

Veterinary phytotherapy in Algeria: *Pistacia lentiscus* as an antimicrobial model

Fitoterapia veterinária na Argélia: emprego da *Pistacia lentiscus* como um modelo antimicrobiano

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ABSTRACT

Currently, the use of alternative medicine is necessary, even in animals. Our study focused on the valorisation of the use of herbal products in Algerian veterinary medicine. This study is based on a 6-month exploratory survey of 257 private practicing veterinarians, followed by a study of the bacteriological activity of the essential oil of the *Pistacia lentiscus* plant. The survey results generally indicate that private veterinarians support phytotherapy as an alternative to conventional drugs in several areas and species. Also, the results of research conducted on the antibacterial activity of *Pistacia lentiscus* essential oil show that it inactivates 50% of the bacterial strains. This confirms the well-defined role of this medicinal plant used as an alternative in veterinary medicine.

Keywords: Antimicrobial activity. Essential oil. Veterinary phytotherapy. *Pistacia lentiscus*.

RESUMO

Na atualidade o emprego da medicina alternativa é uma realidade, inclusive para os animais; este trabalho visa a valorização do emprego de produtos fitoterápicos na medicina veterinária da Argélia. O presente estudo baseou-se em um levantamento realizado durante seis meses com 257 médicos veterinários do setor privado, seguido de uma investigação da atividade bacteriológica do óleo essencial da planta *Pistacia lentiscus*. Os resultados do levantamento indicaram que esses profissionais aceitam o emprego da fitoterapia como uma alternativa para as drogas de uso convencional em diferentes áreas e espécies de animais. A avaliação da atividade antibacteriana do óleo essencial da *Pistacia lentiscus* revelou que 50% das estirpes bacterianas empregadas foram inativadas após seu uso, o que confirmou a possibilidade do emprego desta planta medicinal como uma alternativa para a medicina veterinária.

Palavras-chave: Atividade antimicrobiana. Óleo essencial. Fitoterapia veterinária. *Pistacia lentiscus*.

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Received: November 17, 2020

Approved: April 12, 2021

How to cite: Djebrane C, Belahcene N, Boulebda N. Veterinary phytotherapy in Algeria: *Pistacia lentiscus* as an antimicrobial model. Braz J Vet Res Anim Sci. 2021;58: e178032. <https://doi.org/10.11606/issn.1678-4456.bjvras.2021.178032>

Introduction

Traditional medicines have always held a central place in the world health system and continue to be used as first-line treatments for a wide spectrum of diseases (Lehmann, 2013).

Despite the use of herbal medicines for many centuries, only a relatively small number of plant species have been studied for possible medical applications (Strzemski et al., 2019). Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients, and the preparations containing them (Fibigr et al., 2018).

Today, much of the world's population, particularly in developing countries, is still treated solely with traditional herbal remedies. This source seems inexhaustible since only

about 400,000 known plant species have been chemically and pharmacologically investigated, and each species may contain up to several hundred different constituents (Balick & Cox, 2020).

Conventional medicines and the use of plants in empirical medicine have often been the source of high-level scientific research. In most cases, this research leads to the discovery of original substances of considerable therapeutic interest (Zhiri & Baudoux, 2005).

The Algerian flora is characterized by its floral diversity: Mediterranean, Saharan, and a paleo-tropical flora estimated at more than 3,000 species belonging to several botanical families. Most of these species are spontaneous, with a significant number (15%) of endemic species (Ozenda, 1997), providing an inestimable richness to the traditional pharmacopeia.

Within the perspective of the valorization of plant matter for therapeutic uses, the study of the species *Pistacia lentiscus* (of the *Anacardiaceae* family) is undertaken (Idm'hand et al., 2020).

The *Pistacia lentiscus* plant is known for its use in traditional medicine because of its various anti-inflammatory, analgesic, antipyretic, cardioactive, antifungal, antituberculosis, antitumor, and antioxidant activities (Pachi et al., 2021).

The present work consists of enhancing the therapeutic use of certain Algerian medicinal plants in the veterinary field. This work is carried out in two phases. Phase one is the exploratory phase. It consists of an investigation based on a questionnaire intended for the veterinary surgeons practicing in the territory of Eastern Algeria.

