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Original Article

# Influence of leaf extracts and total flavonoids of *Rhus tripartita (Ucria) Grande* on phytobeneficial bacteria associated with its rhizosphere

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**ABSTRACT**

The article deals with the antimicrobial effect of *Rhus tripartita (Ucria) Grande* leaf extracts and total flavonoids against twelve antagonists Plant Growth Promoting Rhizobacteria of its rhizosphere, characterized in a previous study. The aim of this study is to demonstrate that leaves through their decomposition in the soil, may affect the distribution of bacterial communities in the rhizosphere. Leaves extracts were performed with distilled water, alcohol, methanol, hexane and chloroform as solvent and diluted in concentrations of 0.001, 0.01 and 0.1 mg/mL. The extraction of total flavonoids was carried out from leaves' methanolic extract. The antimicrobial effect of the extracts was evaluated by the agar diffusion method and the determination of the minimum inhibitory concentration was carried out on a liquid medium. Alcohol, chloroform and methanol extracts were found to be the most effective on tested strains. The maximum zone inhibition was 18 mm, and the minimum zone inhibition was 7 mm. Rt 1*: Bacillus licheniformis* appears to be the most sensitive to all extracts. In contrast, Rt 7: *Bacillus megaterium*, seems to be the less sensitive strain. On the other hand, total flavonoids had a significant effect on 25% of the strains tested, mainly *Bacillus* genus. With a broad antimicrobial spectrum, the *Rhus tripartita* leaf extracts can be considered as a control agent for the distribution of the bacterial community in the rhizosphere. Therefore, our work showed that the plant could influence the bacterial diversity of its rhizosphere through its leaves.

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## 1. Introduction

*Rhus tripartita (Ucria) Grande* called "African sumac" is a shrub species of the botanical family of *Anacardiaceae.* This species is distributed in North Africa to Hoggar, Sicily and Western Asia [30]. Therefore, many studies reported the antimicrobial potential of the shrub against a wide range of microorganisms [4; 1; 15; 16; 27; 8].

On the other hand, several phytochemicals were attributed to the antimicrobial potential of the plant [16] such as flavonoids [10; 25; 2; 18]. Therefore, Flavonoids constitute a large group of secondary metabolites in higher plants [3]. Moreover, numerous studies have demonstrated the richness of *Rhus tripartita* in flavonoid compounds [22; 23; 36].

Microorganisms play an essential role in the decomposition of organic matter, nutrient cycling, and plant productivity. Furthermore, soil microbes, mainly bacteria and fungi, are affected by all biochemical processes occurring in soils and play a vital role in maintaining soil productivity. Therefore, the plant strongly

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interacts with its biotic environment through the synthesis of secondary metabolites, most often "diffusible", often exuded secondary metabolites are sources of chemotactism allowing the selection of organisms (pathogens, mutualists or commensals) around the roots [37]. In this way, microorganisms can interact with the mutually beneficial plant, examples include the Plant Growth Promoting Rhizobacteria (PGPR). Other bacteria in the vicinity of plant roots (rhizobacteria) are able to control plant diseases caused by soil pathogens [5] called antagonists bacteria.

Therefore, several parameters influence the distribution or activity of soil microorganisms. Furthermore, the rhizosphere is characterized by various secretions of micro and macromolecular metabolites [7]. The role of root secretions on the functioning and distribution of microbial communities has long been studied [29]. However, the studies of rhizobacteria beneficial to plants did not always take into account factors other than soil composition or root exsudation. Therefore, Lamb et *al*. [20] have reported the plant biomass effects on soil community structure.

To our knowledge, no studies have been carried out on the effect of the leaves' plant on the bacteria of its rhizosphere. The present work showed an exploration angle of *Rhus tripartita's* relationship to its rhizosphere bacterial community through the leaf extracts and total flavonoids effects.

## 2. Materials and Methods

### *2.1 Biological material*

*Rhus tripartita* leaves were collected in December 2018, in the Ilamane region (22° 49′ 59″ north, 5° 19′ 59″ east) which, is located in the Ahaggar Cultural National Park (Algerian Sahara).

