

## Evaluation of Antimicrobial Activity of Aqueous and Ethanolic Extracts of Leaves of *Vitis vinifera* Collected from Different Regions in Morocco

<sup>1,3</sup>Galal Al Askari, <sup>1</sup>Azzeddine Kahouadji, <sup>3</sup>Khadija Khedid, <sup>2</sup>Mahjouba Mousaddak,  
<sup>4</sup>Laila Ouaffak, <sup>3</sup>Réda Charof and <sup>3</sup>Zakaria Mennane

<sup>1</sup>Laboratory of Botany, Mycology and Environment, FSR, Rabat, Morocco

<sup>2</sup>Laboratory of Natural Substances and Extraction Organic, FSR, Rabat, Morocco

<sup>3</sup>Laboratory of Bacteriology Medical, INH, Rabat, Morocco

<sup>4</sup>Laboratory of Mycology Medical, INH, Rabat, Morocco

**Abstract:** Aqueous and ethanolic extracts of leaves of *Vitis vinifera* collected from different regions in Morocco (Fez, Meknes, El Jadida, Skhirat and Marrakech), were evaluated for antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and yeasts. The *in vitro* antimicrobial activity was performed by agar well diffusion method and the minimum inhibitory concentration (MIC) was determined by microtitration technique. The results indicated that, the aqueous extract of *Vitis vinifera* inhibited most of Gram-positive bacteria, as well as two Gram-negative bacteria, while the ethanolic extracts of *Vitis vinifera* leaves generally showed high inhibitory activity against different bacteria, it inhibited Gram-positive bacteria and some Gram-negative bacteria and they showed poor inhibitory activity against *Salmonella* spp and MICs values were between 25-100 µg/ml. Fez extract showed the best inhibition for the tested bacteria. There were no inhibitory effects of the aqueous extracts against all tested yeasts strains. All ethanolic extracts showed antifungal activity against one strain (*Candida famata*) and the diameter of inhibition zone ranged from 9.3 to 14.3 mm, with MIC values for 50 µg/ml. These results indicate some benefits of this plant as antimicrobial, especially against Gram positive bacteria.

**Key words:** *Vitis vinifera* • Leaves • Antimicrobial activity • Morocco

### INTRODUCTION

The history of aromatic and medicinal plants is associated with the evolution of civilization. In all regions of the world, the history shows that these plants have always employed an important place in medicine [1, 2]. Used traditional of medicinal plants produce a variety of compounds of known therapeutic properties [3-5]. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs [6-8].

In Morocco, as well as others countries, the use of plants for medicinal purpose has been a common practices, local population use plants to treat various diseases such diabetes, hypertension, hemostasis, fever and diarrhea [9-11].

Grapes are one of these plants utilized in the traditional medicine in Morocco. Ethno botanical information on this plant is indicated in Table (1) showing some of medicinal uses of the leaves of grapes in different regions in Morocco.

The aim of this study was, investigation the antimicrobial activity (AA) of aqueous and ethanolic extracts of leaves of *Vitis vinifera* collected from different regions in Morocco against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and yeasts.

### MATERIALS AND METHODS

**Plants Materials:** Azzeddine Kahouadji, Professor at the Faculty of Sciences of Rabat and laboratory of Botany, Mycology and Environment. The leaves were shade dried at room temperature for fifteen days.

Table 1: Some of medicinal uses of grapes leave according to ethno botanical studies in different regions in morocco

Authour	Preparation used	Medicinal uses	Region of study
Mohammed [12]	Decoction	Stomachic	Eastern Morocco
Bellakhdar [10]	Cataplasme	Hemostatic	-
Hmamouchi [11]	Decoction	Headache, migraine, hematoma, constipation, gout, as a diuretic, diarrhea.	-
Eddouks <i>et al.</i> [13]	Decoction	Hypertension, cardiac disease	Tafilalet
A. Tahraoui <i>et al.</i> [14]	Decoction	Diabetes, Hypertension	Errachidia
Soufda [15]	Poultice, Decoction	Typhoid, antipyretic. considered spew	Rabat
Hafssa [16].	Decoction, Poultice	Stomach pain, fever	Zaer

### Preparation of Plant Extracts:

**Aqueous Extract (AE):** The powdered of *Vitis vinifera* (50g) were extracted with boiling water (250 ml) for 30 min. After this step, the decoction was filtered and then freeze-dried (aqueous extract) [17].

**Organic Extracts:** Ethanolic extract (EE) was obtained by Soxhlet extraction of 100 g of aerial parts for 24 h in about 700 ml of solvent used. This extract, was concentrated to dryness and the residue was kept at 4°C [17].

**Microorganisms Used:** The test organisms used included: 16 bacteria strains (*Streptococcus* spp, *Streptococcus sanguis*, *Staphylococcus epidermidis*, Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Pseudomonas fluorescence*, *Salmonella* spp1, *Salmonella* spp2, *Salmonella Arizona*, *Proteus mirabilis*, *Hafnia alveie* resistant to  $\beta$ -lactams, *Yersinia* spp, *Escherichia coli* and *Klebsiella pneumoniae* ) and 8 yeast strains: *Candida tropicalis* (2), *Candida famata* (2), *Candida parapsilosis* (2) and *Candida glabrata* (2). These strains were collected from the National Institute of Health (NIH) Rabat - Morocco.

