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## Phytochemical screening and *in vitro* Antibacterial activity of an endemic plant of Morocco; *Artemisia ifranensis* J. Didier essential oil against bacterial strains *Agrobacterium vitis* and *Erwinia amylovora*

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### ABSTRACT

The essential oil of *Artemisia ifranensis* J. Didier (Asteraceae), collected in April (2012) from Timahdite (Moroccan Middle-Atlas), was obtained by hydrodistillation. Moreover, a phytochemical screening of plant leaves was carried out through precipitation and/or colored reactions. *Agrobacterium vitis* and *Erwinia amylovora*, is among the telluric bacteria most aggressive, causing damage in cultures. With the aim of searching other alternatives of the fight against these bacteria, we study in this work the antibacterial activity power of *Artemisia ifranensis* J.D. essential oil against pathogens germs isolated from INRA Meknes, Morocco. Disc-diffusion method in solid medium and macrodilution method in liquid medium were respectively used to determine inhibition diameters and the antibacterial parameters to knowing, minimal inhibitory and bactericidal concentrations (MIC and MBC). Essential oil yield was 0.58% (ml/100g) in April period. Phytochemical screening showed the presence of tannins, flavonoids, sterols/triterpenes, and mucilages. Thus, antibacterial activity reveal that *A. ifranensis* J.D. essential oil has a remarkable efficiency on both strains tested. This one develops the inhibition zones of  $17\pm 0.08\text{mm}$  and  $13\pm 0.08\text{mm}$  respectively against *A. vitis* and *E. amylovora*. The minimal inhibitory concentration (MIC) expressed by the essential oil is 1/2500v/v and 1/250v/v respectively on the both strains tested. These last have the same degree bactericidal at a higher concentration  $\text{MBC} > 1/50\text{v/v}$ . These results, although preliminary, show a good antibacterial activity, to limit and even to stop the development of the pathogen.

**Keywords:** *Artemisia ifranensis* J. Didier, Essential oil, Phytochemical Screening, Antibacterial activity.

### INTRODUCTION

Bacterial pathogens and their control are a serious problem in agricultural practice. Spraying with antibiotics and copper compound, usually suggested to control bacterial diseases, have never been satisfactory. Furthermore, antibiotics are forbidden in many countries and copper compound, because of their general toxicity, exert a negative impact on both yield and the environment. As an alternative strategy to prevent the spread of diseases, natural compounds of plants (essential oil, secondary metabolite) are being tested for their antimicrobial activity. Naturally occurring biologically active plant product can be a source of new pesticides or serve as templates for new, more effective compound. It has been known since ancient times that species and their essential oils (EO's) have varying degrees of antimicrobial activity [1] [2]. Like other plant species, those of the genus *Artemisia*, known for their interesting biological activities and the diversity of chemotypes of their essential oils, are among the most studied plants [3].

Among the known species of *Artemisia*, an endemic of Morocco, it is *Artemisia ifranensis* Didier J. [4] [5]. Commonly called "Wormwood of Ifrane" and called "Ifsi, fessi or shih". This species which is for the moment special in Morocco, is confined in the Middle Atlas (region of Ifrane, plain Selrhert, daya Chiker) and in the Eastern High Atlas (Atlas of Bni Mellel, Jbel Fernissou) between 1600m and 2100m of altitude, in the stages bioclimatic sub-humid and humid continental at cold winter, on muddy soils (argillaceous) that is the colluvium, the pozzines, the rendzinas and assylvatic milieu [6].

The *Artemisia* genus was employed in traditional medicine by many cultures since the ancient periods. The genus was used as a natural pesticide and also in the treatment of few human diseases [7]. They are prescribed as analgesic agents, antibacterial, anti-parasite and hemostats, anthelmintic, anti-diarrheal and diuretic whereas several extracts and EOs showed a certain performace of biological activities such as antihyperglycemic, antioxidant and anti-inflammatory. Moreover, some species of the genus are frequently used like antirabic and for the treatment of certain diseases such as malaria, hepatitis, cancer, and infections by fungi, bacteria, and viruses [8] [9] [10]. Historically, wormwood was a productive kind in the search for new biologically active compounds. The Phytochemical investigations showed that this genre is rich in secondary metabolites such as essential oils (sesquiterpenes and monoterpenes), flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes [11].