The antibacterial effect of the plant is tested on an antagonists population that were related to bacteria that are associated with mechanisms of plant growth promotion from *Rhus tripartita's* rhizosphere [9] : Rt 1 : *Bacillus licheniformis ;* Rt 2: *Bacillus circulans ;* Rt 3: *Pseudomonas aeruginosa ;* Rt 4: *Bacillus megaterium* ; Rt 5: *Bacillus subtilis ;* Rt 6: *Escherichia vulneris ;* Rt 7: *Bacillus megaterium* ; Rt 8: *Kocuria varians* ; Rt 9*: Bacillus subtilis ;* Rt 10: *Bacillus licheniformis ;* Rt 11: *Escherichia vulneris ;* Rt 12: *Bacillus licheniformis.*

### *2.1. Preparation of leaf extracts*

The extracts were prepared using the following solvents: distilled water, methanol, hexane, ethanol and chloroform (Sigma, St Louis, MO, USA). 10 g of dried leaves were grinded in mortar and homogenized with 100 mL of the respective solvents. The raw preparation was macerate overnight in the shaker at room temperature and then filtered through a filter paper. The supernatant is recovered and transferred to a spade and extracted concentrated by evaporation of the solvent at 50 °C. The resulting extract is water to obtain a final concentration of 0.001, 0.01 and 0.1 mg/mL.

### *2.2. Total flavonoid extract*

Total flavonoid was extracted using the method reported by Dewanto et *al*. [12]. It consists to mix 250 µL of leaf methanolic extract with 25 mL of 5 % $NaNO_2$, added with 150 µL of $AlCl_3$ (2 %). After 5 min, 0.5 mL of 1M NaOH is added to the solution and extract was resulting after 10 min of incubation.

### *2.3. Preparation of bacterial strains*

Bacterial cultures were prepared in nutritious broth (bioMerieux sa, Lyon, France), which were incubated at 30 °C for 24-72 hours. Cultivated fresh crops dilutions were adjusted to a concentration of $10^6$ CFU/mL.

### *2.4. In vitro antibacterial activity test*

Direct diffusion method was used to evaluate the leaves antibacterial activity. This method is based on the preparation of 6 mm diameter wells on the Müller Hinton agar (bioMerieux sa, Lyon, France) previously seeded by the bacterial strains to be tested according to the protocol as described by Gurusiddaiah [13]. Then, 15 µL of leaf and flavonoid extracts were deposited in these wells. The antibacterial activity was evaluated by measuring the inhibition zone diameter, formed around the well after an incubation of 24 h at 37 °C.

### *2.5. Antibiotic resistance*

The strains were tested for their susceptibility to Oxacillin (OX) 5 µg (Sigma Chemical Co., St. Louis, Mo.) as a control procedure, according to the Clinical and Laboratory Standards Institute [37].

### *2.6. Minimum Inhibitory Concentration (MIC)*

The Minimum Inhibitory Concentration represents the lowest concentration of a substance to inhibit bacterial growth in an incubation time of 24 h at 37 °C. The MIC was determined using the Delarras method [11] slightly modified. It consisted to dilute the extract lowest dilution which showed an antibacterial potential, according to geometric number of 2. Then, mixing 1 mL of each dilution with 1 mL of 24 hours bacterial inoculum and the result reading was performed after incubation for 24 hours at 37 °C. The MIC corresponds to the concentration of the first tube in which there is no growth visible to the naked eye compared to a control tube (without germ).

## 3. Results

The evaluation of the antimicrobial activity of *Rhus tripartita* leaves extracts was determined by the presence or absence of the inhibition zone. The extracts antibacterial activity was evaluated against 12 antagonists' phytobeneficial bacterial strains from *Rhus tripartita* rhizosphere.

Indeed, the extracts showed distinct inhibitory effects compared to the strains tested. Rt 1 *: Bacillus licheniformis* appears to be the most sensitive with inhibitory zones of aqueous extract (12 mm), alcoholic extract (9±0.23 mm), chloroformic extract (13±0.46 mm), methanolic extract (10±0.84 mm), hexanoic extract (15±0.23 mm) followed-up by Rt 8 : *Kocuria varians* and Rt 11 : *Escherichia vulneris* (Table 1). In contrast, Rt 7: *Bacillus megaterium*, seems to be the less sensitive strain with maximum inhibitory zone of 9 mm (Tab. 1). On the other hand, all tested strains were resistant to oxacitin (5 µg/L) as control procedure (Table 2).

Therefore, chloroformic extract was found to have the broadest spectrum of activity (7-18 mm) but only performed on eight strains of a total of twelve. Moreover, methanol extract showed an antimicrobial effect on all tested strains. However, the extracts have approximately similar effects on both Gram-negative and positive bacteria (Table 3). Therefore, it appears that the antimicrobial activity of hexaoic extracts was the less effective (Table 4). Indeed, aqueous and alcoholic extracts have been able to act on eight out of 12 tested strains.