The second phase involves an assessment of the biological antibacterial effect of certain extracts (essential oils) of a plant from the traditional Algerian pharmacopeia, namely *Pistacia lentiscus*.

Materials and Methods

I- Exploratory phase (Survey)

The use of medicinal plants in the veterinary field in the Wilaya of Souk-Ahras, as in the entire national territory, is not precise and ambiguous. What is the degree of knowledge, vision, and conviction of animal health professionals regarding this practice (called natural) and capital importance in the field of environmental protection, biological species, and, especially, humans?

This is the question that we seek to answer through a survey, based mainly on a questionnaire, that was formulated and distributed to animal health professionals, veterinary doctors, breeders, and owners of different communes in

Eastern Algeria. Included in the questionnaire are several questions on the use of medicinal plants (and/or derivatives) in food-producing animals.

Our investigative work is mainly based on questions about the introduction of plants from traditional Algerian medicine into therapeutic practice in the field of animal health. We try to understand the vision and/or conviction of different actors and professionals in the field of animal health (private and public sector veterinary clinicians), and the reality of this practice of phytotherapy, as seen and lived by the different actors appropriate to this profession.

The elements of the questionnaire (medicinal plants in the veterinary field, real situations as seen, lived, and interpreted by the animal health professional) deal with the following points:

- 1- Prescription and consumption of conventional medicines in livestock of different species.
- 2- Plant use in veterinary medicine in Eastern Algeria.
- 3- Target production (and/or destination) animals.
- 4- Formulations and administration methods of different preparations.
- 5- The different types of indications (pathologies treated).

Results

The data (expressed as a percentage) from the questionnaire answers were classified and/or organized according to their rate and/or frequency of citation of each query on the totality of the collected questionnaires, according to the formula: Number of answers expressed/Number of questionnaires obtained.

II- Experimental phase

- 1- **Plant material:** The harvest of the plant used (*Pistacia Lentiscus*) was carried out during summer 2018 in Mechroha, Souk Ahras (Northeast Algeria). Botany experts classified the plants. The leaves were dried in the open air and the shade, in a ventilated room at room temperature.
- 2- **Bacterial material:** The bacterial material used was composed of four reference strains (Table 1). Its sensitivity to antibiotics was determined by the antibiogram test according to the recommendations of the antibiogram committee of the French Society of Microbiology (Acar et al., 1984).

Table 1 - Origin, culture medium, and growth temperature of the different strains used in the experiments of the antibacterial activity of *Pistacia lentiscus* extracts: Northeast Algeria (December 2018 to May 2019)

Strains	Origin	Growing medium	Optimal growth temperature
<i>Staphylococcus aureus</i>	Microbiology laboratory of the	Chapman	35-41°C
<i>Pseudomonas auruginosa</i>	Iben Roched hospital (Souk	Agar King A	37°C
<i>Escherichia coli</i>	Ahras),	Nutritional agar	40°C
<i>Klebsiella pneumoniae</i>		Nutritional agar	37°C

3- Extraction of essential oils: The extraction of essential oils was carried out by hydro-distillation in a Clevenger-type apparatus (Clevenger, 1928). Three distillations were carried out by boiling 100 g of fresh plant material in a flask with one liter of water for 4 h. The essential oil obtained was kept at +4°C until its use.

4- Evaluation of the antibacterial activity of *Pistacia lentiscus* extracts:

The microbiological method of diffusion on agar:

Pre-tests were carried out to select strains sensitive to the essential oil of the plant under study.

The protocol followed is the one described by Mazari et al. (2010), with some modifications (PCA agar used instead of Mueller-Hinton, and spreading, instead of swabbing, the suspension):

- Preparing the bacterial suspension.
- 0.1 ml of each suspension is spread on the PCA agar with a rake and allowed to dry.
- Using sterile tweezers, soak a disc of Whatman paper (6 mm diameter) in 10 ul of the essential oil. Then, place it in the middle of the seeded agar. After diffusion of the extract for 1 h, steam the tins at 37°C for 24 h.

