

## EVALUATION OF THE ANTIBACTERIAL ACTIVITY AND DETERMINATION OF POLYPHENOLS AND FLAVONOIDS CONTENTS OF MOROCCAN *CORIARIA MYRTIFOLIA* EXTRACTS

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### ABSTRACT

**Objective:** This study aimed to evaluate the antibacterial activity of *Coriaria myrtifolia*'s extracts from two Moroccan regions and to determine their total polyphenol's and flavonoid's contents.

**Methods:** The disk diffusion method was used to evaluate antimicrobial activity. Folin Ciocalteu and aluminum trichloride methods were used to determine the Total polyphenol's and flavonoid's contents respectively.

**Results:** The ethyl acetate extracts induced inhibition zone diameters of 20.3 mm and 22.6 mm for samples collected from Bab Berred (BB) and of 20 and 19 mm for those from Oued Al Koub (OK) respectively in *Mycobacterium aurum* and *Mycobacterium smegmatis*. Inhibition zone diameters of *Pseudomonas aeruginosa* were only 12.3 and 10 mm respectively for BB and OK ethyl acetate extracts. The polyphenols and flavonoids contents were respectively 609 µg/ml and 105 µg/ml in the OK ethyl acetate extract and only 475 and 6 µg/ml in the dichloromethan extract.

**Conclusion:** The antibacterial activity of tested extracts depends on the extract's nature, the bacterial strain and the plant's geographical provenance. The ethyl acetate's extract of *C. myrtifolia* from BB was the most active. *Mycobacterium aurum* and *M. smegmatis* were the most sensitive to *Coriaria myrtifolia*'s extracts and *P. aeruginosa* was the most resistant. The polyphenol's and flavonoid's contents were different depending on the extract's nature and the plant's provenance. Valorization of *Coriaria myrtifolia* and evaluation of its biological and phytochemical activities will enable us to screen and test new natural antibacterial molecules in general and antimycobacterial agents in particular.

**Keywords:** Antibacterial activity, *Coriaria myrtifolia*, *Mycobacterium*, Total polyphenols, Flavonoids.

### INTRODUCTION

For centuries, plants have been used to treat several diseases [1]; hence most eminent doctors were herbalists in the past. Currently, medicinal and aromatic plants have considerable advantages due to the progressive discovery of their applications in health care as well as their uses in other domains of economic interests. Because of these numerous uses they know a stronger demand on the world market. The antimicrobial properties of medicinal plants were recognized for a long time, but were confirmed scientifically only recently. These plants have an enormous therapeutic potential to treat many infectious diseases [2]. Indeed, they contain numerous substances able to protect against microorganisms, insects and weeds [3]. So, the plant's production of secondary metabolites, having an antibacterial activity, took an important place in research studies [4, 5].

Several researchers studied the biological activities of aromatic and medicinal plants from different world's regions. Some extracts were effective to control the growth of a big variety of microorganisms. In this context, this study was conducted in a perspective to evaluate and valorize the phytochemical activity of *C. myrtifolia* even if this plant has no medical or medicinal previously known uses; however, it is used in the north of Morocco in tanning, burning and utensil washing. Hence, the aim of our study was to evaluate the antibacterial activity of *Coriaria myrtifolia*'s extracts against *Mycobacterium smegmatis*, *M. aurum*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Bacillus* spp., *Enterococcus faecalis*, and to determine their total contents of polyphenols and flavonoids. In addition, it represents a contribution for providing prevention or alternative treatment for chronic and/or severe infectious diseases, and for solving the problem of bacterial resistance against existing antibacterial agents. Moreover, the evaluation of antimycobacterial activity of this plant has never been investigated.

### MATERIALS AND METHODS

#### Plant material

*Coriaria myrtifolia* was collected, in February 2011, from two different stations in northern Morocco: Bab Berred (35° 00' 979''

N, 004° 58' 092'' W, 1290 m altitude, South East exposure, 80 % Slope, Limestone dolomite soil) and Oued Al Koub (35° 01' 879'' N, 005° 20' 565'' W, 140 m altitude, North West exposure, 40 % Slope, conglomeratic soil). The samples taken to laboratory were air-dried, then leaves were separated from stems and grind to a suitable particle size for optimal dissolution.

