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In vitro evaluation of antibacterial action of *Caralluma europaea* extracts on *Rhodococcus equi*

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ABSTRACT

The purpose of the present study is to evaluate the antibacterial action of various extracts of Caralluma europaea, a Moroccan indigenous plant, on Rhodococcus equi, a known pathogenic bacterium affecting humans and animals. This gram-positive bacillus is largely known as a pulmonary pathogen in animals, mainly in foals, but affects also immunosuppressed persons, particularly AIDS patients. In this study, we tested three Caralluma europaea extracts (ethyl acetate extract, methanol extract and aqueous extract) of R. equi in a liquid medium and a solid medium as per the propagation method. Six antibiotics were then tested to compare their antibacterial action to that of the plant extracts. Results obtained with the solid medium showed that only the ethyl acetate extract resulted in bacterium growth inhibition. In fact, the average diameter of inhibition zones induced by ethyl acetate was 20mm. The six tested antibiotics showed a positive but different action with the exception of Kanamycin (KMN), which had no effect on this bacterium. The most effective antibiotic against this bacterium was Chloramphenicol (CHL) with an inhibition zone of 28.33mm. For Doxycycline (DOX), this zone was 26.33mm, close to that of ethyl acetate extract which was 20mm. For Amoxicillin (AMX), the inhibition zone was 10.33mm, for Cephalothin (KF) it was 9.66mm, and for Ampicillin (AMP) it was 8.33mm. Additionally, we measured the release of absorptive cellular content at 260 nm in the presence of ethyl acetate extract and of Chloramphenicol. We noted sudden mortality in the early hours of treatment. After 6 hours, we noted the release of 63% of cell contents in the case of ethyl acetate extract and 88% in the presence of Chloramphenicol. We also measured absorbance (DO) at 600 nm to evaluate growth inhibition in the liquid medium of Rhodococcus equi by ethyl acetate extract and by Chloramphenicol. The inhibition was complete after 3 hours for Chloramphenicol while for the ethyl acetate extract, it was observed after 5 hours of treatment.

Keywords: *Caralluma europaea,* plant extracts, antibacterial activity, *Rhodococcus equi*.

INTRODUCTION

Microbial resistance has been evolving over decades and has now come to represent a global threat to public health. The number of antimicrobial agents that can be used against certain pathogens is continuously declining. As a result, drug-resistant microorganisms are becoming increasingly difficult to treat [32]

When resistant microbes are exposed to antimicrobial agents, they are the only ones to survive and reproduce. Their progeny inherits this microbial resistance [21]. Microbial resistance can be either intrinsic or acquired. Microbes can

gain new resistance when a spontaneous mutation occurs in their genes or when a transfer of new genes occurs from another species. Resistance is therefore an adaption mechanism through which microorganisms begin to tolerate a drug concentration that would normally be inhibitory [44] Furthermore, the development of drug resistance may be caused by certain extrinsic factors such as social and technological changes [14]. The excessive use of antibiotics is also one of the main causes behind microbial resistance [40] ; [2] Several studies have shown that bacterial resistance to certain antibiotics is largely attributed to the impermeability of their cell walls [41] In the case of several bacterial species, antibiotic resistance is closely related to the occurrence of mutation [21]. For all the above reasons, efforts have been directed for some years now to the use of natural agents that have no side effects and which can be considered as suitable therapeutic alternatives. Many scientists searching for new antimicrobial agents have turned their attention to plants [21]. Natural resources present a great potential and an opportunity to discover new antibiotics since plants have long been an important source of natural remedies [13]. Natural chemical compounds hold protective and preventive properties against disease. Indeed, besides the known antibiotics, different plants are characterized by synthesis of the molecules that make up the so-called secondary metabolites long known for their antiseptic activity and therapeutic effects in traditional medicine [43]. The plants' secondary metabolism molecules belong to highly diverse chemical classes such as alkaloids, phenols, flavonoids, tannins and others. They are a key source of molecules usable by Man in different areas such as pharmacology and food industry [23].

In many developing countries, large swathes of the population place their trust in traditional healers and their collections of medicinal plants to cure them. According to 2003 statistics from the World Health Organization (WHO) [38] 80% of the world population resorts to traditional medicine to meet their primary health care needs. The recourse to plants can be explained by their accessibility and the availability of traditional medicine in developing countries on the one hand, and the excessive costs and harmful side effects inherent to synthetic drugs [6].

