

Short Communication

Anti-bacterial activity of *Coriaria myrtifolia* against *Agrobacterium tumefaciens*: Plant pathogen responsible for crown gall

Halima BERRADA¹, Abdellah FARAH², Mouhcine FADIL³ and Kawtar FIKRI BENBRAHIM^{1*}

¹Laboratory of Microbial Biotechnology, Faculty of Sciences and Technology, Sidi Mohammed Ben Abdellah University, P. O. Box 2202, Fez, Morocco.

²Laboratory of Medicinal, Aromatic Plants and Natural Substances in the National Institute of Medicinal and Aromatic Plants, Taounate, Morocco.

³Laboratory of Functional Ecology and Environment, Faculty of Sciences and Technology, Sidi Mohammed Ben Abdellah University, P. O. Box 2202, Fez, Morocco.

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The present work aimed to evaluate the antibacterial activity of aqueous and methanolic extracts of *Coriaria myrtifolia*'s leaves against *Agrobacterium* sp. and *Agrobacterium tumefaciens* "plant pathogen responsible for crown gall" in an objective to identify novel antimicrobial agents and to put forward efforts of proving plant's extracts scientific credibility, and determining their spectrum of activity. The bacteria tested were found profoundly sensitive to both of the *C. myrtifolia* extracts. The extent of inhibition was more important by methanolic extract than by aqueous one. The average diameter of inhibition zones ranged from 10.67 to 15.33 mm and 12.68 to 18 mm for aqueous and methanolic extract, respectively. This study was the first to report the antimicrobial activity of extracts obtained from the leaf of *C. myrtifolia* against *Agrobacterium* sp. and *Agrobacterium tumefaciens*. It can be concluded that the observed antibacterial characteristics of *C. myrtifolia* indicate that it might be a promising antimicrobial agent.

Key words: Antibacterial, bacteria, plant extracts, *Coriaria myrtifolia*, *Agrobacterium tumefaciens*.

INTRODUCTION

Pesticides are an essential input for preventing pre and post harvest crop losses (Saksena, 2001). Synthetic pesticides are commonly used to control phytopathogenic microorganisms (Agrios, 1997). Incessant and extensive use of these synthetic pesticides is inducing serious problems to the life supporting systems due to their residual toxicity (Ferrer and Cabral, 1991; Andrea et al., 2000). It is estimated that only 0.1% of the agrochemicals used in crop protection hardly reaches the target pest, leaving the remaining 99.9% in the environment, which produce hazards to non target organisms including humans (Pimentel and Levitan, 1986). The large numbers of synthetic pesticides have been banned in the developed countries because of their

undesirable attributes such as high and acute toxicity, long degradation periods, accumulation in the food chain and extension of their power to destroy both useful and harmful pests (Barnard et al., 1997; Orтели et al., 2005).

In spite of using all available means of plant protection, about 1/3 of the yearly harvest of the world is destroyed by pests and the induced loss is expected to be nearly \$300 billion per year (Chandler, 2005). Moreover, many phytopathogenic bacteria have acquired resistance to synthetic pesticides (Williams and Heymann, 1998; White et al., 2002).

Considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search alternative approaches for the

Table 1. Collection sites of the studied plant.

Site	Altitude (m)	North Position	West Position	Exposition	Slope (%)
Bab Berred (BB)	1290	35° 00' 979"	004° 58' 092"	South East	80
Oued el Koub (OK)	140	35° 01' 879"	005° 20' 565"	North	40

management of plant pathogenic microorganisms (Hostettmann and Wolfender, 1997). Green plants represent a reservoir of effective chemotherapeutic agents and can provide valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effective substitute for chemicals (Verma and Dubey, 1999). Reports are available on the use of several plant by-products, which possess antimicrobial properties, on several pathogenic bacteria and fungi (Dorman and Deans, 2000; Kilani, 2006), but reports are not available on the evaluation of inhibitory action of plants extract on phytopathogenic bacteria particularly in different pathovars of *Agrobacterium* which are known to cause a serious disease at the crown, roots, stems and shoots of many woody and herbaceous plants, causing considerable losses in yield and quality.

Coriaria myrtifolia distributed in the Mediterranean region (France, Italy, Spain, Algeria, Morocco), belongs to the family Coriariaceae and large amounts of tannins from this plant have been used for tanning leather. Hence, the leaves have been used to paint leather in black, and Windholz (1983) reported the use of the fruits in the last century for coloring wine.

The purpose of this study was to evaluate the anti-bacterial activity by the disc diffusion method in the agar medium of the crude extract of *C. myrtifolia* leaves.