Moreover, the leaf extracts MIC was showed promising results, with an effect that varied between 1.25 µg/mL and 1667 µg/mL (Table 3). However, it should be noted that the chloroformic extract was displayd narrow MIC levels (1.67-166.7 µg/mL). Conventionally, Rt 1: *Bacillus licheniformis* was showed sensitivity at the lowest range of MIC (12.5-16.67 µg/mL).

The pure total flavonoids extract showed a significant activity against 25 % of the strains tested (Fig. 1). As demonstrated in the Table 4, the Gram-positive bacteria of *Bacillus* genera were the most sensitive to the pure compounds with MICs in the range of 150 and 650 µg/mL.

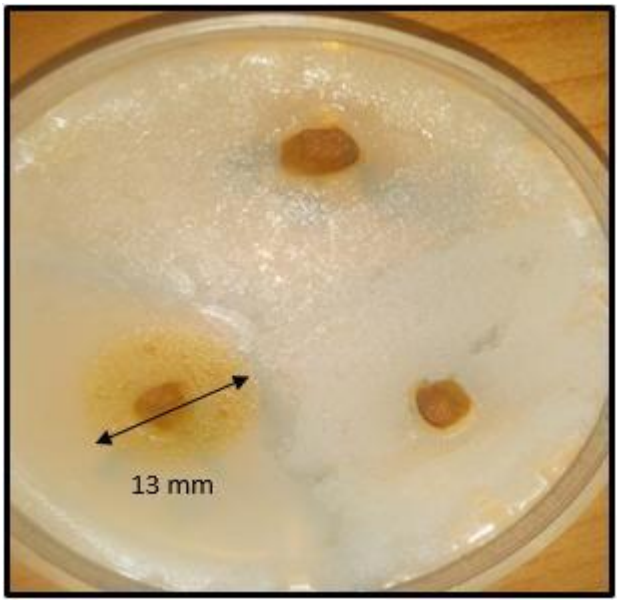


***Figure 1*:** Antimicrobial effect of total flavonoids extracts against *Rt 9 Bacillus subtilis*.

**Table 3:** Minimale Inhibitrice Concentration values recorded for the different extracts (µg/mL)

| Code | Strains | Aqueous extract | Alcoholic extract | Hexanoic extract | Chloroformic extract | Methanolic extract |
|---|---|---|---|---|---|---|
| **Rt 1** | *Bacillus licheniformis* | 12.50 | 12.5 | 125 | 12.5 | 16.67 |
| **Rt 2** | *Bacillus circulans* | 1250 | 125 | 1250 | 16.67 | 25 |
| **Rt 3** | *Pseudomonas aeruginosa* | 166.7 | 250 | 1250 | 125 | 1.25 |
| **Rt 4** | *Bacillus megaterium* | 1250 | 125 | 166.7 | 2.5 | 12.5 |
| **Rt 5** | *Bacillus subtilis* | 2.5 | 125 | 2.5 | 125 | 1.25 |
| **Rt 6** | *Escherichia vulneris* | 1250 | 125 | 1667 | 125 | 250 |
| **Rt 7** | *Bacillus megaterium* | 1250 | 250 | 1250 | 125 | 166.7 |
| **Rt 8** | *kocuria varians* | 1.25 | 1.67 | 16.67 | 1.25 | 1.67 |
| **Rt 9** | *Bacillus subtilis* | 125 | 250 | 1667 | 16.67 | 125 |
| **Rt 10** | *Bacillus licheniformis* | 1250 | 250 | 1667 | 125 | 125 |
| **Rt 11** | *Escherichia vulneris* | 1.25 | 1.25 | 12.5 | 1.67 | 1.67 |
| **Rt 12** | *Bacillus licheniformis* | 12.5 | 125 | 1250 | 166.7 | 125 |

