



Antibacterial Activity of Lyophilized Aqueous Extract of *Coriaria Myrtifolia* from Northern Morocco

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ABSTRACT

This work aims to evaluate the antibacterial activity of lyophilized aqueous extract of *Coriaria myrtifolia* against twenty three bacterial strains belonging to twelve main genera, known to be involved in some pathologies and / or in the food spoilage process: *Bacillus sp.*, *E. coli*, *E. hafnia*, *Enterococcus sp.*, *Klebsiella sp.*, *Listeria monocytogenes*, *Pseudomonas sp.*, *Salmonella sp.*, *Serratia sp.*, *Shigella sp.*, *S. aureus* and *Streptomyces sp.* To achieve this goal, the disk diffusion method was used.

The study revealed that *C. myrtifolia*'s aqueous extract is active against most of the tested strains and that activity is proportional to its concentration. The intensity of inhibition depends on the extract's concentration on one hand and on the bacterial species tested on the other hand.

Indexing terms/Keywords

Coriaria myrtifolia, Aqueous extracts, Antibacterial activity.

Academic Discipline And Sub-Disciplines

Phytopharmacology.

SUBJECT CLASSIFICATION

Microbiology.

TYPE (METHOD/APPROACH)

Quasi-Experimental.

Council for Innovative Research

Peer Review Research Publishing System

Journal: JOURNAL OF ADVANCES IN BIOTECHNOLOGY

Vol 3., No. 3.

www.cirjbt.org , jbteditor@gmail.com



INTRODUCTION

The infectious diseases induce more than 17 million deaths every year throughout the world from which more than half are registered in Africa [1]. They constitute an important concern of the public health because of their frequency and gravity [2]. During the research for alternative and effective medicine against these pathologies, scientists showed an interest more and more growing for medicinal plants [3, 4].

Throughout the world and especially in Morocco, medicinal plants play an important role in therapy, in both urban and rural areas and are an inexhaustible source of new antibacterial agents. Medicinal plants could provide the treatment or prevention of chronic and / or serious infectious diseases and solve the problem of bacterial resistance for current antibacterial agents. To check better the bacterial infections, it is thus urgent to highlight new effective antibacterial agents and it's in this context that this work is considered.

Coriaria myrtifolia is an evergreen shrub, exceptionally in winter, up to 2-3 meters, very common in the Mediterranean region. This plant can be easily adapted to different soil types and climates [9] and can colonize even nitrogen-poor soils because its roots can form nitrogen-fixing nodules [4].

In this study, we evaluated the antibacterial activity of lyophilized aqueous extract of *Coriaria myrtifolia* against twenty three Gram positive and Gram negative bacterial strains involved in several pathologies and / or implicated in the process of food spoilage.

MATERIAL AND METHODS

Plant material

Samples of *Coriaria myrtifolia* were collected from Oued Al Koub region (Altitude: 140 m, North 35° 01' 879", West: 005° 20' 565", Exposure: North, Slope: 40%, Soil: conglomeratic) and air dried. Leaves were then separated from the aerial parts and ground sufficiently to get a fine powder.

Preparation of extract

100g of leaves powder was mixed with 2 liters of distilled water and left for 48 hours to prepare maceration. The obtained solution was carefully filtered several times on hydrophilic cotton then on filter paper Whatman n°1. The filtrate was frozen at -30 ° C and lyophilized at -45 ° C with a Telstar lyophilisator type [7].

400 mg of freeze-dried aqueous extract was dissolved in 1 ml of sterile distilled water and served as a stock solution. A serial dilution ranging from 400 to 25 mg/ml was then realized.

Evaluation of the antibacterial activity of the extract

The screening of antibacterial activity was realized by the disk diffusion method. Whatman paper disks of 6 mm diameter were sterilized and placed on Petri dishes previously inoculated with 100µl of the bacterial strains (10⁸UFC/ml) investigated.

The discs were then impregnated with 10 µl of the lyophilized aqueous extract at different concentrations. A paper disk impregnated with distilled water served as control [8].

All inoculated plates were incubated at 37°C for 24 h, then inhibition zone diameters observed and measured.

Results and Discussion

The study of the antibacterial activity was realized by the disc diffusion technique. Results are expressed by the appearance of inhibition zones around the disks on solid medium (LB). The inhibition zones diameters are presented in Table 2.

The results indicate that the aqueous extract has a dose-dependent antibacterial activity, because the inhibition is proportional to the extract's concentration and that the bacterial sensibility varies from a tested strain to another one.

The extract is active against three species of *Bacillus* studied: *Bacillus cereus*, *B. circulans* and *B. thuringiensis*.

B. circulans with inhibition zones diameters from 20 mm (400mg/ml) to 12.3 mm (25mg/ml) is the most sensitive *Bacillus* tested strain. While the fourth *Bacillus* species, *B. coagulans* was resistant to the same extract.

Salmonella typhimurium (ATCC 14028) and *Salmonella Braenderup* (H9812) from the same origin, show a different behavior against the extract, the first one is resistant while the second presents an important sensibility.