MATERIALS AND METHODS

Collection of plant materials

The plant was collected from two sites in Northern Morocco in April 2011, including Bab Berred and Oued el Koub whose geodesic coordinates and characteristics are presented in Table 1. The identification of the plant was made by Professor A. Ennabili from the National Institute of Medicinal and Aromatic Plants, Taounate (Morocco). The leaves of the plant collected were air dried in shadow for a week and then crushed in a mortar. The obtained powder was then used for preparing the two studied extracts.

Preparation of the aqueous extracts

The aqueous extract of *C. myrtifolia* leaves was obtained by decoction of 50 g of plant material in 100 ml of sterile distilled water. At the end of this phase, the extract was filtered under reduced pressure under aseptic conditions and was concentrated in a rotary evaporator until the syrup was obtained which was lyophilized and stored at 4°C. The extract was then used for the antibacterial activity assay.

Preparation of the methanol extracts

The methanolic extract of *C. myrtifolia* was prepared by sonication

(30°C; 35 KHZ; 30 min). 45 g of powder was added to 200 ml of methanol. After 45 min of extraction, the mixture (powder and solvent) was filtered and the filtrate was evaporated using a rotary evaporator (90 rpm at 40°C). The final residue was stored at 4°C.

Plant pathogenic bacterial cultures

To test the antimicrobial potential of *C. myrtifolia* powder, two bacterial strains of *Agrobacterium* sp.: (S₇F₃: EF 427851.1 Accession number et S₁₃PC₆: EF 427855.1 Accession number), and one strain of phytopathogenic *Agrobacterium tumefaciens*: (S₇F₃ T: X 67223.1 Accession number) isolated from root nodules of bean (*Phaseolus vulgaris*) (S₇F₃; S₇F₃ T) and chickpea (*Cicer arietinum*) (S₁₃PC₆) (Berrada et al., 2012), were used in this test. Well-isolated colonies of each strain were transferred into tubes containing liquid YEM medium and incubated at 28°C for 3 days on a rotary shaker at 160 rpm in order to have a microbial suspension of about 10⁶ bacteria/ml.

Anti-bacterial activity assay by the method of disc diffusion

The disc diffusion method was used to determine the antibacterial activities of the extracts against bacterial strains. Mueller Hinton plates were inoculated with 100 µl of the bacterial strain culture (10⁶ CFU/ml). Sterile 6 mm diameter filter paper discs were impregnated with 10 µl of the extract and placed onto the inoculated plate (Bauer et al., 1966). Sterile distilled water and methanol were used as negative control while ampicillin (50 µg/ml) was used as positive control. All experiments were performed in triplicate. Plates were then incubated at 28°C for three days and size of inhibition zone diameters surrounding filter paper disc was measured in mm.

Statistical analysis

Data were expressed as X + SD. X is the diameter of the inhibition zone in mm and SD is the standard deviation. Two factorial ANOVA test was conducted at $\alpha = 5\%$ level using the Statgraphics software. Significant differences were determined by multiple range test using 95.0% LSD method.

RESULTS AND DISCUSSION

The antibacterial activity of *C. myrtifolia* extracts is shown in Figure 1. The inhibition zones vary depending on bacterial species and on extract's type. The average diameter of inhibition zones ranged from 13 to 15 mm, 10 to 12 mm, 13 to 18 mm and 12 to 17 mm for *C. myrtifolia* aqueous extracts from Bab berred (Aq. BB) and from Oued El Koub (Aq. OK), methanolic extracts from Bab berred (M. BB) and from Oued El Koub (M. OK), respectively. The largest diameter of inhibition zone was observed for methanolic extracts on the growth of S₁₃PC₆ (methanolic extracts of Bab Berred and Oued el Koub)

