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# ANTIFUNGAL AND ANTIFEEDANT ACTIVITY OF *MARRUBIUM VULGARE*

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**ABSTRACT**

*Marrubium vulgare* L. (Lamiaceae) is a largely used medicinal plant. Acetone extract of the aerial parts of this plant was obtained using a Soxhlet apparatus. Total phenolic content was estimated to be 14mg caffeic acid equivalent /100ml extract. Antifungal activity was conducted with decoction and maceration aqueous extracts against *Aspergillus flavus, A. niger, A. fumigatus, Fusarium solani, Penicillium expansum, Ulocladium sp., Candida albicans*, *Pyteromyces orbicular*, *Microsporum canis* and *Trychophyton rubrum*. High effects were observed with the maceration extract against *A. fumigatus* and *F. solani*. Therefore, antifeedant assay was assessed on three herbivorous insects *Spodoptora littoralis, Myzus persicae* and *Ropalosiphum padi* third instar larvae using the acetone extract. A potent antifeedant effect was exhibited against *S. littoralis* whereas the percent settling inhibition of insects (*M. persicae* and *R. padi*) was moderate.

Keywords: *Marrubium vulgare*, antifungal, antifeedant, extracts, insects.

**RESUME**

**ACTIVITE ANTIFONGIQUE ET ANTIAPPETANTE DE *MARRUBIUM VULGARE***

*Marrubium vulgare* L. (Lamiaceae) est une plante médicinale de *large* utilisation. L'extrait acétonique des parties aériennes de cette plante est obtenu au Soxhlet; la teneur en polyphénols totaux est estimé à 14mg équivalents d'acide caféique /100ml d'extrait. L'activité antifongique a été conduite avec les extraits aqueux obtenus par décoction et macération contre *Aspergillus flavus, A. niger, A. fumigatus, Fusarium solani, Penicillium expansum, Ulocladium sp., Candida albicans*, *Pyteromyces orbicular*, *Microsporum canis* et *Trychophyton rubrum*. Les effets les plus forts ont été observés avec l'extrait de macération contre *A. fumigatus* et *F. solani*. Le test d'antiappétance a été conduit avec l'extrait acétonique sur trois insectes herbivores *Spodoptora littoralis*, *Myzus persicae* et *Ropalosiphum padi.* Un effet antiappétant potentiel a été manifesté contre *S .littoralis* (95,5 %) alors que le taux d'inhibition de colonisation par les insectes (*M. persicae et R. padi*) était modéré.

Mots-clés : *Marrubium vulgare*, antifongique, antiappétant, extraits, insectes.

## INTRODUCTION

Medicinal plants are widespread over the world and most of the recorded ones are found in the Mediterranean basin. The Lamiaceae family is one of the largest one with numerous aromatic and culinary species. *Marrubium vulgare* a medicinal plant belonging to this family is largely used in folk medicine to cure several pains such digestive disorders. Many works dealt with the chemical composition and biological activities (antimicrobial, antioxidant....etc.) of the genus *Marrubium* (De Jesus *et al.,*2000; Belhattab *et al*., 2006; Erdogan- Orhan *et al*., 2010).

Vegetable crops are often exposed to microbial contamination and insect invasion in the field which cause economic and organoleptic quality losses. Even antifeedant and repellent plants are known since ancient time, limited works focused on the mechanism of defence supported by secondary metabolites. Synthetic insecticides are largely used in pest control but due to their adverse effects, natural antifeedants of plant origin have been proposed as alternatives (Belles *et al*., 1985). Diterpenoids are well known insect antifeedants isolated from plants of the family Lamiaceae. Furthermore, a few papers document the antifeedant effects of flavones (Sharaby and Ammar, 1997) and other diterpenoids, for example those found in *Plectranthus* leaves (Wellsow *et al*., 2006). Ballesta –Acosta et *al* (2008) showed that diterpens and coumarins exhibited significant antifeedant effects against *S. littoralis* whereas all flavones tested were inactive

A variety of insect models such as the army worm, *Spodoptera littoralis*, an important pest of cotton, tobacco and vegetable crops (tomato, pepper, lettuce, broccoli, cauliflower and artichoke) are used to evaluate the antifeedant activity of secondary metabolites. The larvae cause economically important damage since they feed on the leaves, fruits and buds from summer till the end of autumn in the field (or even year round when warm temperatures prevail during winter and spring) and year round in greenhouses (Ballesta –Acosta *et al*., 2008) 2008).