Tests: Preparation and cellular concentration of the inoculums are done according to traditional methods.

The bacterial concentrations of the inoculum are evaluated by turbidity. They are expressed by measuring the optical density (OD at 600 nm) on a spectrophotometer. An OD of 0.08-0.1 corresponds to 10⁸ CFU/mL (Haddouchi et al., 2009).

The protocol followed is that described by Mazari et al. (2010):

- Inoculate the Mueller-Hinton agar with the suspension using the swabbing method. Allow drying.
- Sterile Whatman paper discs (6 mm diameter) impregnated in essential oil at a rate of 20 µl per disc, are sterilely deposited on the surface of the agar. inoculated. The solvent DMSO was used as a negative control. Standard antibiotics: tetracycline "TE" (30 1/4g / disc), trimethoprim/

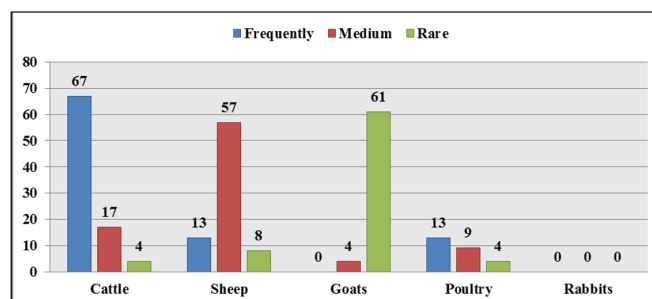


Figure 1 - Prescription rates for conventional drugs in different species of production animals in Northeast Algeria (December 2018 to May 2019)

sulfamethoxazole (SXT) (1.25/23.75) ¼ g/disc) and cefalexin.

“CN” (30 1/4 g/disc) were used as positive controls. Each test is performed in triplicate.

- After diffusion of the extract for 1 h, steam the boxes at 37°C for 18 to 24 h.
- Antimicrobial activity is determined using a ruler to measure the diameter of the inhibition zone.

Data Analysis: Descriptive statistics are used to describe the basic features of the data in a study.

Results and Discussion

I- Exploratory phase (Survey)

The main survey lasted 6 months (December 2018 to May 2019). We solicited 257 veterinarians for the survey.

1-Prescription rates for conventional drugs in different species of production animals:

The present results show a relatively high level of prescriptions and/or consumption of conventional (and/or chemically synthesized) drugs, frequently present in cattle, with a favorable rate of around 67% of the responses expressed (Figure 1). Cattle are by far the most frequent consumers of drugs, ahead of other species of animal production, namely sheep, goats, poultry, and rabbits. The development of the cattle production chain has improved significantly in the region in recent years (Lassaletta et al.,

2014; Berrahal & Eulmi, 2017), and requires adequate and consistent medical care.

According to the response from professionals, sheep are considered as average consumers of medicines with a prescription rate of 57% (of responses expressed). This consumption is better than that recorded for goats, poultry, and especially rabbits, whose high consumption rate is statistically negligible according to the data provided by the solicited veterinarians. This may explain the nature and/or type of livestock farming that is less present and/or less developed in the regions explored in the wilaya of Souk Ahras (Berrahal & Eulmi, 2017).

The results indicate a relatively rare prescription of conventional drugs in goats. Some 61% of the veterinarians pronounced rarely prescribing and/or using plants and/or derivatives for this caprine species. Several justifying factors can be put forward, such as the relatively small number of animals, the less developed type of livestock farming in the region, often reserved for traditional livestock farming located in difficult-to-access mountainous areas, and socio-cultural factors including the use of traditional remedies, etc.

Despite a developed production, thanks to the support of the public authorities and the increasing consumer demand for white meat, according to the data provided by the veterinary clinicians interviewed, the prescription of (so-called conventional) drugs are statistically less present and/or less important in poultry compared to local cattle and sheep producing animals. This relatively unexpected response (paradoxically qualified) may be justified by the relatively small number of practicing clinicians involved in this production chain and therefore the number of pronounced responses (relatively low) in this field; the relatively short life span (50 days on average in broiler chickens); the regular and massive prescription (often aimed at prophylaxis) of certain drugs more present in the early stages of growth (Berghiche et al., 2017, 2018), and the respect of zootechnical recommendations such as hygienic conditions, feeding, etc. (Berghiche et al., 2017, 2018).