#### Bacterial strains tested

Several gram-positive and gram-negative bacteria were used to test the antibacterial activity of *Coriaria myrtifolia* extracts: *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Salmonella* sp., *Enterococcus faecalis*, *Mycobacterium smegmatis* MC<sup>2</sup>155 and *Mycobacterium aurum* A+(Microbial Biotechnology Laboratory, Faculty of Science and Technology Fez, Morocco).

The tested bacteria are pathogenic and are known for their invasiveness and toxicity to humans. They are frequently encountered in many infections like tuberculosis (*Mycobacterium*), food borne illness (*Salmonella*), urinary infections (*Enterococcus*); skin, respiratory, endovascular infections (*Staphylococcus aureus*) and also Nosocomial infections (*Staphylococcus*, *Pseudomonas*). Some ones are also responsible of food alteration (*Bacillus*).

#### Preparation of extracts

Extraction was performed by Soxhlet or by sonication of the *Coriaria myrtifolia*'s leaves powder. Hence, successive application of four analytical solvents (hexane, ethyl acetate, dichloromethane and methanol) to 45 g of powder put in the cartridge of the Soxhlet, yielded various extracts using the protocol described by Farrapo *et al.* (2011) [6]. For the second method, 45 g of powder was immersed in 200 ml of methanol and then subjected to ultrasonication (30 °C, 35 KHz) [5]. At the end of each extraction (Soxhlet, ultrasonication), the solvents were evaporated using a rotary evaporator (90 rpm, 40 °C). Each collected extract was named depending on the solvent used during its extraction.

### Evaluation of the extract's antibacterial activity

The study of antibacterial activity was performed by the agar diffusion method to select the most active extract. Sterilized Whatman paper discs of 6 mm diameter were placed in Petri dishes previously seeded with the tested strain, then impregnated with 10 µl of one of the previously prepared extract.

The inhibition zone diameters were measured after incubation at 37 °C, 72 h for *Mycobacterium* species and 24 h for the other strains. Solvent impregnated discs were used as controls. Which were performed in the same experimental conditions of the tests. Three repetitions were performed for each test [7, 8].

### Determination of the minimum inhibitory concentration (MIC)

The agar dilution technique was used to determine the Minimum Inhibitory Concentration (MIC) of the tested extract [8]. This technique consists in seeding, by a standardized inoculum, a serial dilution of extracts contained in a liquid suitable medium. After incubation, growth observation enabled us to determine the MIC, which is the lowest concentration of extract able to inhibit bacterial growth. Tubes containing 2 ml of sterile LB-agar media were prepared and maintained in a water bath at a temperature of 48 °C to 50 °C. 2 ml of the extract's dilution prepared in LB agar medium was added to each tube, and after homogenization the mixture was poured into sterile Petri dishes. 5 µl of each inoculum (10<sup>8</sup> UFC/ml) were seeded as a spot on the surface of the agar well dry. The dishes were then dried at room temperature until the medium completely absorbs inoculums then incubated at 37 °C. The range of final extract dilution used was 160 to 2.5 mg/ml.

### Determination of flavonoids and polyphenols contents

The determination of total polyphenol's contents in extracts was made according to the Folin Ciocalteu method [9] by measuring the absorbance at 765 nm against a control without extract. The total polyphenol's content in the four extracts (at final concentration 1 mg/ml) was calculated from a linear calibration curve ( $y = ax+b$ ), established with gallic acid (0-500 µg/ml) as a standard reference in the same conditions as the sample.

While the aluminum trichloride method was used to determine the total flavonoids content in the tested extracts [10]. Hence, 1 ml of

each extract sample at (1 mg/ml) was added to 1 ml of AlCl<sub>3</sub> solution (2% in methanol) then, the mixture was vigorously stirred and incubated 10 min. Finally the absorbance was read at 430 nm. A calibration curve ( $y = ax+b$ ) established by quercetin (0-40 µg/ml) was performed under the same operating conditions as for the samples and was used for flavonoids quantification.

### Statistical analysis

The results were expressed as mean of the three repetitions and standard deviations were calculated. Statistical comparisons were made using the Student's test and  $p < 0.05$  was considered as significant [12].

## RESULTS

### Antibacterial activity of the extracts

Results of the antibacterial activity of *C. myrtifolia*'s tested extracts, against seven bacterial strains implicated in several pathologies and/or implicated in food alteration process, resulting in the appearance of inhibition zones around the disks are shown in Tables 1 and 2.