Furthermore, the determination of potential antimicrobial activity of *Artemisia ifranensis* EO could be more informative for the future use in controlling phytopathogens and also in clinical treatment as natural antimicrobial agents. The organisms like *Erwinia amylovora* and *Agrobacterium vitis* were reported to be severe phytopathogens, causing damage in culture. In fact, these phytopathogens cause diseases in any plant tissue it invades, mainly by wilts and rots (*Erwinia amylovora*), and a tumor known as crown gall disease (*Agrobacterium vitis*) [12].

In the present study, a phytochemical screening and the antimicrobial potency of *A. ifranensis* EO were investigated. This work is also a test for the use of this species as a bioconservation product. In our knowledge, this wormwood never was the object of an antimicrobial study apart from some rare works on phytochemistry.

## MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1.Plant material

Wild samples of *A. ifranensis* J.D. were manually harvested in Timahdite region in the April 2012 period. The biomass (leaves) were dried in the shade for 10 days before extraction process. The botanical identification of the species was performed at the scientific institute of Rabat.

#### 2.1.2.Microbial strains

Microbial material consists of vegetal bacterial strains (gram negative) isolated from the Laboratory of Bacteriology and Plant biocontrol of the National Institute of Agronomic Research (INRA) Meknes, Morocco: *Erwinia amylovora* (strain 1446-4) and *Agrobacterium vitis* (strain 1685-5). The bacterial strains were maintained in yeast peptone glucose agar medium (YPGA) for 24h at 30 °C before each series of tests.

### 2.2. Methods

#### 2.2.1.Phytochemical screening

The phytochemical study was conducted from aqueous and organic extracts of *A. ifranensis* J.D leaves.

Dry drug was milled in an electric grinder in order to obtain a thin green powder. Selective extractions were made specifically for each family of studying compounds. The extracts have been obtained by extraction with solvents (petroleum ether, methanol, ethanol, chloroform and distilled water).

Various phytochemical tests were performed using the methodology described by Harborne [13]. These qualitative tests were based on colored and/or precipitation reactions.

#### 2.2.2.Antibacterial tests

##### *Disc- diffusion method on solid medium*

The extracted EO was tested against bacterial strains through the disc-diffusion method [14] [15]. Inocula of 10<sup>8</sup> CFU/ml were prepared with isotonic sterile water for 24 hours-bacterial pure culture. The inoculate was spread on Petri dishes (90mm) containing yeast peptone glucose agar medium (YPGA). Petri dishes were allowed to dry. Then sterile discs (6mm) of watchman paper filter loaded with 2µl of the EO were placed in the centre of the plate. The tests were done in triplicate. Inhibition diameters were reported after 18 to 24 hours of incubation at 37°C. Antramycine (10µg) was used as positive controls.

*Macrodilution method in liquid medium*

The aim is to determine the minimal inhibitory and bactericide concentrations MIC and MBC [16]. Tests were performed in tubes containing 4ml of yeast peptone glucose (YPG). Fresh bacterial inocula of  $10^7$  UFC/ml were first prepared in YPG. Only extracts with significant inhibition diameters against bacterial strain ( $D \geq 8$ mm) were selected. Extracts were emulsified in Dimethylsulfoxid (20% DMSO) solution. In 4ml- YPG test tubes, 40 $\mu$ l of initial inoculum ( $10^7$ UFC/ml) were added. Emulsified extracts were then added to obtain a spectrum ranged from C1 to C8 corresponding to 1/5000, 1/2500, 1/1000, 1/500, 1/250, 1/200, 1/100, 1/50v/v final concentrations of extract and  $10^5$  UFC/ml final bacterial concentration. Tests were performed in triplicate. Tubes without extract were used as negative controls and test tubes containing Antramycine (10 $\mu$ g/ml) were considered as positive controls. MIC was determined after 18-24 hours of incubation at 37°C. MBC is determined after plating 100 $\mu$ l of all tubes without any visible bacterial growth on YPGA medium. Petri dishes were incubated at 37°C for 24 hours.