Figure 2 presents the data provided by the different prescribers trying to explain and/or justify the variability in the rate of drug consumption in different animal production systems. This included frequent and significantly high prescribing in cattle, medium prescribing in sheep, low and rare prescription in poultry and goats, and none in rabbits.

The dominant type of livestock farming (concerning numbers and animal production), namely cattle farming, has become characteristic of the study region. It is an essential factor that determines not only the rate of consumption, prescription, and sale of conventional veterinary medicines

but also the nature of the function and the sector of intervention of veterinary practitioners in the different regions (Berrahal & Eulmi, 2017).

Another factor that may justify the frequency and/or prescription rate of conventional medication is the susceptibility and frequency of disease in target animals. A recent study carried out among veterinary practitioners in the wilaya of Souk Ahras (Berrahal & Eulmi, 2017) shows a relatively high prevalence of pathological dominants in cattle compared to other animal species in the region. Other factors that may favor the use of conventional (and/or chemically synthesized) medicines are the therapeutic efficacy of the latter and the absence and/or unavailability of a suitable alternative (natural or non-synthetic).

2-Herbal remedies and the criteria for choosing herbal medicine in veterinary medicine

The answers to the questions on the current and actual situation of medicinal plant use illustrated in (Figure 3) indicate a relatively important initiative to use phytomedicines and the introduction of medicinal plants and/or their derivatives in veterinary medicine. Approximately 53% of practitioners report having prescribed herbs in their daily practice.

According to the statements of the stakeholders (78% of responses from doctors and non-clinicians) (Figure 4),

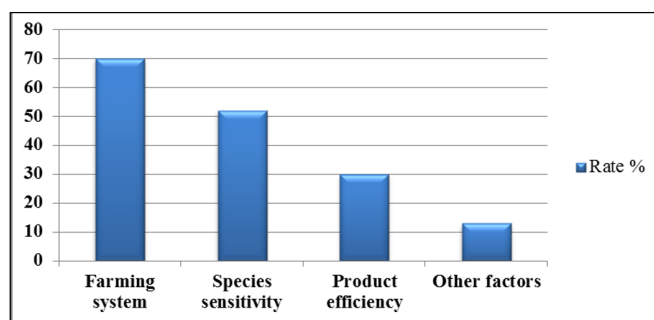


Figure 2 - Factors influencing the rate of consumption of veterinary drugs in Northeast Algeria (December 2018 to May 2019)

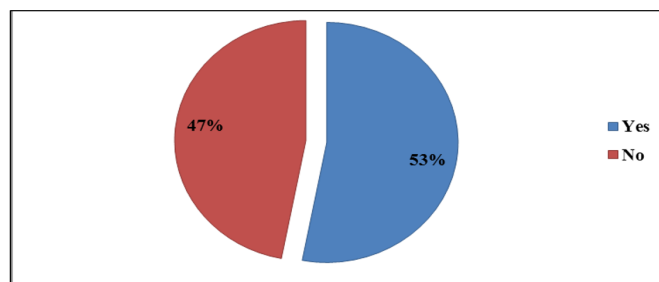


Figure 3 - Recourse to the utilization of medicinal plants in veterinary medicine in Northeast Algeria (December 2018 to May 2019).

