

In Vitro Study of the Bacterial Anti-Bioresistance and the Use of Some Medicinal Plants in Avian therapy

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Abstract

This study addresses the problem of pathogenic bacteria resistant to antibiotics isolated from infected chickens and the use of medicinal plants and their bioactive substances *in vitro*.

On 75 strains isolated from a dead chickens belonging to the three families the Enterobacteriaceae; the Staphylococaceae; the Pseudomonaceae bacteria. The antibiogram tests were used to select ten resistant bacteria strains in this study.

Ethonobotanical study was done to select the most medicinal plants used for the therapy of animals in Algeria. The essential oils of six medicinal plants were extracted by Hydro distillation (Clevenger). The plants with their yields in essential oils are: *Thymus vulgaris* (2.75%), *Salvia officinalis* (2.50%), *Rosmarinus officinalis* (2.43%), *Thymus capitatus* (1.82%), *Ruta chalepensis* (0.93%), *Artemisia herba alba* (0.90%).

By measuring the activity of the oils on agar medium, this test has provided the following results: The essential oils of different plants gave the diameters of inhibition zones between 0 mm and 53.33 ± 1.53 mm, for the 5 µl discs, and between 0 mm and 52.33 ± 2.52 mm, for the 10 µl discs, while discs of 15 µl, those diameters vary between 0 mm and 56.67 ± 1.15 mm.

The results of MICS of oils studied are encouraging, oils of *Thymus capitatus*, *Rosmarinus officinalis* and *Salvia officinalis* share a minimum inhibitory concentration (MIC) between 1.25 and 20 (µL.mL⁻¹) with an effect bactericidal/ bacteriostatic, except the oil *Salvia officinalis* presents a bactericidal effect. The oils of *Thymus vulgaris*, *Artemisia herba alba* and *Ruta chalepensis* have a MIC respectively of 1.25 to 10 (µL.mL⁻¹), 5 to 40 (µL.mL⁻¹), 1.25 and 40 (µL.mL⁻¹). The effect is bactericidal effect for these oils.

Keywords: Essential oils; Antibacterial activity; Bacterial antibioresistance; Minimum Inhibitory Concentration (MIC)

Introduction

Algeria is a country of the North of Africa; recently indicated a worrying situation of antibiotic resistance, these last ten years have been trade mark by the emergence and dissemination of new resistance genes in particular in the north of the country [1]. Any use of antibiotics, either for the man, animal, plant or the technology of food processing, is likely to lead to a certain point in time, a bacterial resistance. Although many publications are beginning to appear, little is known on the different conditions of use in which the antibiotics preferably select, or select to a lesser extent, for bacteria resistant [2]. Bacterial resistance to antimicrobial agents is a problem of increasing importance in medical practice [3]. The history of aromatic and medicinal plants is associated with the evolution of civilizations. In all regions of the world, the history of peoples shows that these plants have always occupied an important place in medical therapy [4].

The 1990s of the last century have been marked by a general awareness in favor of the health of the Man and of the quality of the environment. Organic agriculture, the herbal medicine and the aromatherapy have sparked a renewed interest for the culture of

aromatic and medicinal plants for use in a fresh, dried or in the form of an extract. The world demand for aromatic medicinal plant and their derivatives for the Agri-Food, phytotherapy, perfumes and natural cosmetic products have in fact increased. The aromatic medicinal plant, in the developing countries of Asia, Africa and Latin America, play an important role in the traditional pharmacopoeia and the power [5]. In phyto-therapy, essential oils are used for their antiseptic properties against infectious diseases of bacterial origin [6].

Our work is part of a contribution to the valorization of medicinal plants widely used by traditional Algerian breeders. To this end, our study encompasses two aspects, the first of which is based on microbiological character, isolation, identification and susceptibility. The second aspect of extraction, screening, antimicrobial activity.

Materials and Methods

The strains of germs causing respiratory diseases, digestive and nutritional status remain a major problem in the chicken farming sector, in addition to therapeutic failure in the face of the antibiotics. In this sense the pathogenic bacteria causing these diseases were isolated and identified. The levies are made according to the recommendations of the organization of International Epizootics (O.I.E). The chickens died recently were necropsied. Macroscopic observation aid has

distinguished the infected body and that the return to the parameters of aspect, colors, the smell and sometimes presences of stains. The macroscopic examination of tissues and organs in order to detect possible changes lésionnelles. It is including the liver, heart, spleen, and intestine [7].