Thanks to its geographical location, Morocco enjoys a climate and environment that are conducive to the growth of a rich and diverse flora. Out of the existing 7,000 species and subspecies, about 537 are endemic to this country [4]; [17]. Given this wealth, Morocco has cultivated a centuries-old know-how of natural medication. A treasure-trove of traditional therapeutic recipes and practices exist in our country. The practice of traditional medicine is linked to several factors: age, gender, educational level, marital status, type of disease, family of plants used, and knowledge of plant toxicity [5]; [31]. In Morocco, medicinal herbs hold a prominent place in traditional medicine, itself widely used against a multitude of ailments. Remedies using medicinal herbs are seen as less expensive and free of side effects and tend to be used for chronic diseases such as diabetes and rheumatism and for cancer and digestive disorders.

One of the medicinal plants used in Morocco is the *Caralluma europaea*. This species is used in traditional medicine as a remedy against diabetes and cancer [11]. It belongs to the *Caralluma* genus, which contains about 133 species and belongs to the *Apocynaceae* class [47].

In Morocco, the plant can be found inland, in the Anti-Atlas, the Middle Atlas and the Rif. For the present study purposes, we tested three *Caralluma europaea* extracts of Moroccan origin against *Rhodococcus equi: in* an ethyl acetate extract, a methanol extract and an aqueous extract. *R. equi* is a gram-positive *coccobacillus*, primarily known as a pulmonary pathogen in animals, foals and in immunosuppressed humans, especially AIDS patients [15]. Many symptoms of this condition have been described: an acute respiratory form, pneumonia, and the chronic respiratory form with abscesses. Infections also occur in the gastrointestinal tract causing enteritis. The musculoskeletal form often takes shape in damages to the musculoskeletal system.

Treatment of *Rhodococcus equi* infections in animals relies on the administration of antibiotics such as Erythromycin, Chloramphenicol and Rifampin. However, *R. equi* strains resistant to antibiotics were recently observed, rendering these antibiotics ineffective.

As with foals, *R. equi* can be fatal for AIDS patients. It mainly causes lung infections in the form of chronic abscesses. Patients can have a wide range of problems from lung disease to lobular pulmonary necrosis [15].

R. equi morphology is similar to the main agent of Tuberculosis, *Mycobacterium tuberculosis*, commonly called Koch bacillus or BK. This pathogen attacks human beings only. The HIV pandemic has provided a favorable

environment for the resurgence of the disease. The incidence of tuberculosis in HIV-infected people is about 500 times higher than among the general population.

Africa continues to have the highest incidence number, with over 281 cases per 100 000 inhabitants in 2014 [37]. Therefore, both the *R. equi* and *M. tuberculosis* bacteria, with their similar morphology, are indistinguishable and cause similar symptoms [35].

The objective of this work is to study the antibacterial effects of *C. europaea* on *R. equi,* a bacterium widely encountered in various diseases affecting humans and animals [46]. This study is of particular interest because the *R.* species are taxonomically related to mycobacteria, including the tuberculosis agent.

EXPERIMENTAL SECTION

Plant material

The aerial part of the *C. europaea* used in our study was harvested in the Sidi Bettache forest in the region of Rabat in the spring of 2015. The samples used were dried in the shade for 15 days, then in an oven at 60 °C for one week. The dried plant was ground and stored away from light until use.

Preparation of plant extracts:

The extraction is continuous and takes place with a Soxhlet device. This method enables us to infinitely repeat the extraction cycle with the help of a solvent until the total exhaustion of the solute in the raw materials.

100g of powder were placed in a paper filter cartridge in the extractor. For the same cartridge, one liter of solvent was added in the soxhlet flask following the 2005 French Pharmacopoeia method. The plant material was extracted simultaneously with three solvents, which are in order of increasing polarity: ethyl acetate, methanol and finally water. The separation of the extract solvent is carried out using Buchïrotavapor R200. The obtained concentrated extracts will be used for biological tests.

The total yield is expressed by the following ratio:
R (%) = (extract mass/ plant powder mass) × 100.

Bioassay test

Bacterial culture

The bacterium used in our tests is the *Rhodococcus equi* (GK1 CIP 105335).