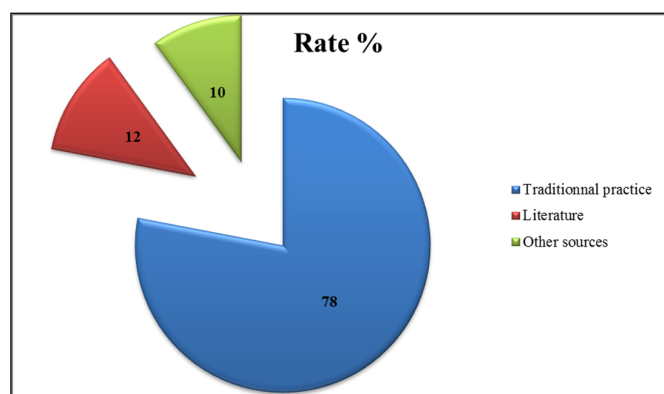


Figure 4 - Information explaining the justification for the use of herbal medicines in veterinary practice in Northeast Algeria (December 2018 to May 2019).

the use of these plants can be justified by their therapeutic efficacy in traditional human medicine. Other practitioners spoke of the influence of professional literature (specializing in ethnobotany and traditional medicine) on the initiation process and the development of this phytotherapeutic practice in the animal world.

The use of medicines in animals is historically linked to their use in humans since the late 1930s. Animal health, as the first term understood in animal husbandry, seeks to ensure better qualitative and quantitative productivity, while maintaining animals in the best possible state of health. Since animal health is also an important factor in the competitiveness of livestock farming, it is, therefore, a challenge (Krieger et al., 2020).

Alternative veterinary practices include phytotherapy, aromatherapy, homeopathy, observation, and any other form of alternative curative or preventive medicine (Hellec et al., 2016).

In recent years, there has been renewed interest in these medicines in livestock farming, as consumers consider them to be less aggressive and more respectful of nature than the usual medicines.

3-Part of the plant used and forms of use

According to the results of the ethnobotanical survey on the use of plants in veterinary medicine, it appears that most of the veterinarians surveyed recommend the use of leaves (69% favorable responses) and roots (49%). According to the same data, only 25% of veterinarians use fruits and stems. Only 18% of them also use the aerial part and seeds of plants (Figure 5).

The (relatively) high frequency of use of leaves can be explained by the fact that they are the site of photosynthesis and sometimes storage of secondary metabolites responsible for the biological properties of the plant (Bigendako-Polygenis & Lejoly, 1990). Also, using leaves make harvesting easier and faster (Bitsindou & Bouquet, 1996).

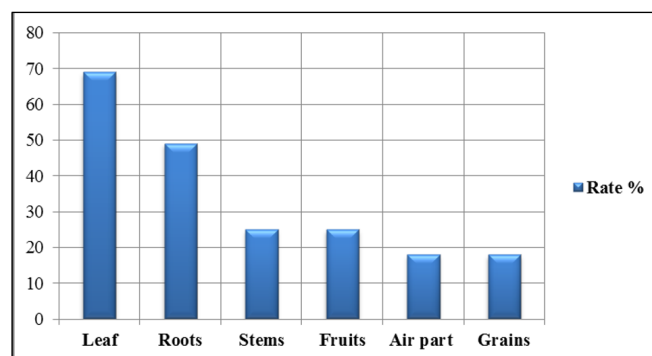


Figure 5 - The most-used parts of plants in veterinary practice in Northeast Algeria (December 2018 to May 2019).

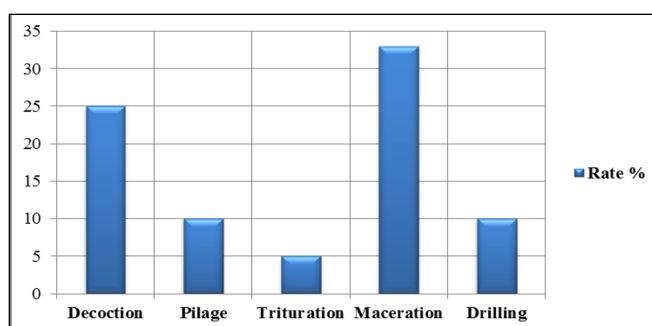


Figure 6 - Form and preparation of plant administration in Northeast Algeria (December 2018 to May 2019).