According to Pilet C, et al. [8], identification begins with the determination of the family, then of the genus (oxidase, Catalase) and finally of the different species by the classic gallery.

Bacteria are isolated from sick and dead animals. The media were selected according to the desired bacterial groups [9]. Representative colonies were selected in a random manner and subcultured by streaking on appropriate medium. The purification of the strains was performed by repeated cultures up to the obtaining of a pure culture [10]. Gram staining was a key trait for classifying families of bacteria.

Identification of strains of Enterobacteria: Colonies representative of Enterobacteria were selected randomly and transplanted by streaking on appropriate medium. The isolated bacterial strains were characterized using the API20 E [11]. The strains were identified by comparing their characteristics with those of known taxa as described in the Bergey's Manual for Determinative Bacteriology [11].

All strains were identified using standard bacteriological methods (production of catalase and coagulase and biochemical characteristics using the API20 staph [12]. The oxidase test is the differentiating character between *Pseudomonas* and other *Enterobacteriaceae*.

The bacterial strains isolated were characterized using the biochemical Gallery tests for *Staphylococcus*, *Pseudomonas* and *Enterobacter* bacteria with a high frequency of contamination and sensitivity.

The sensitivity of the isolated strains to antibiotics has been determined by the method of dissemination in agar as recommended by the Committee of the sensitivity of the French Society of Microbiology [13].

Aromatogramme test

The aromatogramme is a method of *in vitro* measurement of the antibacterial power of chemotyped essential oils. Different types of aromatogrammes, in solid, liquid, are exploitable [14].

The choice of our plants is based on a survey conducted earlier in the axis of the Ethno-Veterinary approach of the medicinal plants used in the region of Sidi Bel Abbes-Algeria [15]. The essential oils are obtained by Hydro distillation with a device of type Clevenger [16] for a period of three hours.

A bacterial suspension of equivalent density to the Standard 0.5 of Mac Farland (108 cfu.mL^{-1}) is prepared and then diluted to the 1/100 fortified by 10% DMSO [17].

The method of dilutions of Kirby-Bauer

Minimum Inhibitory Concentration (MIC): one proceeds to a successive dilution by Progress. The following dilutions 1/2, 1/4 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 [18]. The MIC (% v/v) of the essential oil tested is deducted from the first tube of the range devoid of bacterial growth.

Minimum concentration bactericidal (MBC): The nutrient agar poured into petri dishes is seeded with ridges by 100 μL of the contents of the tubes with a concentration\MIC in the series of previous

dilution [19]. The MBC is determined after an incubation period of 24 hours at 37°C. It is the lowest concentration which inhibits totally the growth.

The MBC (% v/v) of the essential oil is deducted from the first box devoid of bacteria (Guinoiseau, 2010). The antibacterial effect was considered bactericidal or bacteriostatic in function of the report: MBC/MIC. Indeed, if MBC/MIC=1 to 2, the effect is bactericidal and if MBC/MIC=4 to 16.

Results

The isolated bacteria

75 strains were isolated from the liver, heart, spleen, and intestine. Bacterial strains tested were the following: Enterobacteriaceae 65% (n=49), Staphylococcaceae 21% (n=16) Pseudomonaceae 13% (n=10) (Figure 1).

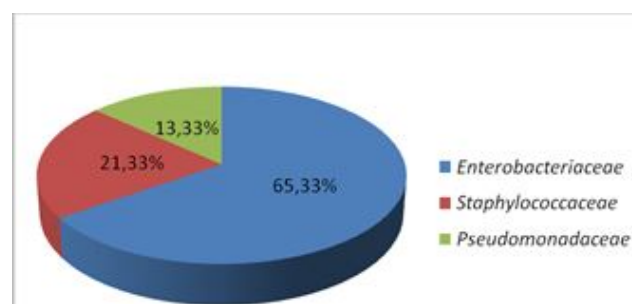


Figure 1: Distribution of isolated bacterial groups.