From an 18-hour culture in the broth growing medium trypto Casein-Soy (TSA): pancreatic digest of casein (15 g), soybean treptone papain (5g), sodium chloride (5g) and distilled water (1000ml), a bacterial suspension was prepared and incubated at 30 °C for 24 hours. This solution represents the bacterial inoculum, which will be used throughout the study.

Antibacterial test

Antibacterial activity is evaluated using the diffusion technique in a TSA agar medium in Petri dishes. The media were seeded with a few milliliters of inoculum to cover the entire agar surface. The tests were performed according to the Vincent (Aromatogram) method [11]. The latter consists of depositing paper filter disks impregnated with the plant extracts to be studied on the agar-covered medium in Petri dishes previously seeded with the inoculum.

On other dishes previously seeded with the inoculum we deposited disks impregnated with the six antibiotics tested: Ampicillin, Amoxicillin, Kanamycin, Chloramphenicol, Cephalothin and Doxycycline. The dishes were then incubated in an oven at 30 °C for 48 hours. The same operation was performed on the culture medium containing inoculum alone as a control sample. Antibacterial action tests on the ethyl acetate, methanol and aqueous extracts were carried out in an agar medium by comparing their action with that of the various antibiotics tested. This test is at the same time qualitative and quantitative. First, it provides an idea about the inhibiting power of the tested substance (presence or absence of inhibition), and then it measures the diameter of the inhibition zone in mm (light areas around the disks impregnated with the substances under study).

Detection methods of pharmacological substances:
Qualitative phyto-chemistry is based on color reactions, or precipitation by specific chemical reagents introduced to the plant extract. For the purposes of a first estimate, it provides preliminary data on the crude extract components.

Characterization of alkaloids:
100 mg of plant extract (ethyl acetate, methanol and aqueous) were added to each test tube containing 3 ml of 1% sulfuric acid. The mixture was boiled in a water bath (100 °C) for 5 minutes. After cooling and filtration, 5 drops of the Meyer reagent were added. The formation of a white precipitate indicates the presence of alkaloids [33].

Characterization of tannins and phenolic compounds:
In a test tube, 10 mg of the plant extract were dissolved in distilled water and a few drops of 1% FeCl 3 ferric chloride were added [24]. The appearance of a blue-black color indicates the presence of gallic tannins while a green-blackish color indicates the presence of catechic tannins.

Characterization of flavonoids:
In a test tube holding the alcohol filtrate the following substances were added: 10mg of plant extract, 1 ml of distilled water, 1 ml of concentrated hydrochloric acid and a chip of magnesium turnings. We held the tube with a wooden clamp, dipped in a (250 ml) beaker of cold water to avoid raising the temperature. The appearance of an orange color indicates the presence of flavones. A cherry red color indicates the presence of flavonols and an orange color indicates the presence of flavanones.

Measuring bacterial lysis with DO 260 and the presence of *R. equi* with DO 600 in the incubation medium.
Both methanol and aqueous extracts have shown no effects on *R. equi.* The *C. europaea* an acetate extract was therefore selected for further testing.

Lysis of the bacterial cell wall is quantified by measuring absorbance (DO) at 260 nm in parallel with the bacterium death. The contact between the ethyl acetate extract and the bacteria at 37 °C in a water bath with stirring, is carried out for variable durations (0H, 1H, 2H, 3H, 4H 5H, 6H). At these various time intervals, we measured the DO 260 nm of the supernatant of bacterium suspension treated with the ethyl acetate extract. The same process was conducted with tubes containing the bacterium in the presence of a given antibacterial agent concentration: Chloramphenicol, and tubes containing only the bacterium as a control sample. In fact, in order to better assess the release of absorptive cell contents at 260 nm, a 500µl suspension of the bacterium was treated with 100 µl of the ethyl acetate extract and an equivalent concentration of Chloramphenicol and then incubated at 37 °C for 24 hours. The tubes were then centrifuged for 2 minutes at 4 °C and 12000 T / S.

The supernatant was then collected, diluted in 500µl of a PBS buffer solution (phosphate-buffered saline) and then placed in a quartz cuvette for reading absorbance (DO) at 260 nm after removing the DO from distilled water, from the culture medium and from the ethyl acetate. The second test was performed with the bacterium only and a third test was carried out with the bacteria in the presence of Chloramphenicol. The same protocol was used to read absorbance at 600 nm of the supernatant that can reveal the presence of bacteria in the medium.