Bacterial strains

Several Gram-positive and Gram-negative bacteria from different origins were used (Table 1)

Table 1: Gram + and Gram- bacteria used in the study of the antibacterial activity of the aqueous extract freeze-dried.

Souches	Origine	Gram
<i>Bacillus cereus</i>	Collection (LMBA)	Gram positive Bacilli
<i>Bacillus circulans</i>	Collection (LMBA)	Gram positive Bacilli
<i>Bacillus thuringiensis</i> (BT41)	Collection (LMBA)	Gram positive Bacilli
<i>Bacillus coagulans</i>	Collection (LMBA)	Gram positive Bacilli
<i>Escherichia coli</i> (ATCC 25922)	Clinique	Gram negative Bacilli
<i>Escherichia coli</i> (DH5 α)	Collection (LMBA)	Gram negative Bacilli
<i>Escherichia coli</i> (I ₂ CF ₁)	Collection (LBM)	Gram negative Bacilli
<i>Enterobacter hafnia</i>	Collection (LBM)	Gram negative Bacilli
<i>Enterococcus irae</i> (ATCC 29212)	Collection (LMBA)	Gram positive Cocci
<i>Enterococcus faecalis</i> (292/1)	Collection (LMBA)	Gram positive Cocci
<i>Enterococcus faecium</i> (ATCC 19436)	Collection (LMBA)	Gram positive Cocci
<i>Klebsiella sp.</i> (I ₄ CT ₂)	Collection (LBM)	Gram negative Bacilli
<i>Listeria monocytogenes</i>	Collection (LMBA)	Gram positive Cocci
<i>P. aeruginosa</i> (ATCC 27853)	Clinique	Gram negative Bacilli
<i>Pseudomonas aeruginosa</i>	Collection (LMBA)	Gram negative Bacilli
<i>Pseudomonas sp.</i>	Collection (LBM)	Gram negative Bacilli
<i>Salmonella braenderup</i> (H9812)	Clinique	Gram negative Bacilli
<i>Salmonella typhimurium</i> (ATCC14028)	Clinique	Gram negative Bacilli
<i>Serratia sp.</i> (L ₁ CF ₃)	Collection (LBM)	Gram negative Bacilli
<i>Shigella sp.</i> (shig 23)	Collection (LBM)	Gram negative Bacilli
<i>Stapylococcus aureus</i> (ATCC25923)	Clinique	Gram positive Cocci
<i>Streptomyces sp.</i> (3.8)	Collection (LMBA)	Gram positive Filamentous bacteria
<i>Streptomyces sp.</i> (3.4)	Collection (LMBA)	Gram positive Filamentous bacteria

LMBA: Laboratory of Microorganisms and Active Biomolecules in the Faculty of Sciences of Tunis (Tunisia);

LBM: Laboratory of Microbial Biotechnology, Faculty of Science and Technology of Fez (Morocco).

These results show that for the same genus, a bacterial interspecific variability was observed for inhibition by *C. myrtifolia* aqueous extract used.

Both strains of *Streptomyces sp.* tested present different behavior against the studied extract. *Streptomyces sp.* (3.8) is the most sensitive of all tested strains, with an inhibition zone diameter of 35 mm at 400mg/ml. *Streptomyces sp.* (3.4) sensibility is less than that observed in the first *Streptomyces* strain (inhibition zone diameter of 25 mm at 400mg/ml) but more important than all other tested strains.

Three strains of *E. coli* from different origins were used. The extract has an important antibacterial activity against *E. coli* (DH5 α) while it has no effect against *E. coli* (ACC25922) and *E. coli* (I2CF1). This suggests that the antibacterial activity of the aqueous extract of *C. myrtifolia* varies according to the tested strain and that mutation can lead to loose of a strain resistance.

These last observations can let us suggest the existence of bacterial intraspecific variability for the extract's inhibition power.



Table 2: Results of evaluation of the antibacterial activity of lyophilized aqueous extract of *Coriaria myrtifolia*