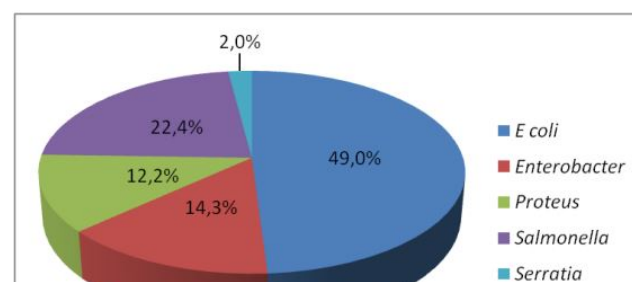


Figure 2: Distribution of Enterobacteriaceae strains.

On a total of 49 strains of Enterobacteriaceae, we note *E. coli* 49% (n=24), *Enterobacter* 14% (n=7), *Proteus* 12% (n=6), *Salmonella*, 22% (n=11), *Serratia* 2% (n=1) (Figure 2).

Antibiogramme

Selection of the most resistant strains: Isolates which follow this study of identification and aromatogramme are the one who presents a resistance to a many antibiotics tested (Figure 3).

Escherichia coli N (31) showed a resistance of 83.33% to all antibiotics tested except for gentamicin, whereas *Escherichia coli* N (54) had a 50% resistance to all the antibiotics tested except for ampicillin, Colistin and gentamicin (Figure 3).

The testicular antibiotics are ceftiofur, colistin and gentamicin. The antibiotic resistance of *Pseudomonas aeruginosa* N (61) is 66.66%, resistant to all antibiotics except gentamicin (Figure 3).

Enterobacter aerogenes N (49), *Enterobacter faecalis* N (22), *Proteus vulgaris* N (20), *Salmonella thyphi* N (18) and *Salmonella para thyphi* N (35) are sensitive to colistin and gentamicin at 66.66% with sensitivity to gentamicin (Figure 3).

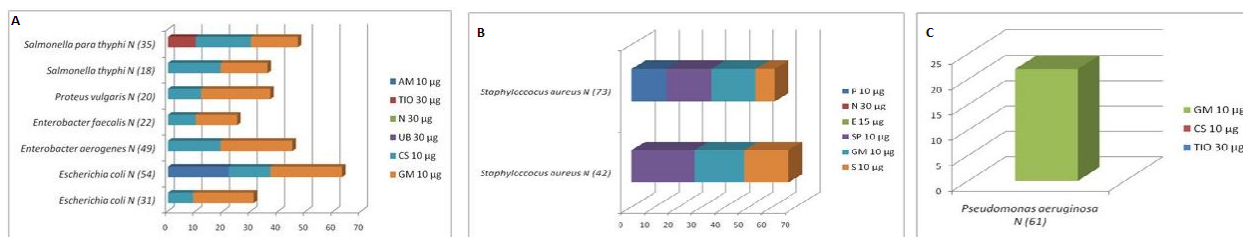


Figure 3: Diameter zones of inhibition in mm of the most resistant strains to the antibiotics.

AM: Ampiciline 10 µg; TIO: Ceftiofur 30 µg; N: Neomycine 30 µg; UB: Fluméquine 30 µg; CS: Colistine 10 µg; GM: Gentamicine 10 µg; P: Peniciline 10 µg; E: Erythromycine 15 µg; SP : Spiramycine 10 µg ; S: Streptomycine 10 µg.

The antibiotic testes are penicillin, neomycin, erythromycin, spiramycin, gentamicin and streptomycin. *Staphylococcus aureus* N (24) and *Staphylococcus aureus* N (73) have a minimum resistance of 50%, susceptible to all antibiotics except spiramycin, gentamicin, streptomycin, While *Staphylococcus aureus* N (73) susceptible to Spiramycin and gentamicin.

The Results of Essential Oils

The results of the activity of the oils on agar medium

The results obtained are illustrated in the histogram by the (Figure 4). We note that the DMSO has no antibacterial activity, therefore a zone of inhibition around the disc soaked by DMSO, is totally absent.