As a part of ongoing study of Lamiaceae species from North Africa (Algeria), here we report on the polyphenol content, antifungal and antifeedant activities of *Marrubium vulgare* since to the best of our knowledge no data is available on antifeedant properties of the genus *Marrubium*, more over this plant is known for its bitter taste due to secondary metabolite, marrubiin (and premarrubiin), a labdane diterpene.

## MATERIAL AND METHODS

### Material

#### Plant material

*Marrubium vulgare* aerial parts were collected in Setif region (Algeria) and stored in the shade till use. A voucher specimen is deposited at the Department Laboratory.

#### Insects

*Spodoptera littoralis* Boisd. (Lep: Noctuidae), *Myzus persicae*, and *Ropalosiphum padi* were reared on artificial potato foliage and bell pepper (*Capsicum annum*) plants, respectively, and maintained at 24±1 °C, 60-70% relative humidity, with a 16 :8h (l: d) photoperiod in a growth chamber.

#### Fungi

Fungi were obtain from the Laboratory of Applied Microbiology (Setif-1 University, Setif, Algeria) and from Laboratory of Parasitology and Microbiology (''Centre Hospitalo-Universitaire'' Setif, Algeria).

The antimicrobial activity was determined by agar diffusion assay against eight fungi (*Aspergillus niger, A. flavus, A. fumigatus, Penicillium expansum, Fusarium solani, Candida albicans and Pyteromyces orbicular*) and in tubes against *Microsporum canis* and *Trychophyton. rubrum.*

### Methods

#### Preparation of extract

20 g of grinded leaves were extracted with acetone using a Soxhlet apparatus for 6 h. The volume was completed to 100 ml in a volumetric flask. Decoction and maceration aqueous extracts were performed according to Belhattab *et al.* (2004).

**Determination of phenolic content**

The content of total phenols in the plant extract was measured by the Folin- Ciocalteu assay (Singleton *et al.*, 1999). Briefly, 20 µl of each extract are combined to 1,58 ml distilled water and 100 µl of diluted Folin- Ciocalteu reagent; the mixture is stirred vigorously and let to react for 6 min then 300µl of sodium carbonate (7.5%) are added. After incubation for 2 h at ambient temperature in the shade, the absorbance of the tubes is recorded using U-2000 Hitachi (Tokyo, Japon) spectrophotometer at 760 nm.

An analog procedure was followed for the standard polyphenol, caffeic acid, dissolved in methanol-water: 60/40(v/v), to prepare the calibration curve.

The blank consists of methanol, Folin- Ciocalteu reagent and sodium carbonate. Results (means of triplicate) were expressed as caffeic acid mg/ 100g extract.

The phenol content was determined according to the following calibration curve:

$A = 0.0097\ C - 0.019$, $R2=0.9945$

Where:

A: the absorbance at 725 nm and

C: total phenol content µg / 10 ml.

**Antifungal activity**

This activity was evaluated according to Belhattab *et al*. (2004) with slight modifications. Before the test, mould strains were grown on Potato Dextrose Agar (PDA) for 6 days and *Candida albicans* was grown on Sabouraud + chloramphenicol for 48h. Briefly, a suspension of the tested microorganism was spread on the Petri dishes containing the culture media. Sterile filter paper discs (Whatman N°1, 6 mm diameter) were individually soaked with 20 and 40 µl of the extracts then placed onto the media. Distilled water and acetone were used as negative control whereas amphotericin, clotrimazol and nystatin were used as positive control. The Petri dishes were incubated at 37°C for 48 h for yeast, and at 27°C for 72 h for moulds. After incubation, the diameters of the inhibition zones were measured in mm including the diameter of discs. Each assay was run in triplicate.

**Feeding assays**

Dried acetone extract was dissolved in methanol (10mg/ml). The assays were conducted according to methods developed by Poitout and Bues (1974) and Reina *et al.* (2001).