Strains	Inhibition zone's diameters depending on tested extract concentration				
	400mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
<i>Bacillus cereus</i>	8±1.32	7±0	6±0.5	6±0	6±0.2
<i>Bacillus circulans</i>	20±2	16±0.2	15±0.4	14±0.5	12.30.17±
<i>Bacillus thuringiensis</i> (BT41)	15±0.5	13±0	11.2±0.1	10±2	9±0
<i>Bacillus coagulans</i>	-	-	-	-	-
<i>Enterobacter hafnia</i>	15±2	13.3±0.7	12.7±0.2	11.6±0.2	10.1±0.3
<i>Enterococcus irae</i> (ATCC 29212)	-	-	-	-	-
<i>Enterococcus faecalis</i> (292/1)	-	-	-	-	-
<i>Enterococcus faecium</i> (ATCC 19436)	-	-	-	-	-
<i>Escherichia coli</i> (ATCC 25922)	-	-	-	-	-
<i>Escherichia coli</i> (I ₂ CF ₁)	-	-	-	-	-
<i>Escherichia coli</i> (DH5α)	18.3±0.1	13.3±0.3	10.6±3.1	6.5±0.5	6±0.1
<i>Klebsiella sp.</i> (I ₄ CT ₂)	-	-	-	-	-
<i>Listeria monocytogenes</i>	23±1	20.3±1	19±0	17.6±0.1	16±1
<i>P. aeruginosa</i> (ATCC 27853)	15±1	12±0	9.5±0.7	7.1±0.1	6±0
<i>Pseudomonas sp.</i> (Ps2)	16.6±0.6	13.6±0.3	12.3±0.2	11.6±0	9.8±0.4
<i>Pseudomona aeruginosa</i>	21.5±0.5	19±1	16±0.5	15.3±0.2	14.3±0.1
<i>S. aureus</i> (ATCC25923)	13.9±0.1	11.3±0.1	10±0	9±0.1	6.6±0.3
<i>S. typhimurinum</i> (ATCC 14028)	-	-	-	-	-
<i>Salmonella braenderup</i> (H9812)	19.5±0.4	18.5±0.1	16±0	13±0.5	9.5±0.17
<i>Serratia sp.</i> (L ₁ CF ₃)	16±0	15±0.7	13±0.4	10±0.5	8.8±0.4
<i>Shigella sp.</i> (Shig 23)	16±1.73	12±0	10.6±0.4	8.6±0.2	7.1±0.1
<i>Streptomyces sp.</i> (3.8)	35±1	28±2	25±0	23±1	12±0
<i>Streptomyces sp.</i> (3.4)	25±1	21±0.5	17±0.17	14±0	10±0.6

The results show a considerable sensibility of *S. aureus* against the extract, which has been translated in inhibition diameters ranging from 13.9 mm (400 mg/ml) to 6.6 mm (25 mg/ml).

Boudkhili et al (2012) [9] demonstrated the important sensibility of *E. coli*, *Salmonella sp.*, *Bacillus subtilis* and *Staphylococcus sp.* against the methanolic extract of *C. myrtifolia*. This result is concordant with the inhibitory effect obtained by aqueous extract on *Staphylococcus aureus*. While comparison can't be realized neither for *Salmonella* nor for *E. coli*, because the species of *Salmonella* and the origin of *E. coli* used in their study were not indicated.

The aqueous extract presents no effect against *Enterococcus* strain tested, which is consistent with a previous study that showed resistance of *Enterococcus faecalis* against methanol extract of *Padina pavonica* [10]. However, ethanolic and aqueous extracts of *Acacia aroma* [11] and methanolic extract of *Rhus corriaria* are active against this strain [12].

The *C. myrtifolia* aqueous extract is active against *Pseudomonas aeruginosa*, *Enterobacter hafniya*, *Listeria monocytogenes*, *Shigella sp.* and *Serratia sp.* Previous studies showed that these strains were also sensitive towards other plant's extracts [10, 13].



The activity of the aqueous extract disappears against *Klebsiella*. This result is concordant with a previous study that indicated resistance of this strain against *Cuminum cyminum* extracts [14] and discordant with the study of Hammoudi et al., (2012) who found high sensitivity of this strain for *Teucrium pollium* extracts. But, this can be attributed to the nature of substances and chemical compounds found in each tested extract.

Hyper sensibility noticed for Gram + strains can be explained by the presence of a single peptidoglycan layer in their cell wall and the absence of outer double membrane [15]. Moreover, they are easily influenced by the external environmental changes such as temperature and pH [16]. Furthermore, the Gram-negative strains are more resistant due to the nature of their outer membrane, containing lipopolysaccharids and proteins, that is impermeable to most of the biocides [17].

CONCLUSION

Medicinal plants could provide prevention or alternative treatment for chronic and / or severe infectious diseases, and solve the problem of bacterial resistance against existing antibacterial agents.

This work is a contribution to the study of antibacterial activity of lyophilized aqueous extract of *Coriaria myrtifolia* from the northern Morocco. The study revealed that the extract is active against most tested strains.

The results of this work show that *Coriaria myrtifolia* can be exploited as an inexhaustible source of new natural antibacterial agents.

Moreover, they open large perspectives to study other *Coriaria myrtifolia* extracts inhibitory effect and to try to determine the most active bio molecule implied in this antibacterial activity.

AUTHOR'S CONTRIBUTIONS

KH executed the laboratory work in Tunisia. MH executed the laboratory work in Morocco, contributed to plant material collection and drafted the manuscript. AF revised the manuscript. IS contributed to plant material collection and to extract preparation. MG contributed to choose the plant material and provided a part of required chemicals and laboratory consumables. KFB collected the plant, designed the protocol, provided the other part of required chemicals and laboratory consumables, read and substantially revised the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGMENTS

The authors thank the Morocco-Tunisian Co-operation project (MT 14/10), to provide Research Training Fellowship for two of the authors (KH and IS). They also thank Pr. Saad IBNSOUDA, the Head of Microbial Biotechnology laboratory of Sciences and Technology Faculty – Fez (Morocco) for providing research infrastructures and for receiving the training authors in his laboratory ; and Pr Abdellatif BOUDABBOUS for his contribution and help concerning the part realized in the Laboratory of microorganisms and bioactive molecules in the Sciences Faculty of Tunis (Tunisia).

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

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