RESULTS AND DISCUSSION

The yield of the ethyl acetate extract was 26.45%, the methanol extract was 8.21% and the aqueous extract was 4.25%.
To assess antimicrobial activity, we used the scale of Mautrait *et al.* (2009), which classified bacterial growth inhibition zone diameters (D) into 5 classes:

- Extremely inhibitory: D greater than 30mm
- Highly inhibitory: D between 20mm and 29mm
- Moderately inhibitory: D 16mm and 19mm
- Slightly inhibitory: D between 11mm and 16mm
- Not inhibitory: D <10

From the results obtained and using the scale of Mautrait *and al.*, the ethyl acetate extract of *C. europaea* was the only one that effectively inhibited the growth of *R. equi.* The other two extracts, aqueous and methanolic, showed no effect on this bacterium (Table 1).
The ethyl acetate extract produced the best results with an inhibition zone of 20 mm. This indicates that the molecules behind antibacterial activity are more extractable with ethyl acetate than with other solvents. The acetate ethyl extract *C. europaea* was therefore selected for further tests.

Table 1: Impact evaluation of aqueous and methanolic extracts and of ethyl acetate extract of *C. europaea* on *R. equi*

Impact on *Rhodoccocus equi*	Ethyl acetate	Methanol	Aqueous extract
	+	-	-

+: Inhibition *-: Absence of inhibition*

As for *R. equi* growth, we found a relatively large zone of inhibition with ethyl acetate extract (Table 2). The *C. europaea* extract remarkably inhibited the bacteria compared to the effect of certain antibiotics tested. An extract is considered active if it induces an inhibition zone greater than or equal to 10 mm [26]. In our study, the average diameters of inhibition zones induced by the ethyl acetate-based extract on *R. equi* are significantly large compared to those resulting from these antibiotics: Chloramphenicol, Amoxicillin, Ampicillin and Cephalothin. The most effective antibiotic on this bacterium was Chloramphenicol (CHL). It presented a 28,33mm inhibition halo, the closest to that of the ethyl acetate extract, which averaged 20mm. Meanwhile, these inhibition zones were much lower for Amoxicillin (AMX) at 10,33mm, Cephalothin (KF) at 9,66mm and Ampicillin (GPA) at 8,33mm diameter. Kanamycin (KMN), on the other hand, showed no effect on this bacterium (Table 2). Therefore, the six antibiotics acted differently with respect to *R. equi,* which appears to be sensitive to Chloramphenicol (CHL) and doxycycline (DOX).

It was hardly sensitive to the three other tested antibiotics: cephalothin (KF), amoxicillin (AMX) and ampicillin (AMP).

Table 2: Impact evaluation of ethyl acetate extract and the six antibiotics tested on *R. equi*

Evaluation of antibiotic action	Tested substances						
	Ethyl acetate extract	**DIX**	**KF**	**CHL**	**KMN**	**AMX**	**AMP**
Action on *R. equi*	+	+	±	+	-	±	±
Average diameter of inhibition zone (in mm)	**20**	**27.33**	**9.66**	**28.33**	**0**	**10.33**	**8,33**

+: Inhibition; ±: weak inhibition; -: No inhibition

Bacterial lysis as per duration of treatment with ethyl acetate extract of *C. Europaea*
The results shown in Figure 1 indicate the existence of a direct correlation between the duration of bacteria treatment by ethyl acetate extract or Chloramphenicol and the release of absorbent cell contents at 260 nm. These results show that three hours are sufficient to obtain a good lysis of the bacteria by ethyl acetate extract of *C. europaea* compared with the results obtained with Chloramphenicol. Indeed, after a three-hour exposure of the bacterium to the tested products, lysis reached 25% of release with ethyl acetate extract and 50% with Chloramphenicol. After 6 hours, we noted 63% of cellular content release for the plant extract and 88% for Chloramphenicol (Figure 1). These results show a very rapid initial release during the first three hours, followed by a slow release during the subsequent processing. Mortality is sudden from the early hours of treatment. These results reflect in a better way the type of damage caused to the bacterial envelope through analysis of the molecule absorbent at 260 nm which is DNA and of which the release is indicative of a complete lysis of the cell wall.