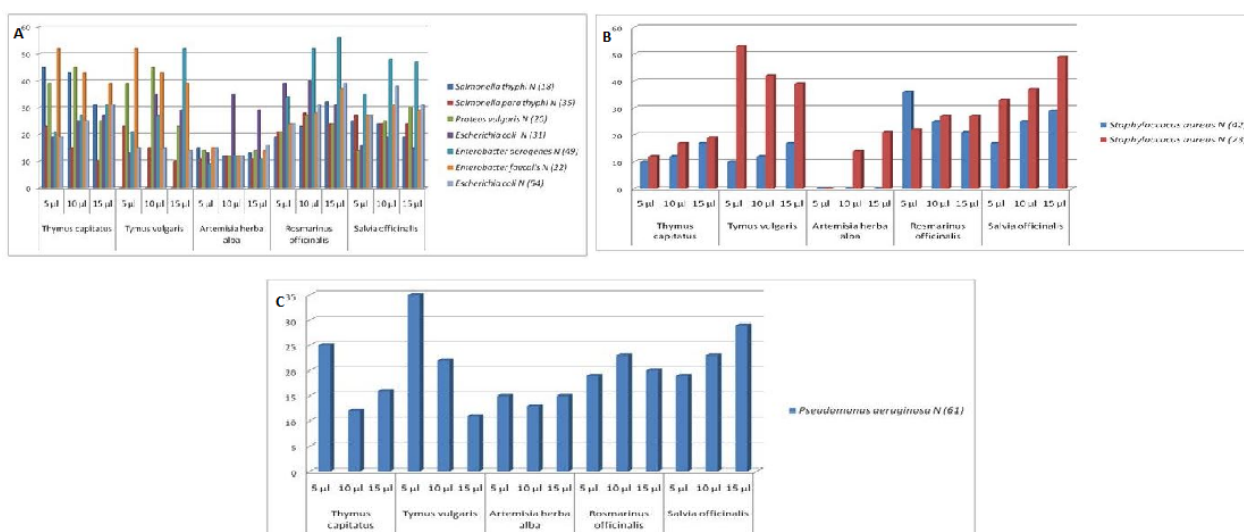


Figure 4: A) The diameter of the inhibition zone in mm of enterobactereacea at different concentrations of essential oils; B) The diameter of the inhibition zone in mm of staphylococci of different concentrations of essential oils; C) The diameter of the inhibition zone in mm of pseudomonas of different concentrations of essential oils.

For the pure OE it generates zones of important inhibition what first appears is the sensitivity of the bacterial strains belonging to the three

families Enterobacteriaceae, Staphylococaceae, Pseudomonaceae with respect to the majority of the essential oils studied of concentration 5 μL , 10 μL and 15 μL .

The essential oils of *Thymus capitatus* and *Thymus vulgaris* show a powerful effect but not in relation to other plants, then that we note a clear resistance of *Salmonella para thyphi* with the oils of *Thymus capitatus* has 15 μL for a diameter of 10.33 ± 0.58 mm unlike *S. aureus*

to same concentration. *S. thyphi* N (18) with the oils of *Thymus vulgaris* and this for the three concentrations.

The Results of the MIC and MBC

The determination of the minimum inhibitory concentration and the minimal bactericidal concentration are in (Table 1a and 1b).

($\mu\text{L.mL}^{-1}$)	Thymus capitatus			Thymus vulgaris			Artemisia herba alba		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
<i>Salmonella parathyphi</i> N (35)	20	40	2	10	10	1	40	40	1
<i>Salmonella thyphi</i> N (18)	5	5	1	/	/	/	20	40	2
<i>Proteus vulgaris</i> N (20)	2.5	5	2	2.5	2.5	1	10	20	2
<i>Escherichia coli</i> N (31)	2.5	5	2	5	5	1	20	40	2
<i>Enterobacter aerogenes</i> N (49)	1.25	2.25	1.8	5	10	2	5	10	2
<i>Staphylococcus aureus</i> N (42)	10	40	4	1.25	1.25	1	/	/	/
<i>Enterobacter faecalis</i> N (22)	1.25	5	4	2.5	5	2	20	20	1
<i>Pseudomonas aeruginosa</i> N (61)	10	40	4	10	20	2	40	40	1
<i>Escherichia coli</i> N (54)	2.5	5	2	5	10	2	10	20	2
<i>Staphylococcus aureus</i> N (73)	10	40	4	1.25	2.5	2	10	40	4

Table 1a: The MIC and the MBC of essential oils of different strains.