The ethyl acetate extracts induced inhibition zone diameters (IZ φ) ranging from 17.3 to 22.6 mm and from 9.3 to 20 mm for *C. myrtifolia* harvested respectively from Bab Berred (B. B) and from Oued El Koub (O. K). While the soxhlet methanol extract induced IZ φ from 8.6 to 17.6 mm and 7 to 16 mm for BB's and OK's harvested plants respectively. Dichloromethan extracts induced, for all the tested strains, the lowest IZ φ (6 to 10.6 mm) which were very close to controls.

Moreover, the ethyl acetate (EA) extracts induced IZ φ of 20.3 mm and 22.6 mm for samples collected from Bab Berred and of 20 and 19 mm for those from Oued Al Koub respectively in *Mycobacterium aurum* and *M. smegmatis*. While, IZ φ of *Pseudomonas aeruginosa* were only 12.3 and 10 mm respectively for BB and OK ethyl acetate extracts.

The IZ φ of *S. aureus* and *E. faecalis* by the EA extract from Bab Berred's plants were respectively 15.3 and 17 mm, while those of OK's EA extract didn't exceed 11 mm. But they ranged between 11 and 12 mm with the soxhlet methanol extract for the same strains.

**Table 1: Antibacterial activity of the extracts of *Coriaria myrtifolia* collected from Bab Berred (Inhibition zone diameters in mm)**

	T1	T2	T3	A	B	C	D
<i>Salmonella</i>	7±0.09	9±0.10	8±0.11	17, 3±0.10**	11±0.08	11, 6±0.09	8, 6±0.12
<i>Pseudomonas aeruginosa</i>	8±0.13	6±0.04	7±0.10	12, 3±0.15*	8±0.05	8, 6±0.06	7±0.11
<i>Staphylococcus sp.</i>	7±0.08	8, 3±0.10	7, 3±0.1	15, 3±0.11**	10±0.12	11±0.09	9±0.10
<i>Bacillus sp.</i>	7±0.09	7±0.10	7±0.10	16, 3±0.10**	10±0.10	12±0.07	10±0.12
<i>Enterococcus faecalis</i>	6±0.10	6±0.09	6±0.25	17±0.05**	11, 6±0.10	12±0.10	9±0.20
<i>Mycobacterium aurum</i>	9±0.11	10±0.08	8±0.95	22, 6±0.20**	15±0.95*	15, 6±0.1*	10, 3±0.10
<i>Mycobacterium smegmatis</i>	10, 6±0.09	10±0.10	10±0.19	20, 3±0.17**	17, 3±0.10*	17, 6±0.12*	10, 6±0.20

**Table 2: Antibacterial activity of the extracts of *Coriaria myrtifolia* collected from Oued Al Koub (Inhibition zone diameters in mm)**

	T1	T2	T4	A	B	C	D
<i>Salmonella</i>	7±0.10	9±0.10	8±0.12	9.3±0.08	9±0.12	12±0.10*	8±0.10
<i>Pseudomonas aeruginosa</i>	8±0.13	6±0.05	7±0.09	10±0.10*	6±0.09	12±0.07**	7±0.10
<i>Staphylococcus</i>	7±0.08	8.3±0.12	7.3±0.18	11±0.15**	10±0.10	11±0.09*	7.3±0.2
<i>Bacillus sp</i>	7±0.08	7±0.09	7±0.20	10±0.20	7±0.20	7±0.09	7±0.10
<i>Enterococcus faecalis</i>	6±0.09	6±0.10	6±0.30	11±0.90**	8.5±0.09	11±0.20*	6±0.19
<i>M. aurum</i>	9±0.12	10±0.10	8±0.12	20±0.18**	10±10	15±0.75*	8±0.19
<i>M. smegmatis</i>	10.6±0.10	10±0.09	10±0.23	19±0.09**	10±0.12	16±0.15*	10±0.25

T1: Ethyl Acetate Control, T2: Methanol Control, T3: Dichloromethan Control, A: Ethyl acetate extract, B: Methanol extract obtained by sonication, C: Methanol extract obtained by Soxhlet, D: dichloromethane extract.