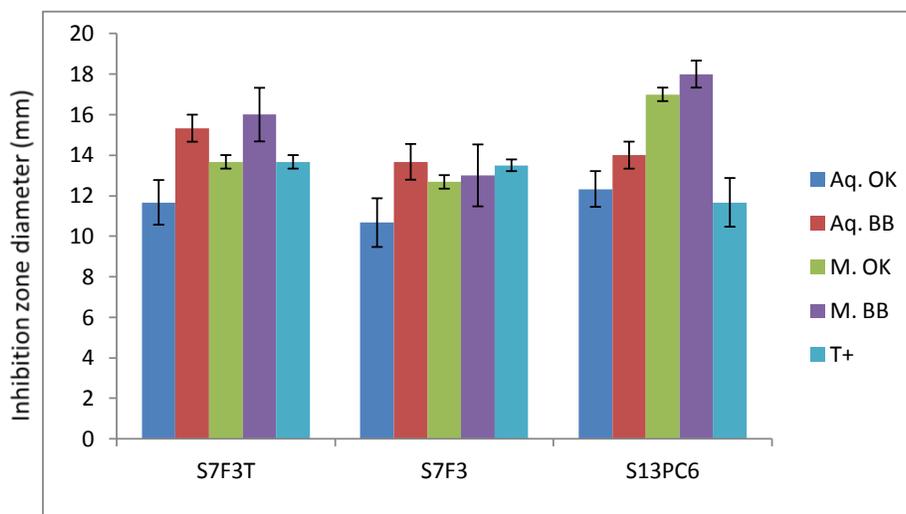


Figure 1. Inhibition zone diameter (in millimetre) of different *Coriaria myrtifolia*'s extracts and antibiotic against the phytopathogenic *Agrobacterium tumefaciens* and *Agrobacterium* sp. Aq.: Aqueous extract, M.: Methanolic extract, B.B.: Bab Berred, O.K.: Oued El Koube, T+: Antibiotic

and S₇F₃T (methanolic extracts of Bab Berred).

The comparison of the inhibition zone diameters for aqueous and methanolic extracts (Figure 1) indicate that methanolic extracts has a greater antibacterial activity in both stations, especially for strains S₇F₃T and S₁₃PC₆. This suggests that methanol and water have different solubilising capacity for the bioactive *C. myrtifolia* components which seem to be better extracted with methanol than with water. In general, the activities against test bacterial culture used have shown good activity compared with antibiotic tested. Similar results were found by Boudkhili et al. (2012) for methanolic extracts against some human pathogenic bacteria.

The analysis of variance of the effect of different extracts showed a statistically significant difference in the three strains tested ($P = 0.0127$) and that S₁₃PC₆ was the most sensitive to the tested extracts. Moreover, the results (Figure 1) indicate that different extracts of *C. myrtifolia* inhibit significantly ($P = 0.003$) the growth of different strains studied compared to the positive control. The highest inhibition was mostly provided by the methanol extract of Bab Berred followed by methanol extract of Oued el Koube then the aqueous extract of Bab Berred and lastly by the aqueous extract of Oued el Koube. The inhibition activity of the antibiotic (T+) was not significantly different from that of the aqueous extract of Oued el Koube.

These results indicate that under the same operating conditions and for the same strain, the antibacterial activity of *C. myrtifolia* extract from Bab Berred was much greater than that of *C. myrtifolia* extract from Oued el Koube, as well as for aqueous extract or methanolic extract. These differences were statistically significant

especially for S₇F₃T and S₁₃PC₆. This difference in activity may be due to the influence of environmental conditions on the chemical composition, and hence the concentration of active compounds, of the two origins of this plant. Furthermore, our results show that the antibacterial activity depends on the nature of the solvent used and the bacterial strain tested.

In recent years, field existences of antibiotic resistant phytopathogenic bacteria are increasing (Mandavia et al., 1999). The World Health Organization has banned many pesticides, even if recognized as very important in agriculture, due to their wide range of toxicity to non-target organisms, including humans (Barnard et al., 1997). Some developing countries still use these pesticides despite their harmful effects.

The use of naturally available chemicals from plants, which retards the reproduction of undesirable micro organisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides (Verma and Dubey, 1999; Gottlieb et al., 2002). Many reports of antibacterial activity of plants extract against human pathogens and their pharmaceutical application are available (Newman et al., 2000; Gibbons, 2005; Mohana et al., 2008), but not much has been done on the antibacterial activity of plants extract against plant pathogens (Satish et al., 1999). This is mainly due to lack of information on the screening/evaluation of various plants for their antibacterial potential. Thus, the present study reveals that *C. myrtifolia* is a potential candidate that could be successfully exploited for the management of diseases caused by different pathogens of *Agrobacterium*.

Conclusion

In conclusion, this study contributes to the knowledge of the antimicrobial potential of *Coriaria myrtifolia* in vitro. The data presented shows that the plant's extracts studied exerted good antibacterial activity. It appears from this study that:

- 1) The methanolic extracts of *C. myrtifolia* were more active than the aqueous ones.
- 2) In the same operating conditions and for the same strains, the antibacterial activity of *C. myrtifolia* extracts from Bab Berred was generally much greater than that of *C. myrtifolia* extracts from Oued el Koub, both for the aqueous and the methanol extracts. This activity is reported for the first time against *Agrobacterium* genus.
- 3) Whatever the extraction method used, the amount of compounds synthesized in the secondary metabolism of *C. myrtifolia* of Bab Berred is greater than *C. myrtifolia* extracts of Oued el Koub.

Finally, we can say that *Coriaria myrtifolia* of Moroccan origin is an inexhaustible source of natural bioactive substances and compounds. Further studies are needed to focus on the isolation and characterization of biological and chemical bioactive compounds of this important plant, as well to assess their safety.

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