**Culture Media and Antimicrobial Assay:** Muller Hinton agar (MH) and Sabouraud Dextrose agar (SD) (Hi-Media, Bombay, India) were used for bacteria and yeasts growth, respectively. Microbial cultures, freshly grown at 37°C/24h for bacteria and 30°C /48h for yeast were appropriately diluted in sterile normal saline solution to obtain the cell suspension at  $10^5$  CFU/ml.

To evaluate AA, an agar well diffusion method was used as described by Nongpanga *et al.* [18], the organisms were spread on MH: SD agar plates by cotton swab. Wells of 6 mm diameter were punched into the agar medium and filled with 50  $\mu$ ml of plants extracts. The plates were incubated for 24 h at 37°C for bacteria and 48h at 30°C for yeast. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

**Minimum Inhibitory Concentration (MIC):** The determination of MIC of the plants extracts against microbial strains was performed according to the microtitration technique described by Eloff [19].

## RESULTS

The results of antimicrobial activity of the aqueous and ethanolic extracts of *Vitis vinifera* Leaves are indicated in Table 2. The results have showed, that the aqueous extracts of *Vitis vinifera* Leaves from different region has a good activity against Gram positive more than Gram negative bacteria. Fez aqueous extract was the most active, it was active against 7 strains from 16 strains tested and it was found to have maximum zone of inhibition against *Streptococcus sanguis* (16 mm) while the minimum zone of inhibition was against *Yersinia* spp (9 mm).

The ethanolic extracts studied showed inhibition of growth of most of the tested microorganisms with various degrees. The results showed that the extract of samples collected from Fez exhibited high antimicrobial activities against (15/16) strains tested and the inhibition diameter of this extract was between 8 – 23 mm, with average 15.5mm, also this extract was the only effective against *Salmonella* spp1, *Hafnia alveie* and *Klebsiella pneumoniae*. Also there was no activity of all the extract tested against *Salmonella* spp2.

Minimum inhibitory concentration results of *Vitis vinifera* leaves extracts are presented in Table 3, the aqueous extracts showed MIC between 50-100  $\mu$ g/ml. Most ethanolic extracts presented similar MIC against tested bacterial strains (50 $\mu$ g/ml), except Fes extract exhibited higher degree of anti-microbial activity (25 $\mu$ g/ml) against *Staphylococcus* as compared with the other extracts.

Antifungal activity of leaves extracts of *Vitis vinifera* are indicated in Table 4, the results showed no inhibitory effects of all aqueous extracts against any strain of yeasts tested. All ethanolic extracts showed antifungal activity against one strain (*Candida famata*1) and the diameter of the inhibition zones ranged from 9.3 to 14.3 mm, with MIC values of 50  $\mu$ g/ml.

Table 2: Screening of antibacterial activity of *Vitis vinifera* leaves collected from different regions in morocco

Diameters of inhibition in mm											
Bacterial strains tested	Fez extract		Meknes extract		Al Jadida extract		Skhirat extract		Marrakech extract		Tetracycline 30µg/ml
	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	
SR	10	9.6	8	10.3	8.6	12	8	14	10	13	16
SS	16	17	0	13	12	14	10	15	11	15	18
SE	14	23	10	17	11	16	10	15	8	12	14
MSA	10	18	8	11	0	14	0	16	0	15	12
SA	14	14	8	13	12	13	11	14	0	12	13
PA	0	10	0	9	0	11	0	10	0	9	19
AB	0	15	0	12	0	12	0	13	0	11	16
PF	0	10	0	10	0	9	0	9	0	10	18
SL1	0	9.3	0	0	0	0	0	0	0	0	16
SL2	0	0	0	0	0	0	0	0	0	0	18
SLA	0	8	0	10	0	11	0	9	0	10	20
PM	0	12	0	12	0	11	0	13	0	11	16
HA	0	8	0	0	0	0	0	0	0	0	14
Y	9	14	0	10.7	11	10	0	13	0	14	13
EC	10	18	0	17	9.6	14	10	15	0	14	11
KP	0	8	0	0	0	0	0	0	0	0	17

SR: *Streptococcus* sp, SS: *Streptococcus sanguinis*, SE: *Staphylococcus epidermis*, MSA, multiresistant *Staphylococcus aureus*, SA: *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, AB: *Acinetobacter baumannii*, PF: *Pseudomonas fluorescens*, SL1: *Salmonella* sp1, SL2: *Salmonella* sp2, SLA: *Salmonella arizonas*, PM: *Proteus mirabilis*, HA: *Hafnia alveie*, Y: *Yersinia* spp, EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, AE: aqueous extracts, EE: ethanolic extracts, 0: no activity