Results of the most active extract's Minimum Inhibitory Concentration, determined by the agar dilution method, showed that the highest MIC value (80 mg/ml) was obtained for *Pseudomonas*

*aeruginosa* by the ethyl acetate extract of BB, followed by *Salmonella* sp. (40 mg/ml) then the other strains (20 mg/ml), while the lowest value was obtained for *Mycobacterium smegmatis* (10 mg/ml) (table 3).

Table 3: MIC of the Ethyl Acetate extract of *Coriaria myrtifolia* from Bab Berred

Strains	2.5	5	10	20	40	80	160
<i>Enterococcus faecalis</i>	+	+	+	-	-	-	-
<i>Salmonella sp.</i>	+	+	+	+	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	-	-	-	-
<i>Bacillus</i>	+	+	+	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	-	-
<i>Mycobacterium aurum</i>	+	+	+	-	-	-	-
<i>Mycobacterium smegmatis</i>	+	+	-	-	-	-	-

The concentrations are expressed in mg/ml, +Presence of bacterial growth, -Inhibition of bacterial growth.

Table 4: Total content of phenols and flavonoids in different extracts of *C. myrtifolia* (in µg/ml)

Extracts	Total polyphenols concentration (µg/ml)	Total flavonoids concentration (µg/ml)
1B	480±4.80	17±0.54
2B	609±5	105±0.60
3B	490±4.80	34±0.54
4B	475±4.80	6±0.54

1B: Ethyl acetate extract of *C. myrtifolia* from Bab Berred, 2B: Ethyl acetate extract of *C. myrtifolia* from Oued Al Kouba, 3B: Methanol extract obtained by Soxhlet (from Bab Berred), 4B: Dichloromethane extract (from Bab Berred).

### Flavonoids and polyphenols content determination

Results of Flavonoids and polyphenols contents in the four extracts selected according to their antibacterial activity are shown in table 4. The highest polyphenols and flavonoids concentrations were found in the ethyl acetate extract from Oued Al Kouba's harvested plants (about 609±5 µg/ml and 105±0.6 µg/ml respectively), followed by those of the methanolic extract obtained by Soxhlet from Bab Berred (490±4.8 and 34±0.54 µg/ml respectively), while the lowest concentrations were observed for the dichloromethane extract from Bab Berred (475±4.8 and 6±0.54 µg/ml respectively).

### DISCUSSION

The ethyl acetate (EA) extracts of *C. myrtifolia* induced the greatest inhibition zones diameters, followed by methanol extracts obtained by Soxhlet method. While the inhibition zone diameters obtained with the Dichloromethane extracts were very low for *C. myrtifolia* collected from Bab Berred and very close to the controls for those from Oued El kouba. Moreover, we noted that the antibacterial activity of *C. myrtifolia*'s extracts from Bab Berred was higher than that of Oued Al Kouba's extracts, tested in the same operating conditions.

The antibacterial activity of different *C. myrtifolia*'s extracts found against several gram positive and gram negative strains confirm that the studied plant have an important activity and can be used as an inexhaustible source of new natural antibacterial agents, which is concordant with our previous work on its aqueous extracts [11].

*Staphylococcus aureus* and *Enterococcus faecalis* were highly sensitive to the EA extracts and slightly sensitive to the methanolic extract of *Coriaria myrtifolia* from both stations. *Mycobacterium aurum* and *M. smegmatis* were the most sensitive tested strains to all the studied extracts (most inhibited), with a strong activity observed for the ethyl acetate extract ( $p < 0.05$ ). A previous study conducted by Boudkhili et al. (2012) [12] also showed that *Coriaria myrtifolia* has an important antibacterial effect against *Staphylococcus sp.* and *Salmonella sp.* However, the *Coriaria*'s antimycobacterial activity and its activity against the remaining tested bacterial strains have never been tested nor demonstrated.

Furthermore, another preliminary study, measuring the antimycobacterial effect of species belonging to different families such as *Asparagus stipularis*, *Cyperus longus*, *Scirpoides holoschoenus*, *Charybdis maritima*, *Dipsacus fullonum*, *Teucrium fruticans*, indicated that these plant's extracts at a concentration of 160 mg/ml were inactive against *Mycobacterium aurum* and *M. smegmatis* [7]. While the same dose of *Coriaria myrtifolia*'s ethyl acetate extract of Bab Berred was found in our study to be bactericidal against these

strains and showed the lowest MIC value for *M. smegmatis*. These results show a promising antimycobacterial effect of the tested extract which should be more studied and valorized to determine the bioactive phytochemicals involved in this activity.