**Test 1: Feeding inhibition**

Bell pepper leaves (*Capsicum annuum*) cut into small pieces were put into wells punched in agar medium (2%). The plant sheets previously soaked with 10 µl of the extract (test) or methanol (control) are filed onto the surface of Petri dishes (10 plates) containing agar then incubated for 24 h at 25°C in the presence of the chewing insect newly emerged *S. littoralis* L6 larvae. The experiment is stopped as soon as the 3/4 of the leaves is consumed (test or control). The remaining leave pieces are taken off the Petri dishes and bonded to a sheet of paper using scotch tape. Then, results are recorded.

Percent feeding reduction (%FR) was determined for each plate by the equation:

%FR= [1- (treatment consumption/control consumption)] x 100.

**Test 2: Settling inhibition**

This test was conducted with two aphids, *Myzus persicae* (polyphagous) and *Ropalosiphum padi.* The former insects used usually settle on the inferior side of the leaves of crops (pepper) to avoid UV light whereas the latter fed on cereals (wheat leaves). For this purpose, leaves from pepper are cut to form half circles and those from wheat to form 1cm long sheets, then soaked with *M. vulgare* acetone extract (test) or methanol (control) and put into Petri dishes containing agar. Afterwards, ten aphids (*M. persicae* and *R. padi*) are introduced into the Petri dishes (5 to 10 plates) and 24h later the number of aphids settled on each leaf is determined and a settling inhibition index (%SI) was calculated for each aphid:

%SI = 1- (%T/%C) x 100

**RESULTS**

Acetone was chosen as less polar solvent, often used to extract active phenolic constituents from plants (Exarchou *et al*., 2002). The extraction yield was 6,3% w/w. Total phenolic content of the extract was measured according to the following calibration curve (Fig.1) and found to be 14mg CAE/100ml (Tab. I)

Fig.1: Courbe d'étalonnage de l'acide caféique
Calibration curve of caffeic acid

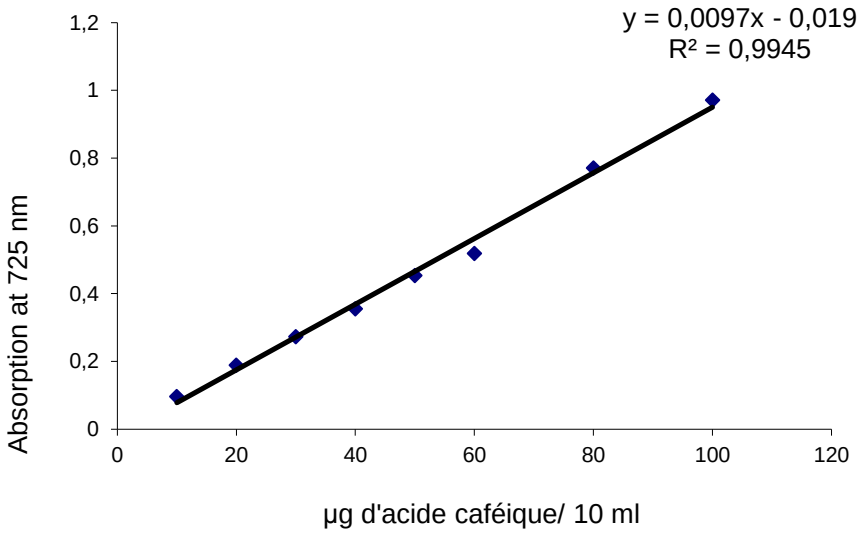


Tableau I: Teneur de l'extrait acétonique de *M. vulgare* en polyphénols totaux
Table I: Total phenolic content of *M. vulgare* acetone extract

| solid Residue (%w/v) | Total phenol content (caffeic acid mg /100ml) | Solid residue total phenol content (% w/w) |
|---|---|---|
| 0.22 | 14.0 | 6.3 |

**Antifungal activity of *M. vulgare* extracts**

Inhibition effects of *M. vulgare* extracts against several isolated fungal strains have been evaluated (Tab. II ). The most effective effects were exhibited by the decoction and maceration extracts against *A. fumigatus* (10mm and 35mm respectively) followed by *F. solani* (8mm and 25mm respectively).
Decoction extract showed no effect against dermatophytes as well as against *A. flavus*, furthermore no inhibition effect was observed with the maceration extract when tested against *P. expansum* and *Ulocladium sp.* Dermatophytes*, M. canis* and *T. rubrum*, cultivated in tubes containing a cultivation medium supplemented with 50µl of the maceration extract have shown no growth after 15 days incubation at 37°C at meanwhile control tubes were positive.

