro, *Braz. J. Microbiol., 43(4), 1269–1273*. doi:10.1590/S1517-83822012000400005.
14. Sathiyaseelan J., Jiang X., & Baldwin C. L. (2000). Growth of *Brucella abortus* in macrophages from resistant and susceptible mouse strains, *Clin. Exp. Immunol, 121(2), 289–294*. doi:10.1046/j.1365-2249.2000. 01295.x
15. Motamedi H., Darabpour E., Gholipour M., & Seyyed Nejad S.M. (2010). In vitro assay for the anti-*Brucella* activity of medicinal plants against tetracycline-resistant *Brucella* melitensis, *J. Zhejiang Univ. Sci., 11(7), 506–511*. doi:10.1631/jzus.B0900365.
16. Wendell H.H. (1990). Modern Chemotherapy for Brucellosis in Humans, Reviews of Infectious Diseases, *12: (6): 1060–1099*, https://doi.org/10.1093/clinids/12.6.1060.
17. Valderas M.W., Barrow W.W. (2008). Establishment of a method for evaluating intracellular antibiotic efficacy in *Brucella abortus* infected Mono Mac 6 monocytes, *J Antimicrob Chemother, 61:128-134*.
18. Baykam N., Esener H., Ergonul O., Eren S., Celikbas A.K., Dokuzoguz B. (2004). In vitro antimicrobial susceptibility of *Brucella* species, Intern. *J. Antimicrob. Agents, 23:405–407*.
19. Hashemi S.H, Gachkar L, Keramat F, Mamani M, Hajilooi M, Janbakhsh A, Majzoobi M.M, Mahjub H. (2012). Comparison of doxycycline-streptomycin, doxycycline-rifampin,and ofloxacin-rifampin in the treatment of brucellosis: a randomized, clinical trial. *Int J Infect Dis, 16(4): e247-e251*.
20. Akova M., Gür D., Livermore D.M., Kocagöz T., & Akalin H.E. (1999). *In vitro* activities of antibiotics alone and in combination against *Brucella melitensis* at neutral and acidic pHs, *Antimicrob. Agents Chemother, 43(5), 1298–1300.*
21. Hooper D.C. (2000) New uses for new and old quinolones and the challenge of resistance. *Clin Infect Dis, 30:243-54*.
22. Lang R., and Rubinstein E. (1992). Quinolones for the treatment of brucellosis, *J. Antimicrob. Chemother, 29:357–363*.https://doi.org/10.1093/jac/29.4.357.
23. Cefprozil Susceptibility and Resistance Data (PDF). (2013). *Retrieved 23 July*.
24. Rossetti C.A., Arenas-Gamboa A.M., Maurizio E. (2017). Caprine brucellosis: A historically neglected disease with significant impact on public health, *PLoS Negl Trop Dis, 11(8): e0005692*.
25. Rubinstein E., Lang R., Shasha B., Hagar B., Diamanstein L., Joseph G., Anderson M., and Harrison K. (1991). *In vitro* susceptibility of *Brucella melitensis* to antibiotics, *Antimicrob. Agents Chemother, 35:1925*-1927.
26. Qadri S.M., Akhtar M., Ueno Y., and Sibai M.B. (1989). Susceptibility of *Brucella melitensis* to fluoroquinolones, *Drugs Exp. Clin. Res, 15:483*-485.
27. Matthew E.F, Ioannis A.B. (2005). Quinolones for Treatment of Human Brucellosis: Critical Review of the Evidence from Microbiological and Clinical Studies, Antimicrobial *Antimicrob. Agents Chemother., 50 (1)*

22-33; DOI: 10.1128/AAC.50.1.22-33.2006.