The IZ  $\phi$  values obtained for *Staphylococcus aureus* and *Enterococcus faecalis* showed their important sensibility to *Coriaria myrtifolia*'s extracts, especially the ethyl acetate one, which was confirmed by the low MIC values (20 mg/ml). This sensitivity is concomitant with the antibacterial effect of the methanol extract of *Rhus coriaria* against these two strains [13].

Based on the comparison between the different extracts action's results, we can note that the ethyl acetate extract and methanol extract obtained by Soxhlet method were the most active, while the dichloromethane extracts have the lowest inhibitory activity in plants collected from both stations. Indeed, the EA extracts of the two samples have a wide action spectrum both on gram-positive and gram-negative bacteria. This result is concordant with those of a previous study investigated by Bolou et al. (2011) [14] on the EA extracts of *Terminalia glaucescens*.

Antimicrobial activity differences observed against tested germs between these extracts could be explained by the nature and/or the concentration of molecules contained in each one of them. This confirms the existence of differences in solubilization capacity of solvents used in the extraction of active phytochemicals that have been reported in a previous study [14].

The significant difference in antibacterial activity, ( $p < 0.05$ ), between extracts of the tested plants collected from two stations can be explained by the influence of environmental conditions on the plant's metabolism according to Aganga and Mosase's findings [15].

Comparison of MIC results of Ethyl acetate *C. myrtifolia*'s extract against all tested strains showed that *Pseudomonas aeruginosa* was the most resistant one, while *Mycobacterium smegmatis* was the most sensitive.

The polyphenol's and flavonoid's contents determination showed that their concentrations in the ethyl acetate's extract from Bab Berred were lower than those from Oued Al Kouba. This content's difference between the two samples can be explained by the environmental condition's influence on the plant metabolism [15, 2].

The difference in flavonoid's and polyphenol's contents obtained between the three tested extracts suggest that ethyl acetate and methanol can solubilize the maximum of chemical compounds in comparison with dichloromethane which showed the lowest concentrations of the two compounds. Indeed, previous studies have shown that the extraction of bioactive components depends not only

on the extraction's time and temperature, but mainly on the nature of extraction solvent used [16; 17]. In addition, the great antibacterial activity of ethyl acetate and methanol extracts could be related to their important content of polyphenols and flavonoids which could correspond to the main active compounds responsible of this activity.

Furthermore, we found that the ethyl acetate extracts were more active than the methanolic ones, although both were rich in polyphenols and flavonoids. This suggests the presence of a synergic effect between these phenolic compounds and other plant components, according to previous findings and studies showing that the plants antibacterial activity depends not only on the presence of phenolic compounds, but also the presence of a variety of secondary metabolites [18; 19]. Moreover, this can be confirmed by the low activity of OK plant's extracts in spite of their high polyphenol's and flavonoid's contents which suppose that they're not the only active compounds. Indeed, *C. myrtifolia* leaves are known to contain tannins which can also contribute to their antimicrobial activity.

## CONCLUSION

In this study, we evaluated the antibacterial activity of *Coriaria myrtifolia*'s extracts from two different stations in northern Morocco, against seven gram positive and negative bacterial strains implicated in several pathologies and/or implicated in food alteration process on one hand. On the other hand, we determined the contents of polyphenols and flavonoids of these *Coriaria myrtifolia*'s extracts

The tested extracts presented variable antibacterial activity against the seven pathogenic tested strains (*Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* sp., *Enterococcus faecalis*, *Mycobacterium aurum* and *Mycobacterium smegmatis*).

The ethyl acetate's extract of *C. myrtifolia*, collected from Bab Berred, was the most active and was selected for determining its Minimum Inhibitory concentration.

The polyphenols and flavonoids concentrations were different depending on the extract's nature and the plant's provenance and were important in the ethyl acetate and the methanolic extracts.

The significant antibacterial activity of the studied extracts can be explained by their active compounds.

Finally, highlighted performances of *Coriaria myrtifolia* extracts encourage the valorization of this plant, by evaluating other biological and phytochemical activities and by revealing his chemical composition to determine the active biomolecules. Moreover, it opens a perspective for screening and testing new antimycobacterial agents.

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## CONFLICT OF INTERESTS

Declared None

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