**Table 1:** Antibacterial activity of *Rhus tripartita's leaves* extracts on PGPR antagonists' strains, expressed by diameter inhibition zones (mm)

| Extracts | Concentrations | PGPR antagonists strains | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rt 1 | Rt 2 | Rt 3 | Rt 4 | Rt 5 | Rt 6 | Rt 7 | Rt 8 | Rt 9 | Rt 10 | Rt 11 | Rt 12 |
| Aqueous | 0.1 g/L | 12 | 0 | 13±0.46 | 10±0.46 | 8±0.23 | 0 | 0 | 9±0.58 | 10 | 0 | 12±0.84 | 13±0.46 |
| | 0.01 g/L | 0 | 0 | 10±0.46 | 0 | 7 | 0 | 0 | 7±0.58 | 0 | 0 | 12 | 10±0.84 |
| | 0.001 g/L | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| Alcoholic | 0.1 g/L | 9±0.23 | 0 | 0 | 11±0.46 | 8±0.23 | 11±0.46 | 0 | 7±0.58 | 0 | 8±0.46 | 12±0.84 | 14±0.46 |
| | 0.01 g/L | 7±0.46 | 0 | 0 | 0 | 6±0.46 | 0 | 0 | 7 | 0 | 6±0.23 | 8±0.23 | 0 |
| | 0.001 g/L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloroformic | 0.1 g/L | 13±0.46 | 10±0.23 | 0 | 11±0.56 | 7 | 7±0.84 | 0 | 18±0.46 | 0 | 12 | 16 | 13 |
| | 0.01 g/L | 0 | 6±0.46 | 0 | 8±0.46 | 6±0.46 | 6±0.71 | 0 | 13±0.46 | 0 | 0 | 0 | 0 |
| | 0.001 g/L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7±0.84 | 0 | 0 | 0 | 0 |
| Methanolic | 0.1 g/L | 10±0.84 | 14±0.69 | 9±0.44 | 16±0.23 | 7±0.78 | 13±2.12 | 9±0.46 | 9±0.46 | 10±0.84 | 16±0.46 | 12±0.46 | 13±0.46 |
| | 0.01 g/L | 0 | 9±0.46 | 7±0.46 | 8 | 0 | 7±0.78 | 6±0.46 | 7 | 0 | 0 | 10 | 0 |
| | 0.001 g/L | 0 | 0 | 0 | 0 | 0 | 6±1.08 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hexanoic | 0.1 g/L | 15±0.23 | 0 | 0 | 0 | 13±0.46 | 0 | 0 | 10±0.46 | 0 | 0 | 12±0.46 | 0 |
| | 0.01 g/L | 8±0.46 | 0 | 0 | 0 | 9 | 0 | 0 | 7 | 0 | 0 | 9±0.46 | 0 |
| | 0.001 g/L | 6±0.23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Diameter of well (6mm) is included

**Table 2:** Antibiogram of strains tested by disc diffusion method

| | Rt 1 | Rt 2 | Rt 3 | Rt 4 | Rt 5 | Rt 6 | Rt 7 | Rt 8 | Rt 9 | Rt 10 | Rt 11 | Rt 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oxacitin (5 µL) | R | R | R | R | R | R | R | R | R | R | R | R |

**R :** resistant ; **Rt 1** : *Bacillus licheniformis* ;**Rt 2**: *Bacillus circulans* ;**Rt 3**: *Pseudomonas aeruginosa* ;**Rt 4**: *Bacillus megaterium* ; **Rt 5**:*Bacillus subtilis* ; **Rt 6**: *Escherichia vulneris* ; **Rt 7**: *Bacillus megaterium* ; **Rt 8**: *Kocuria varians* ; **Rt 9***: Bacillus subtilis* ;**Rt 10**: *Bacillus licheniformis* ;**Rt 11**: *Escherichia vulneris* ;**Rt 12**: *Bacillus licheniformi.*

**Table 4:** Antibacterial activity of total flavonoids (extracted from *Rhus tripartita's* leaves) on PGPR antagonists strains, expressed by diameter inhibition zones (mm) and Minimal Inhibitory Concentration values recorded (µg/mL)

| Total flavonoids | Rt 1 | Rt 2 | Rt 3 | Rt 4 | Rt 5 | Rt 6 | Rt 7 | Rt 8 | Rt 9 | Rt 10 | Rt 11 | Rt 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inhibition Zone | 0 | 12 | 0 | 0 | 7 | 0 | 12 | 0 | 13 | 0 | 0 | 0 |
| MIC (µL/mL) | - | 240 | - | - | 650 | - | 240 | - | 150 | - | - | - |

**Rt 1** : *Bacillus licheniformis* ;**Rt 2**: *Bacillus circulans* ;**Rt 3**: *Pseudomonas aeruginosa* ;**Rt 4**: *Bacillus megaterium* ; **Rt 5**: *Bacillus subtilis* ; **Rt 6**: *Escherichia vulneris* ; **Rt 7**: *Bacillus megaterium* ; **Rt 8**: *Kocuria varians* ; **Rt 9***: Bacillus subtilis* ;**Rt 10**: *Bacillus licheniformis* ;**Rt 11**: *Escherichia vulneris* ;**Rt 12**: *Bacillus licheniformis* *Diameter of well (6 mm) is included.