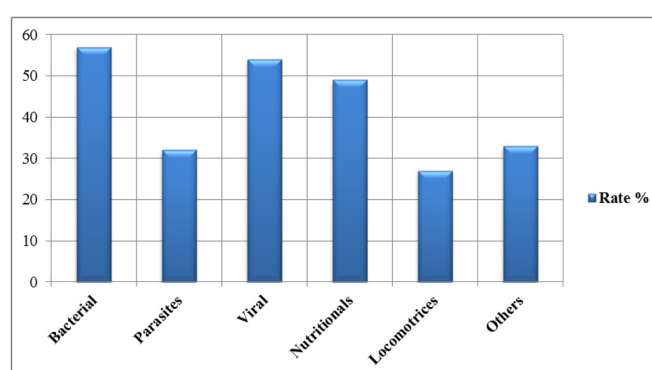


Figure 7 - Frequency of diseases treated by plants in veterinary medicine in Northeast Algeria (December 2018 to May 2019).

About the form of use (Figure 6), the most frequent is herbal tea (prepared by maceration 33% and/or decoction 25%). According to the veterinarians surveyed, this facilitates the administration of the plant and provides rapid and effective results.

The plants are also used in the form of drilling (10%), piling (10%), and rarely used in the form of drilling with only 5% favorable responses.

4-Indications (or diseases treated) of plants in veterinary medicine

The results shown in Figure 7 indicate the nature of the diseases treated with medicinal plants in animals in Eastern Algeria (all regions combined).

The survey conducted among herbalists enabled us to identify a number of the diseases treated by plants. These are mainly bacterial (57%), viral (54%), and parasitic (32%) infectious diseases. Also, 49% of the respondents use plants for the treatment of nutritional diseases, and 27% for locomotor diseases.

The present results show an irregular use, with phytotherapy widely practiced with certain diseases (e.g., digestive diseases) on the one hand, and rarely and/or only slightly present in the treatment of other diseases. This often-variable practice of phytotherapy can be explained by certain factors such as the frequency of the dominant pathologies, the type of breeding characteristic of the region (Berrahal & Eulmi, 2017), the type and/or etiology of the disease, and that safe and better-known medicines are often preferred in the treatment of serious and/or severe diseases including infectious and respiratory diseases, etc.

Other factors include the lack of guaranteed efficacy and availability of herbal products, not understanding the rules, and/or little to no legislative coverage of the use of certain plants in veterinary medicine (Brown, 2017).

II- Experimental phase

1-Yield of the Essential Oil:

The content of essential oil obtained from the leaves of the explored plant (*Pistacia Lentiscus*) is about 0.07%. These quantities are extremely low compared to those described in the literature (Bonsignore et al., 1998; Magiatis et al., 1999; Delazar et al., 2004).

The reasons for this variability can be explained by differences in environmental conditions (climate and

geographical location), the harvest period, and the distillation technique (Lahlou, 2004). It should also be noted that the production of essential and aromatic oils from the plant results from a series of physiological, biochemical, metabolic, and genetic regulations (Prins et al., 2010).

2-Antimicrobial activity

The antimicrobial activity of essential oils is evaluated on reference microbial strains. This activity is evaluated by the aromagram method. The antimicrobial power of this oil is obtained by measuring the diameter of the inhibition zone in mm.

The scale for the estimation of antimicrobial activity is given by Mutai et al. (2009), who classified the diameters of inhibition zones (D) of bacterial growth into 5 classes:

- Very strongly inhibitory: $D > 30$ mm
- Highly inhibiting: D between 21 mm and 29 mm
- Moderately inhibitory: D between 16 mm and 20 mm
- Slightly inhibitory: D between 11 mm and 16 mm
- Non-inhibitory: $D < 10$

3- Preliminary test results

The results of the preliminary tests are shown in Table 2.

4- Test results

The results of the antibacterial activity of *Pistacia Lentiscus* EO (Table 3) using the disc method on agar medium showed that EO exhibits activity against three bacterial strains on four germs tested despite their morphology and Gram. Indeed, EO exerts a strong activity on Gram-positive bacteria. As shown in the previous table, the *Staphylococcus aureus*

Table 2 - Pre-test results of antibacterial activity of *Pistacia Lentiscus* Essential Oils collected in summer 2018 in Northeast Algeria