In addition, we measured DO at 600 nm to assess growth inhibition of *R. equi* in a liquid medium by the various products tested as per treatment duration. It was observed that bacteria growth was slowed down in the presence of ethyl acetate extract and Chloramphenicol. The ethyl acetate extract proved active on bacteria growth compared to Chloramphenicol during the first three hours (Figure 2). After 3 hours of treatment, inhibition was complete with Chloramphenicol, while with *C. europaea* ethyl acetate extract; we noted a growth arrest after 5 hours. In the medium containing bacteria only, growth was linear and even rising after 6 hours.

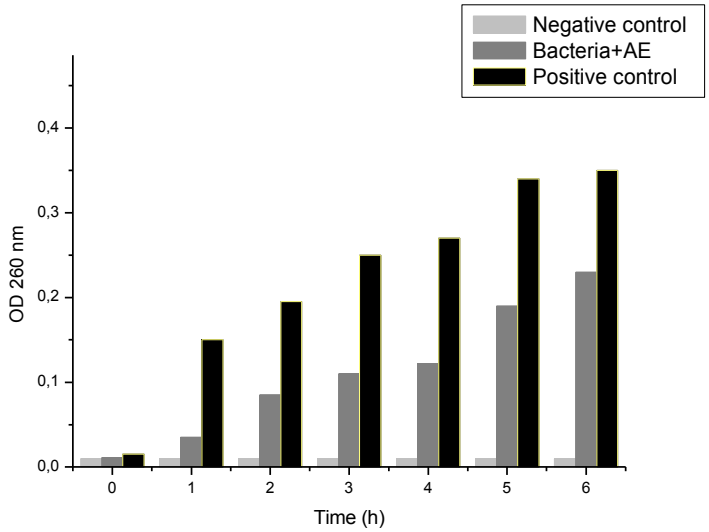

Figure 1: Absorbance at 260 nm of the extracellular medium as a function of the treatment duration of *R. equi* by the different tested products

*Legend: * Negative control -: Untreated bacteria*
** Positive control +: Bacteria + Chloramphenicol*
** Bacteria + AE: bacteria in the presence of ethyl acetate extract.*

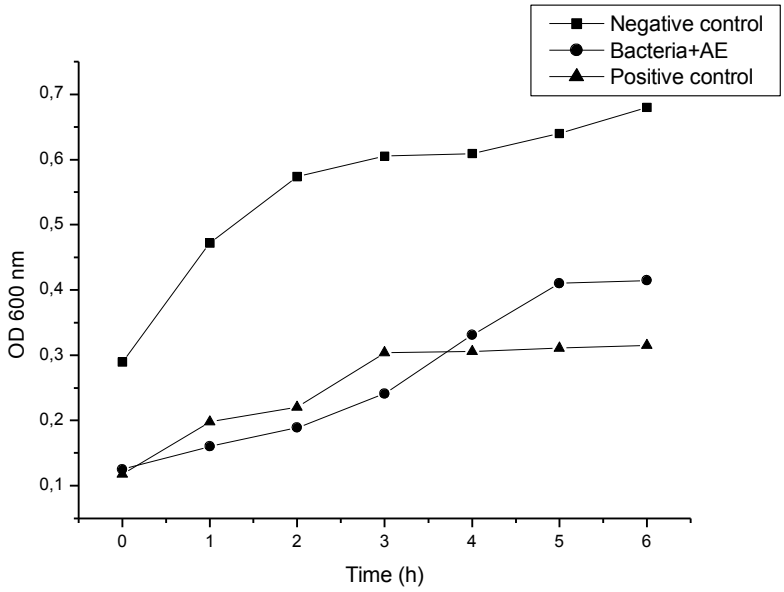

Figure 2: Evaluation of *R. equi* growth inhibition in a liquid medium by the different tested products

*Legend: * Negative control - : Untreated bacteria*
** Positive control +: Bacteria + Chloramphenicol*
** Bacteria + AE: bacteria in the presence of ethyl acetate extract.*

The *in vitro* results of gram-positive bacterium (*R. equi)* lysis obtained with the absorbance increase at 260 nm of the extracellular medium after treatment of the bacteria by ethyl acetate extract, and Chloramphenicol and without forgetting its growth inhibition, indicates that the action of ethyl acetate extract on the bacterium is linked to its ability to cause damage to the bacterium's wall.