28. Tekkok I.H., Berker M., Ozcan O.E., Ozgen T., and Akalin E. (1993). Brucellosis of the spine, *Neurosurgery*, *33:838-844*.
29. Kilic S., Dizbay M., Hizel K., Arman D. (2008). *In vitro* synergistic activity of antibiotic combinations against Brucella melitensis using E-test methodology. *Braz J Microbiol*. *39(2):233-7*. doi: 10.1590/S1517-83822008000200006. Epub 2008 Jun 1. PMID: 24031207; PMCID: PMC3768412.
30. Manosuthi W., Thummakul T., Vibhagool A., Vorachit M., and Malathum K. (2004). Brucellosis: a re-emerging disease in Thailand, Southeast Asian *J. Trop. Med Public Health*, *35:109-112*.
31. Arshad N., Khan S., Tariq H., & Samreen S. (2014). Characterization of Contact Lens Associated Bacteria and Their Responses to Botanical Essential Oils, *Int J Agric Biol*, *16. 1171-1176*.
32. Viuda-Martos, M. Ruiz-Navajas Y. Fernández-López J. & Pérez-Álvarez J. (2008). Antibacterial activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils, *J. Food Saf., 28. 567-576*. 10.1111/j.1745-4565.2008.00131.x.
33. Nostro A., Sudano R.A., Bisignano G., Marino A., Cannatelli M.A., Pizzimenti F.C. (2007). Effects of oregano, carvacrol and thymol on Staphylococcus aureus and Staphylococcus epidermidis biofilms, *J Med Microbiol*, *56:519–23*. doi: 10.1099/jmm.0.46804-0. PubMed PMID: 17374894.
34. Lambert R.J., Skandamis P.N., Coote P.J., and Nychas G.J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol, *J. Appl. Microbiol*, *91:453–462*.
35. Ismail M.M, Essam T.M, Mohamed A.F, Mourad F.E. (2012). Screening for the antimicrobial activities of alcoholic and aqueous extracts of some common spices in Egypt, *Int J Microbiol Res, 3:200-207*.
36. Al-Mariri A., Saour G., & Hamou R. (2012). In vitro antibacterial effects of five volatile oil extracts against intramacrophage *Brucella abortus* 544, *Iran J. Med. Sci, 37(2), 119–125*.
37. Al-Mariri A., Safi M. (2013). The antibacterial activity of selected Labiatae (Lamiaceae) essential oils against *Brucella melitensis*, *Iran J. Med. Sci*, *38(1): 44-50*.
38. Al-Bayati F.A. Isolation and identification of antimicrobial compound from Mentha longifolia L. leaves grown wild in Iraq. (2009), *Ann Clin Microbiol Antimicrob*, *12(6), 8-20*. doi: 10.1186/1476-0711-8-20. PubMed PMID: 19523224; PubMed Central PMCID: PMC2707363.
39. Mkaddem M., Bouajila J., Ennajar M., Lebrihi A., Mathieu F., Romdhane M. (2009). Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oil extracts, *J Food Sci*, *74:358-63*. doi: 10.1111/j.1750-3841.2009.01272.x. PubMed PMID: 19895481.
40. Safi M. & Al-Mariri A. (2014). *In vitro* antibacterial activity of several plant extracts and essential oils against *Brucella melitensis*, *Herba Pol., 60. 10.2478/hepo-2014-0003*.
41. Mayaud L., Carricajo A., Zhiri A., Aubert G. (2008). Comparison of bacteriostatic and bactericidal activity of 13 essential oil extracts against strains with varying sensitivity to antibiotics, *Lett Appl Microbiol*, *47:167–73*. doi: 10.1111/j.1472-765X.2008.02406.x. PubMed PMID: 19552780.
42. Ooi L.S., Li Y., Kam S.L., Wang H., Wong E.Y., Ooi V.E. (2006). Antimicrobial activities of Cinnamon oil and cinnamaldehyde from the Chinese medicinal herb Cinnamomum cassia Blume, *Am J Chin Med*, *34:511-522*.
43. Senhaji O., Faid M., Kalalou I. (2007) Inactivation of *Escherichia coli* O157:H7 by essential oil from *Cinnamomum*

zeylanicum. *Braz J Infect Dis*, *11(2):234-6*. doi: 10.1590/s1413-86702007000200013. PMID: 17625768.

44. Probst I.S., Sforcin J.M., Rall V.L.M., Fernandes A.A.H., Fernandes JúniorA. (2011). Antimicrobial activity of propolis and essential oil extracts and synergism between these natural products, *J Venom Anim Toxins incl Trop Dis*, *17:159–67*.
45. Nanasombat S., Wimuttigosol P. (2011). Antimicrobial and antioxidant activity of spice essential oil extracts, *Food Sci Biotechnol*, *20:45–53*.
46. Wagnera H., Ulrich-Merzenich G. (2009). Synergy research: Approaching a new generation of phytopharmaceuticals, *Phytomedicine*, *16:97–110*. doi: 10.1016/j.phymed.2008.12.018.

Cite this article as:
Mariri A.A., Alobeid B., Dayuob N. and Hallab L. Al. (2025). Antibacterial Effects of Some Antibiotics and Essential Oils Against *Brucella abortus* Inside Goat's Macrophages and New Promising Treatments. *Int. J. of Pharm. & Life Sci.*, 16(9):27-40.

Source of Support: Nil
Conflict of Interest: Not declared
For reprints contact: ijplsjournal@gmail.com