Table 3: Minimum inhibitory concentration of *Vitis vinifera* leaves extracts collected from different regions in morocco

Bacterial strains tested	Fez extract		Meknes extract		Al Jadida extract		Skhirat extract		Marrakech extract	
	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE
SR	+	++	+	++	++	+	+	++	+	++
SS	++	+	-	++	++	++	+	++	+	++
SE	++	+++	+	++	+	++	+	++	+	++
MSA	+	+++	+	-	-	++	-	++	-	++
SA	++	+++	+	++	++	++	+	++	-	++
PA	-	+	-	++	-	++	-	++	-	++
AB	-	+	-	++	-	++	-	++	-	++
PF	-	++	-	++	-	++	-	++	-	++
SL1	-	++	-	-	-	-	-	-	-	-
SL2	-	-	-	-	-	-	-	-	-	-
SLA	-	++	-	++	-	++	-	++	-	++
PM	-	+	-	-	++	-	-	++	-	++
HA	-	++	-	-	-	-	-	-	-	-
Y	+	+	-	++	+	++	-	++	-	++
EC	+	+	-	-	+	-	+	++	-	++
KP	-	+	-	-	-	-	-	-	-	-

MIC: Minimum Inhibitory Concentration, +:100 µg/ml, ++: 50 µg/ml, +++: 25µg/ml, -: no activity.

Table 4: Antifungal activity of *Vitis vinifera* leaves extracts collected from different regions in morocco

Diameters of inhibition in mm											
Yeasts strains tested.	Fes extract		Meknes extract		Al Jadida extract		Skhirat extract		Marrakech extract		Streptomycin 12µg/ml
	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	
CT1	0	0	0	0	0	0	0	0	0	0	18
CT2	0	0	0	0	0	0	0	0	0	0	22
CF1	0	10	0	9.3	0	14.3	0	12	0	10	14
CF2	0	0	0	0	0	0	0	0	0	0	17
CP1	0	0	0	0	0	0	0	0	0	0	16
CP2	0	0	0	0	0	0	0	0	0	0	10
CG1	0	0	0	0	0	0	0	0	0	0	12
CG2	0	0	0	0	0	0	0	0	0	0	15

CT1: *Candida tropicalis*, CT2: *Candida tropicalis*, CF1: *Candida famata*, CF2: *Candida famata*, CP1: *Candida parapsilosis*, CP2: *Candida parapsilosis*, CG1: *Candida glabrata*, CG2: *Candida glabrata*, 0: no activity.

## DISCUSSION

The aqueous extract of *Vitis vinifera* in this study inhibited the most of Gram-positive bacteria, as well as two Gram-negative bacteria *Yersinia* and *Escherichia coli*. Similar results of antibacterial activity of aqueous extracts of this plant were found by Jigna and Sumitra [20]. The Gram-negative bacteria seemed to be more resistant to the aqueous extract. The activity of the aqueous extract of the plants against Gram-negative bacteria was not remarkable; many workers [21- 24] have generally reported that water extracts of plants do not have much activity against bacteria, especially Gram negative bacteria.

The ethanolic extracts of *Vitis vinifera* leaves generally showed high inhibitory activity against different bacteria, it inhibited Gram-positive bacteria and some Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Proteus mirabilis*. However, poor antimicrobial activity has been found against *Salmonella* spp [25]. Identical results of *Vitis vinifera* leaves extracts were found by Jigna and sumitra [20] and Didem *et al.* [26] who reported that the organic extracts of *Vitis vinifera* leaves were active against Gram positive than Gram negative bacteria. Conflicting results were found by Oskay and Sar [27], who reported that the ethanol extract of *Vitis vinifera* leaves showed antimicrobial activity against Gram-positive and Gram-negative bacteria.

In general, these plant extracts inhibited the Gram-positive bacteria better than the Gram-negative ones; this is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than Gram negative bacteria [24, 28]. Higher resistance of Gram-negative bacteria against plant extracts is credited to the presence of lipopolysaccharides in their outer

membranes [29, 30]. Also these observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics [31].

Grape leaves are rich in phenolic compounds such as myricetin, ellagic acid, kaempferol, quercetin, gallic acid, all of these compound have antimicrobial activity [32, 33], especially against Gram-positive bacteria [34]. Our results supported, to a certain degree, the traditional medicinal use of the plants appraised for human disease therapy [35], especially diseases caused by Gram positive bacteria, such as *S. aureus* which is involved in several humans' disease [36], also this antimicrobial properties could be used to increase the shelf-life of food [37].

Antifungal activity results showed no activity of any aqueous extract against yeasts strains tested, however, the ethanolic extracts were active against one strain. Didem *et al.* [26] reported that, there is no significant activity of extracts of leaves of grapes against fungi (*C. albicans* and *C. parapsilosis*). Many researches reports have stated that several plant extracts did not exhibit *in vitro* antifungal activity [38-42]. Further the absence of active compounds and many reasons could explain the lack of antifungal activity, containing the parts of the plants, used method of extraction and the type of solvent and possibly the time of collection [43].

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