($\mu\text{L.mL}^{-1}$)	Rosmarinus officinalis			Salvia officinalis			Ruta chalepensis		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
<i>Salmonella parathyphi</i> N (35)	2.5	5	2	5	10	2	40	40	1
<i>Salmonella thyphi</i> N (18)	5	5	1	10	10	1	2.5	5	2
<i>Proteus vulgaris</i> N (20)	5	10	2	20	20	1	1.25	1.25	1
<i>Escherichia coli</i> N (31)	1.25	5	4	5	10	2	5	5	1
<i>Enterobacter aerogenes</i> N (49)	20	20	1	1.25	2.5	2	5	10	2
<i>Staphylococcus aureus</i> N (42)	2.5	5	2	5	5	1	10	10	1
<i>Enterobacter faecalis</i> N (22)	10	10	1	2.5	5	2	1.25	2.5	2
<i>Pseudomonas aeruginosa</i> N (61)	5	10	2	5	10	2	5	5	1
<i>Escherichia coli</i> N (54)	2.5	5	2	5	5	1	5	10	2
<i>Staphylococcus aureus</i> N (73)	1.25	1.25	1	2.5	2.5	1	2.5	5	2

Table 1b: The MIC and the MBC of essential oils of different strains ($\mu\text{L.mL}^{-1}$).

Thymus capitatus

This This oil is very active by their strong inhibition on all bacteria Gram- tested and are belong to the family Enterobacteriaceae with a minimum inhibitory concentration between 1.25 and 5 ($\mu\text{L.mL}^{-1}$) with a bactericidal effect/bacteriostatic, by against the pseudomonacea and Staphylococcus have an MIC of 10 ($\mu\text{L.mL}^{-1}$) with a bactericidal effect/bacteriostatic.

Thymus vulgaris

This oil is active by their strong inhibition on all bacteria tested except *Salmonella typhi* 6539 with a minimum inhibitory concentration (MIC) of 2.5 to 10 ($\mu\text{L.mL}^{-1}$) for the Enterobacteriaceae and 1.25 ($\mu\text{L.mL}^{-1}$) for Staphylococci and 10 ($\mu\text{L.mL}^{-1}$) for the pseudomonacea. A bactericidal effect is given against these strains studied.

Artemisia herba alba

This oil carries active on all the three families studied with the exception Staphylococci, Enterobacteriaceae shows a sensitivity of (MIC) between 5 to 40 ($\mu\text{L.mL}^{-1}$), Whereas the Pseudomonaceae reached a MIC of 40 ($\mu\text{L.mL}^{-1}$) with a bactericidal effect/ bacteriostatic.

Rosmarinus officinalis

This oil is very active by their strong inhibition on all bacteria tested. MIC for the Enterobacteriaceae between 1.25 to 10 ($\mu\text{L.mL}^{-1}$) except the Enterobacters an MIC of 20 ($\mu\text{L.mL}^{-1}$), a minimum inhibitory concentration (MIC) between 1.25 to 2.5 ($\mu\text{L.mL}^{-1}$) for the Staphylococcus and Pseudomonas MIC between 2.5 to 5 ($\mu\text{L.mL}^{-1}$), with a bactericidal effect/bacteriostatic.

Salvia officinalis

This oil is very active by their strong inhibition on all strains tested with a minimum inhibitory concentration (MIC) between 1.25 and 20 ($\mu\text{L.mL}^{-1}$) for the Enterobacteriaceae, 2.5 to 5 ($\mu\text{L.mL}^{-1}$) for Staphylococci 5 ($\mu\text{L.mL}^{-1}$) for the Pseudomonas, with a bactericidal effect on all of the strains.

Ruta chalepensis

This oil is very active by their strong inhibition on the Enterobacters, Proteus, Staphylococci and Salmonella with a minimum inhibitory concentration (MIC) between 1.25 and 5 ($\mu\text{L.mL}^{-1}$), the Pseudomonas show an MIC of 5 ($\mu\text{L.mL}^{-1}$), with a bactericidal effect on all of the strains.

Discussion

Flumequine resistance and sensitivity to gentamicin are the same findings [20], and disagree with the result of the lesser sensitivity of ceftiofur. Of the most commonly tested antibiotics, *E. coli* isolated from poultry have the least resistance to gentamicin [20]. The proportions of *E. Coli* are more than 90% stable and stable between 2003 and 2009 [21]. The high name *Escherichia coli* isolated from carcasses was probably related to the natural presence of this bacterial species in the digestive tract [22]. Ampicillin has become the less active antibiotic on *E. coli* [23].