Qualitative phytochemical analysis of *C. europaea* extracts:
The outcome of *C. europaea* extract analysis revealed the presence or absence of secondary metabolites. In fact, the aqueous extract contains only flavonoids and alkaloids, while the methanol extract contains, besides alkaloids, phenolic compounds. As for the ethyl acetate extract, it contains the four groups of bioactive compounds tested: alkaloids, flavonoids, phenolics and tannins (Table 3). However, we noted that the ethyl acetate extract was the only one containing tannins and also the only one among the three extracts tested that resulted in a significant inhibition of *R. equi.*

Table 3: Results of qualitative phytochemical analysis of crude extracts: ethyl acetate, methanol and aqueous extracts of *C. europaea*

***C.europaea* extracts**	Phenolic components	Flavonoids	Alkaloids	Tannins
Acetate ethyl	+	+	+	+
Methanol	+	-	+	-
Aqueous	-	+	+	-

The number of antibiotic resistant pathogens has been on a constant increase in the past years. The discovery of new antimicrobial agents would be the solution to combat this problem.

Fortunately, natural resources hold great potential and provide an opportunity to discover new antibiotics. Using traditional medicine and ethno botany and screening phytochemicals in plant extracts can lead to the discovery of these antibiotics [19].

Antibiotics occur at the level of certain structures or through different metabolic reactions of microorganisms, at highly specific levels known as action sites or molecular targets intrinsic to each of them. Four microorganism metabolic structures or processes are impacted at different levels: some antibiotics inhibit the bacterial wall synthesis; others alter the cytoplasmic membrane causing lethal permeability imbalances. Many others disrupt protein synthesis at the ribosome level while some inhibit the synthesis of nucleic acids. This means that not all antibiotics are indiscriminately active on all pathogens. Thus, for every new antibiotic, the 'activity spectrum' that corresponds to the microorganisms on which an antibiotic has an effective action must be defined.

Besides known antibiotics, different plants are characterized by the synthesis of molecules known as secondary metabolites, renowned for their antiseptic therapeutic effect in folk medicine [36].

In vitro, the microbicidal impact of certain aromatic and medicinal plants was even found to be higher than that of antibiotics [3], in addition to having a very large scope of action. In fact, phytochemicals have a very broad inhibition spectrum that covers gram-positive and gram-negative bacteria as well as fungi [9]. The modus operandi of these compounds is only partially studied: more phenolic molecules such as asxylenol, cresol and carvacrol exert a bactericidal action by causing alterations in the bacterial membranes and loss of intracellular metabolites [45]. These authors showed that carvacol has an inhibitory effect on *Bacillus cereus*. This effect is explained by a sharp decrease in intracellular ATP, reduced membrane potential and intracellular pH and also a disrupted flow of potassium (intra- and extracellular). This reflects the degree of damage caused to the cytoplasmic membrane.

Other studies have shown that the essential oils of certain aromatic plants (rosemary, thyme, oregano and wormwood) have an antibacterial effect on gram+ and gram- bacilli [42]. Key phenolic compounds, tested either alone or associated with antibiotics, act on bacteria by binding to its cell envelope. Essential oils, similar to their components, were able to induce cell lysis. This action was manifest in their lease of absorbents at 260 nm. This release of substances associated with rapid bacterial death could be the result of lesions on cell envelopes induced by antibacterial agents. The use of an electron microscope revealed that the essential oils attacked at the same time cell membranes and walls. However, [45] those certain phenols create an imbalance in membrane permeability and block oxidative phosphorylation, which represents the source of the cell respiration. Other studies by [49] on *C. europaea* essential oils in Spain showed that the phenolic compounds contained in these oils have an antibacterial effect.

In addition, antimicrobial resistance is mainly related to antibiotics while non-bacterial resistance to essential oils has been reported or demonstrated. This is probably due to the different compounds contained in these oils and their mode of action, which affects simultaneously different structures of the bacterial cell [29]. Thus, thanks to essential oils, the impermeability of the bacteria wall to antibiotic action would no longer constitute an obstacle.