**RESULTS AND DISCUSSION****3.1. phytochemical screening**

The results of the phytochemical screening showed that *A. ifranensis* leaves are rich in catechic tannins, flavonoids, sterol, triterpens, and mucilage (Table 1). Similar results were obtained as in the aerial parts of Libyan *A. herba alba* [17]. In Algeria, the *A. herba alba* species are devoid of tannins [18]. Phytochemical tests, performed in India on the *A. absinthium* and *A. Vulgaris*, revealed the presence of the saponins accompanied to the chemical groups cited in our results [19] [20] [21]. However, the leaves of *A. annua* from Nigeria are rich in alkaloids and flavonoids, but they are devoid of the tannins and saponins [22]. Withal, the *A. annua* and *A. parviflora* from India and Turkey are characterized by the presence of the majority of chemical groups [23] [24] [25]. The aerial parts of *A. macrocephala* of Pakistan are also rich in alkaloids, flavonoids and saponins; negative tests proved the absence of tannins [26]. However, phytochemical screening works of *A. Nilajirika* collected from two different stations of India are distinguished by the presence or absence of alkaloids [27] [28].

In contrast, tests for coumarins research of *A. ifranensis* gave negative results. They agree with the work of Naili et al. (2010) [29], already made onto Libyan *A. campestris*.

*A. ifranensis* therefore appears to be rich in secondary metabolites. These results prove the therapeutic properties of the wormwood.

**Table 1. Phytochemical screening reactions of *A. ifranensis***

Chemical groups	Reagents or Reaction name	Results	
Tanins	total	FeCl <sub>3</sub>	++ (Dark green color)
	catechic	Stiasny reagent	+ (red precipitate)
	gallic	Reaction with sodium acetate	-
Flavonoïdes	flavonoles	Cyanidin reaction	-
	flavones	Cyanidin reaction with Mg shavings	+ (Pink-orange color)
Alkaloids	Valser - Mayer Reagent		-
	Dragendorff Reagent		-
Sterols and triterpenes	Anhydre acétique + chloroforme + H <sub>2</sub> SO <sub>4</sub> Liebermann Buchard Reaction		+ (Red ring and brownish violet color of the supernatant layer)
Coumarines	fluorescence Reaction		-
Mucilage	précipitation Reaction		+ (Flake)

Highly positive reaction: ++; positive Reaction : +  
Moderately positive reaction: + / -; Negative Reaction: -

**3.2. Antibacterial activity***Aromatogram performed by disc-diffusion method*

The results of susceptibility testing are shown in table 2. The antibacterial activity of the yellow essential oil of *A. ifranensis* (0.58% yield) against germs used was qualitatively and quantitatively evaluated by the presence or absence of zones of inhibition. For this method, an extract is considered active when it induces an inhibition zone greater or equal to 9mm [30]. Thus, the analysis of the results shows that EO of *A. ifranensis* exerts an antibacterial effect which is manifested by more pronounced inhibition zone against *A. vitis* (17 $\pm$ 0.08mm). However, the activity of the *A. ifranensis* EO toward *Erwinia amylovora* microbial strain showed also a remarkable efficiency (13 $\pm$ 0.08mm). These strains are susceptible to the extract. This effect is low compared to that of Antramycine used as antibiotic reference. Different results were obtained by Kordali et al. (2005), antibacterial tests of the EO's of *Artemisia* Turkish species (*A. absinthium*, *A. dracunculus*, *A. santonicum* and *A. spicigera*) against tested germs, are less important (8 $\leq$ D $\leq$ 10mm) [31]. In fact, *Agrobacterium vitis* was inhibited by EO of Clove and Lemon balm, and exhibited zones of 13 and 10mm receptively [32]. In other work on *Artemisia absinthium* EO, from USA, exert a significant inhibitory effect against *Erwinia amylovora* 12.5 $\pm$ 1.5mm [33]. Thus, *A. absinthium* EO's from Germany

and *Artemisia afra* EO's from South Africa were shown a medium inhibitory efficiency toward *Erwinia chrysanthemi* (10.1mm; 11.0mm respectively) [34] [35]. In other works, The diameter of inhibition zones of the shoot and root tissues of *Astragalus sinicus*, *Brassica napus*, *Dactylis glomerata*, *Lolium perenne*, *Lolium multiflorum* and *Vicia villosa* extracts in water and ethyl acetate solvents against *A. vitis* was found to be in the range of 9.8±1.3 to 18.0±2.0mm [36]. EOs from *Origanum Compactum* and *Saturja montana* were more effective against *E. amylovora* (25.17 ± 5.10 and 25 mm respectively) [37] [38].