## 2. Discussion

The focus of this study was to establish the biological activities of organic, aqueous and flavonoid extracts on R.tripartita leaves on phytobeneficial bacteria of its rhizosphere by comparing their antimicrobial properties on antagonists PGPR associated to plant rhizosphere.

These results showed that extracts made with organic solvents have not a significant effect than aqueous extract. In contrast, it has been previously reported that organic extracts had shown a better antibacterial effect than aqueous extracts [32]. Indeed, several parameters affect the effectiveness of bioactive substances, it depends on bacterial species, whether resistant or sensitive and the solvent type. It is interesting that the aqueous extract would have an antimicrobial on the majority of strains tested. Theoretically, it is assumed that leaves in the environment when they are found on the ground are certainly in contact with surface water, which probably over time can extract bioactive substances from the leaves and influence microbial diversity.

Therefore, Rhus tripartita extracts showed a significant broad-spectrum activity against all tested microorganisms. That said, the leaves had a negative effect on the development of these bacteria. Many studies have reported the antimicrobial effect of Rhus tripartita extracts against bacterial Gram negative and positive strains such as Staphylococcus aureus [27], Bacillus subtilis [15], Escherichia coli, Salmonella typhimurium, Salmonella argenosa [8] and Pseudomonas aeruginosa [16]. Furthermore, it is necessary to take into account that the tested bacterial population belongs to the group of antagonist PGPRs, basically beneficial for the plant health and development. On the other hand, it has been hypothesized that a general reduction in soil microbial diversity will result in reduced functional capacity of the soil [13].

In the current study, Bacillus was the most sensitive species to the extracts used, which was reflected in the MIC values. Furthermore, Bacillus genera represent a large fraction of the microbial community living in soil and the rhizosphere, especially the root systems of plants. They are part of the zymogenic flora of the soil and are found in plant endophytes or epiphytes, and the rhizosphere of various cultivated [17]. They have been studied a lot for their beneficial and protective effect on plant [21; 26; 28; 34]. Therefore, it means that the assignment of these genera could cause soil depletion, particularly in arid soil, which has been isolated from an earlier study.

Therefore, this preliminary study demonstrated that the MIC exhibits real antibacterial activity. In fact, solvent nature plays a key role in the plant antimicrobial activity. However, the results found are difficult to generalize before carrying out experiments on the natural environment.

Moreover, the highest antibacterial effect of the methanol extract may be due to its high content on flavonoids. In fact, these compounds were extracted using methanol that suggests a positive correlation between the antibacterial effects of the methanolic extract on the one hand and flavonoids extracted on the other. The term flavonoid includes the following commonly occurring polyphenols: flavanones, flavones, flavan-3-ols, flavonols and anthocyanins [24]. However, these entire compounds produce different levels of antimicrobial effects. In addition, plant extracts generally contain flavonoids in glycosidic form [3], which may explain why total flavonoids had a significant effect only on 25 % of tested strains. Whereas their antimicrobial effect have been reported in some studies [35; 33]. However, it should also be noted that antimicrobial studies of flavonoids have been carried out on human or foodborne infection bacteria. On the other hand, all tested rhizobacteria in the present work, were resistant to oxacitin. Therefore, resistant bacteria have been detected in the environment such as sediments and soils. This resistance can be attributed to the use of antibiotics for livestock entering the environment when manure is applied to fields [19]. Otherwise, the excessive use of fertilizers and pesticides in agriculture has made it possible to promote this resistance. Moreover, Bacteria in the soil live in community, which implies that there is gene transfer between species, especially those of resistance.

## 3. Conclusion

This study is considered a first report on the in-vitro antibacterial potential of Rhus tripartita on their benefical rhizospheric bacteria. Therefore, with a broad antimicrobial spectrum against Gram positive and negative species, the Rhus tripartita' leaf extracts can be considered as a control agent for the distribution of the bacterial community in the rhizosphere. On the other hand, the total flavonoids have a lower effect than the leaf extracts but they do represent a novel leads for future investigation. Therefore, we suggest therefore, in rhizobacteria studies to take into account the fall of dead leaves of the plants on the rhizosphere soil concerning it.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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