Bacterial strains	Resultats (Activity)	D (mm)	E1 (mm)	E2 (mm)	E3 (mm)
<i>Escherichia coli</i>	+	14.0 mm	9mm	9mm	11mm
<i>Klebsiella pneumonia</i>	-	/	/	/	/
<i>Staphylococcus aureus</i>	++	31.78 mm	15mm	10mm	10mm
<i>Pseudomonas auruginosa</i>	++	12mm	10mm	10mm	9mm

+: EO active, ++: EO very active, -: EO inactive, /: No zone. E 1: Inhibition zone diameter of test 1; E 2: Inhibition zone diameter of test 2; E 3: Inhibition zone diameter of test 3; E 1, E 2, E 3: EO diluted 1/10 in Dimethylsulphoxide.

Table 3 - Result of the antibacterial activity of *Pistacia Lentiscus* Essential Oils collected in summer 2018 in Northeast Algeria

Strains	Inhibit zones (D1) in mm	Positive control			Zones of inhibition rate function of dilution (D2) in mm
		TE	CN	SXT	
<i>Escherichia coli</i>	10 mm	26 mm	R	27mm	00 mm
<i>Klebsiella pneumonie</i>	-	NT	NT	NT	NT
<i>Staphylococcus aureus</i>	20,5 mm	24 mm	26 mm	31 mm	19 mm
<i>Pseudomonas auruginosa</i>	9 mm	25 mm	20 mm	25 mm	00 mm

“TE”: Tetracycline, “SXT”: Trimethoprim/sulphamethoxazol and “CN”: Cefalexin.

strain is the most sensitive with a maximum inhibition zone of 19.5 mm, followed by *E. coli* with a minimum zone of 10 mm. We also recorded an absence of activity on *Klebsiella pneumoniae*.

However, areas of inhibition were weaker than those of antibiotics, which showed very strong inhibition of bacterial growth.

Several parameters may be responsible for the presence or absence of antimicrobial activity.

The size of the inoculum: An inoculum that is too dense can lead to false-negative results. An inoculum that is too small can lead to false-positive results. For our study, we chose an inoculum with a density of 0.08 - 0.10, to have a regular carpet. According to the results of antibacterial tests, two out of three bacterial strains show some sensitivity (Figure 8).

These results show more activity towards Gram-positive bacteria than Gram-negative ones. According to Nikaido (2003),

these results could be due to the membrane composition of Gram-negative bacteria. The surface of lipopolysaccharides contains negative charges, which prevent the diffusion of hydrophobic molecules, and pores that block the passage of high molecular weight molecules (Garrett & Grisham, 1995; Vergalli et al., 2020). Some bacteria contain pores of low permeability in their membranes. Unlike Gram-negative bacteria, Gram-positive bacteria are more sensitive.

Our observations are consistent with the results obtained on the antimicrobial activity of essential oil on Gram-positive to Gram-negative bacteria (Bouzouita et al., 2008) and with (Mazari et al., 2010) that used the same essential oil extracts.

The inhibition zones were also found to be smaller than those of antibiotics, which showed large inhibition zones compared to those obtained by testing the essential oil used.