E. aerogenes possesses a natural, inducible chromosomal cephalosporinase of low level of expression. *E. aerogenes* is naturally resistant to first-generation aminopenicillins and cephalosporins [24]. *E. aerogenes* can host, like all Enterobacteriaceae now, a plasmid encoding ESBL [24-26]. The natural resistance to cefalotine (i.e. to ampicillin and AMC) is constant. *P vulgaris*, by its sensitivity to CMA, are the exception [27] compared to other Enterobacteriaceae. Salmonella pose serious problems in both human and veterinary medicine and are all the more formidable as many of them are currently resistant to many antibiotics. Certain serotypes form the outset multiresistant [28]. Multi-resistant strains of *S. typhi* have also been reported in other countries [29]. According to the same author, Salmonella tested to the antibiogram, show a resistant and gentamycin.

Staphylococcus aureus has adaptive power and anti-staphylococcal resistance. More than 50% of strains of MRSA are resistant to gentamicin [12,30].

According to Abdallah et al. the highest rates were observed for gentamicin whereas the most constantly active antibiotics are

resistance, this is the same result in our study. The presence of antibiotic-resistant *Enterobacteria*, *Staphylococci* and *salmonella* in the samples we analyzed; The proportion of resistant bacteria, pathogenic or saprophytic, is highest in animals intensively grown [31].

For the effect of the oil of *Thymus capitatus* our results are similar to those of Amarti et al. [32] who obtained complete inhibition of *Escherichia coli* and *Staphylococcus aureus* for an essential oil concentration of *Thymus capitatus* 1/2000 v/v. According to Amrani et al. this important bioactivity of *Thymus capitatus* oil is related to their high content of carvacrol and thymol.

The oil of *Thymus vulgaris* showed an antibacterial activity important against *Escherichia coli* and *Staphylococcus aureus*. Our results are aligned to Dorman et al. and El Ouali Lalami et al. [33,34].

The essential oils of *Artemisia herba Alba* are less interesting for the anti-bacterial plan and this for different concentrations, *Staphylococci*, *Proteus*, *Salmonella* and *Enterobacter* show a resistance of diameter between 0 and less than 15 mm. According to Ghanmi et al., in the study on the effect of harvest date on performance, the chemical composition and bioactivity of *Artemisia herba-alba* essential oils in the Guerçif region show that samples Collected in September are more active than those of the other months (April, June). We can also explain the difference observed between these three collection dates by the biosynthesis process of these main components [5].

The *Salvia officinalis* oil is very active at 5 μL , 10 μL and 15 μL against *S. aureus* and *P. aerogenosa*, these results do not converge with the work of Billerbeck was a result of 100 μL for *P. aerogenosa*.

Rosmarinus officinalis and *Salvia officinalis* showed efficacy against *S. aureus* and also approved by [35].

It appears that the essential oil of *Ruta chalepensis* has an effect on the strains studied. It has been reported that 2-undecanone was the major constituent of the essential oil of *Ruta chalepensis* and has a strong antibacterial activity [36,37].

We can confirm the importance and power of antibacterial *Rosmarinus officinalis* and *Salvia officinalis* in first position followed by *Thymus capitatus* and *Thymus vulgaris*, it remains *Artemisia herba Alba* of low activity in the face of the bacteria that have a multi resistance to antibiotics.

Conclusion

In conclusion this study which was intended to show the importance of essential oils against pathogenic strains, these strains have a resistance vis-a-vis the antibiotics used in animal husbandry avian, this resistance is the cause of a therapeutic failure is a major problem and disturbing to veterinarians and livestock producers. The sensitivity achieved has confirmed the presence of an undesirable phenomenon of antibioresistance among a variety of strains isolated. The oils of *Thymus capitatus*, *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Artemisia herba alba* and *Ruta chalepensis* share a series of minimum inhibitory concentration (MIC) between 1.25 to 40 ($\mu\text{L.mL}^{-1}$) with a bactericidal effect/bacteriostatic. It is quite in agreement to test the activity of these oils *in vivo*, and thinking has oriented the breeders toward a breeding of less of antibiotics and based on organic products such as medicinal plants in the treatment of different avian pathologies.

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