Regarding our findings, we noted bacterial growth inhibition and the release of intracellular contents in the presence of the ethyl acetate extract. This inhibition of bacterial activity suggests the existence of an interaction between the phytochemicals contained in the ethyl acetate extract of *C. Europaea* and the bacterial membrane [20]. Qualitative evaluation of the antimicrobial activity of the ethyl acetate extract showed an antimicrobial effect by highlighting positive activity with an inhibition zone of 20 mm. Thus revealing a significant sensitivity of *R. equi*. This can be explained by the impact of the secondary metabolites contained in the ethyl acetate extract. These secondary metabolites act simultaneously or differently, borrow the same or different paths, act together or independently on one or more targets, leading to an antifungal and/or effective bacterial activity. [7] and [22] became interested in the study of the action of phenolic compounds on the bacterial wall. According to these authors, phenols target cell walls, cell membranes and the cytoplasm. Their effects on these three sites depend on the concentration degree used: at low concentrations, they produce reversible effects, whereas with high concentrations they produce a general coagulation with cell death. Other researchers attribute the antibacterial function to phenolic compounds [49]. The biological activity of a natural plant is in direct relation and correlation with its chemical composition [27]. In fact, phenolic compounds can interfere with the plasma membrane causing the malfunction or destruction of cells. This is a mechanism through which bacterial growth can be reduced or completely inhibited.

With regard to flavonoids, these are phenolic hydroxylated substances synthesized by plants in response to a microbial infection. Their antibacterial activity is probably owed to their ability to form complexes with soluble proteins and extracellular proteins. *In vitro*, those are broad-spectrum antimicrobials [18].

Tannins, on the other hand, exert their antimicrobial action through various mechanisms such as the destruction of membranes or inhibition of enzymes by adhesion to membrane proteins. They adhere to proteins, interfering with their synthesis and thus block cell membrane renewal [16]. The same authors have shown that a significant antibacterial effect of tannins, extracted from *Marrubium vulgare L*., a common species in the Mediterranean basin, is observed on some strains considered among the most resistant to antibiotics such as *Pseudomonas Aeruginosa* and *Staphylococcus aureus* 7244. Inhibition zones exceed by far those caused by the antibiotic rifampicin. Other authors [25] showed that an ethanolic extract rich in tannins, obtained from the bark of *Maytenus undata*, exhibits an antibacterial and antifungal action on a wide range of pathogenic microorganisms *(staphylococci, enterococci, bacilli* and *streptococci candidiasis).*

In 2002, [29] author has showed that the phytochemical analysis of four plants of the *Combretaceae* family revealed that these plants are very rich in tannins to which an antibacterial activity can be attributed.

Alkaloids have an antimicrobial capacity exercised through interference with the process of DNA replication and RNA transcription, both vital processes for microorganisms. Other mechanisms included impaired stability of bio membranes and protein synthesis and important metabolic enzymes [1].

The antibacterial activity of natural substances has been the subject of a great many studies. There are two types of effects on these microorganisms: the bactericidal activity, which has a lethal impact and the bacteriostatic effect, which causes growth inhibition. However, the bactericidal action of natural substances on bacterial cells is still insufficiently clarified [28]. Several mechanisms are involved: inhibition of glycolysis and depletion of potassium [13], changing the morphology of the bacterial cell, precipitation of proteins and nucleic acids [39], inhibition of selective membrane permeability and membrane deterioration [8], absorption and formation of a film around the bacterial cell with inhibition of the breathing, absorption and excretion processes [39] and inhibition of macromolecules synthesis: DNA, RNA and proteins [12].

CONCLUSION

Our results are very encouraging. The *C. europaea* ethyl acetate extract shows a significant antibacterial effect on *R. equi.* Chemical analysis of its various extracts has revealed several molecules that could be responsible for the observed effects. Ethyl acetate is the only extract containing tannins and though other secondary metabolites exist in other extracts. They have not shown any antibacterial activity. This opens up prospects for isolating and characterizing the active principles responsible for the ethyl acetate extract antibiotic activity and identifying new molecules able to fight *R. equi*. The same strategy would constitute a potential alternative to countering the tuberculosis agent *M. tuberculosis,* in view of its morphological resemblance to *R. equi*.

C. europaea could embody hope in the fight against tuberculosis, a disease that continues to constitute a serious health hazard in Morocco and in Africa.

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