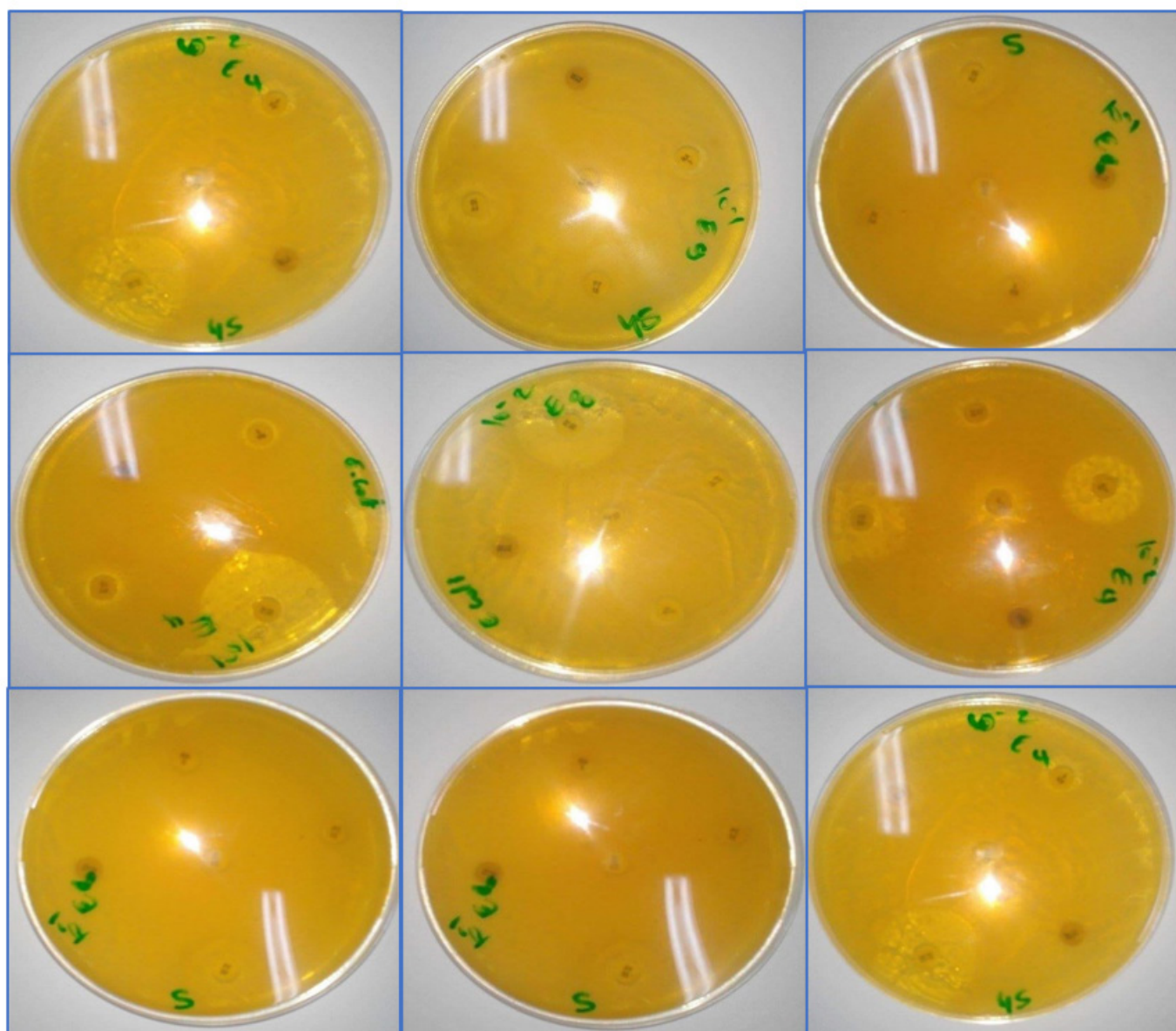


Figure 8 - Aromatogram test of *Pistacia Lentiscus* Essential Oils on the bacteria strains studied collected in summer 2018 in Northeast Algeria.

Various parameters help to explain this difference in effectiveness between antibiotics and essential oils: concentration (may be exaggerated in the discs), the degree of purity, the toxicity.

Besides, various components may also contribute to the antimicrobial activity of essential oils, involving synergy with other active compounds (Mazari et al., 2010).

Conclusion and Recommendations

This study sought to contribute to the knowledge of the role of phytotherapy in veterinary medicine and to evaluate the bioactive potential of essential oils from the leaves of *Pistacia lentiscus*.

According to the exploratory study, the use of phytotherapy in veterinary medicine is a topical issue and the involvement of this alternative method of pharmaceutical medicine for curative and preventive purposes is mandatory.

The extraction of essential oils by hydrodistillation using a Clevenger-type extraction device is carried out. We then tested the antimicrobial activity of the oils using the disk diffusion technique.

The essential oil yield value of *Pistacia lentiscus* leaves was 0.07%. This value is lower than the yields obtained in other species of the same genus.

Conflict of Interest

The authors declare no conflict of interest in the current manuscript.

Ethics Statement

The experiment was carried out according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee.

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Financial Support: None.