When we compared the results previously with those reported in this study, it was found that the antimicrobial activity is strongly influenced by the chemical composition of the essential oil.

**Table 2. Inhibition diameters of *A. ifranensis* EO on bacterial strains presented as Means (mm) ± standard deviation**

	<i>Erwinia amylovora</i> (mm)	<i>Agrobacterium vitis</i> (mm)
Essential Oil of <i>A. ifranensis</i>	13±0.08	17±0.08
Positive Control (Antramycine)T (+)	32±0.81	25±1

*Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tested samples*

MIC and MBC of *A. ifranensis* EO have been determined only for those that have previously exhibited significant antibacterial effects. From aromatogram results, the both strains *E. amylovora* and *A. Vitis* were all susceptible to the EO of wormwood.

In fact, our results show that *A. ifranensis* EO is endowed with inhibitory activity against *A. Vitis* and *E. amylovora* at a minimum concentration of 1/2500v/v and 1/250v/v respectively. Nevertheless, the bactericidal effect (CMB) of *A. ifranensis* EO against bacterial strain tested can be at a higher concentration of >1/50v/v (Table 3 and 4). In literature, the EO of *Chenopodium ambrosoid* was reported as strong antibacterial agents against phytopathogenic bacteria *Erwinia herbicola* (MIC= 0.25µl.ml<sup>-1</sup>; MBC= 0.2µl.ml<sup>-1</sup>) [39]. In fact, the essential oil of *Satureja adamovicii* and *satureja hotensis* shown the highest inhibitory and bactericidal activity against *E. amylovora* (MIC=MBC=0.09µl.ml<sup>-1</sup> and MIC/MBC=0.025/0.05µl.ml<sup>-1</sup> respectively) [38] [40]. However, Badawy *et al.* (2014) [41] tested the EO MIC of Egyptian *Artemisia judiaca* and *Artemisia monosperma*, they reported that the extract exhibited MIC towards the target bacteria (*Erwinia carotovora* and *Agrobacterium tumefaciens*) in the range of 525 to 675mg.l<sup>-1</sup>. In other works, the MIC tests of *Artemisia nilagirica* organic chloroform extract showed maximum activity with MIC=32 µg.ml<sup>-1</sup> against *Erwinia* sp. [42]. Thus, The minimum inhibitory concentration (MIC) values of shoot and root extracts of *Astragalus sinicus*, *Brassica napus*, *Dactylis glomerata*, *Lolium perenne*, *Lolium multiflorum* and *Vicia villosa* extracts in water and ethyl acetate solvents against *A. vitis* were in the range of 3.12 to 12.5mg.ml<sup>-1</sup> [36].

**Table 3. Susceptibility of tested germs with *A. ifranensis* EO and MIC determination**

Tests	Concentrations (v/v)							
	1/5000	1/2500	1/1000	1/500	1/250	1/200	1/100	1/50
Negative Control T(-)	+	+	+	+	+	+	+	+
Positive Control T(+)(Antramycine, 10ug/ml)	-	-	-	-	-	-	-	-
EO/ <i>E. amylovora</i>	+	+	+	+	-	-	-	-
EO/ <i>A. vitis</i>	+	-	-	-	-	-	-	-

(-) antibacterial action.

(+) no antibacterial action.

**Table 4. Antibacterial parameters of *A. ifranensis* (MIC and MBC)**

	<i>E. amylovora</i>		<i>A. vitis</i>	
	MIC (v/v)	MBC (v/v)	MIC (v/v)	MBC (v/v)
Essential Oil	1/250	>1/50	1/2500	>1/50

## CONCLUSION

In this work, we have contributed to a phytochemical screening and evaluation of antibacterial activity of *Artemisia ifranensis* from Timahdite, endemic to Morocco. The phytochemical screening has identified different secondary metabolism (tannins, flavonoids, sterols and triterpenes and mucilage). The results of antibacterial tests have shown that the essential oil tested *in vitro* has showed significant inhibitory effects against the tested phytopathogen. Thus, this method appears to be a good alternative for the extraction of EO from middle Morocco for their applications in the food, pharmaceutical, and cosmetic industries.

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