Tableau II: Zones d'inhibition (mm)* manifestées par les extraits de décoction et de macération de *M. vulgare* contre les espèces fongiques

Table II: Inhibition zones (mm)* developed by *M. vulgare* decoction and maceration extracts against fungal species.

| Microorganisms | Décoction extract (µl/disc) | | Macération extract (µl/disc) | |
|---|---|---|---|---|
| | 20 | 40 | 20 | 40 |
| *A. flavus* | - | - | 7 | 20 |
| *A. niger* | 6 | 6 | 6 | 17 |
| *A. fumigatus* | 10 | 10 | 35 | 35 |
| *F. solani* | 8 | 8 | 20 | 25 |
| *P. expansum* | 7 | 7 | 6 | 6 |
| *Ulocladium sp.* | 6 | 7 | 6 | 6 |
| *C. albicans* | 6 | 6 | 8 | 8 |
| *P. orbicular* | 6 | 6 | 7 | 7 |

* Mean value of triplicate

Standard antifungal compounds showed strong inhibition effects when tested against the same species. The most potent effect was developed against *A. flavus* with 23, 17 and 20 mm inhibition zone diameter when subjected to clotrimazol, amphotericin B and nystatin respectively. *A. niger* and *P. expansum* were resistant to clotrimazol and amphotericin B while *Ulocladium sp. w*as resistant to nystatin (Tab.III).

Tableau III: Zones d'inhibition (mm)* manifestées par les composés antifongiques standards contre les espèces fongiques

Table III: Inhibition zone (mm)* developed by standard antifungal compounds against fungal species.

| | Clotrimazol | Amphotéricin B | Nystatin |
|---|---|---|---|
| *A. flavus* | 23 | 17 | 20 |
| *A. niger* | 6 | 6 | 9 |
| *A. fumigatus* | 17 | 12 | 15,5 |
| *F. solani* | 15 | 13 | 6 |
| *P. expansum* | 6 | 6 | 12 |
| *Ulocladium sp.* | 11 | 14,5 | 6 |
| *C. albicans* | 20 | 11 | 23 |
| *P. orbicular* | ND | ND | ND |

* Mean value of triplicate, ND= not determined

**Insect bioassay**

The acetone extract (10 µl) of *M. vulgare* aerial parts inhibited the feeding activity of *S..littoralis* larvae. Percent feeding reduction (%FR) was determined for each plate (Tab. IV). According to the above equation, % FR was determined to be 95.5 %.

Tableau IV: Taux de consommation des feuilles de poivron traitées avec l'extrait acétonique de *M. vulgare* (test), le méthanol (témoin), par *S. littoralis*

Table IV: Percent of consumption of bell pepper leaves treated with *M. vulgare* acetone extract (test) and methanol (control) by *S .littoralis*

| % consumption | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 |
|---|---|---|---|---|---|---|---|---|
| Test | 00 | 25 | 00 | 00 | 00 | 00 | 00 | 6,25 |
| Control | 100 | 87.5 | 93.75 | 87.5 | 87.5 | 87.5 | 75 | 75 |
| T/C | 0 | 0.28 | 00 | 00 | 00 | 00 | 0 | 0.08 |
| % FR | 100 | 72 | 100 | 100 | 100 | 100 | 100 | 92 |

A difference between the behavior of the sucking insects, *M. persicae* and *R. padi* when subjected to the settlement inhibitory test in the presence or absence of *M. vulgare* acetone extract, was observed. The mean number of *M. persicae* larvae settled on the treated and control leaves was 0.7 and 2.1 respectively which correspond to a settling inhibition index of 33 %, whereas that of *R. padi* was 2.35 and 4.5 respectively which correspond to a settling inhibition index equal to 48 %.

**DISCUSSION**

The antifungal and antifeedant activity of *Marrubium vulgare* extracts suggest a plant defensive role played by the secondary metabolites beside other effects. The antifungal test showed stronger effects of the maceration extract comparing to decoction extract. In addition, antifeedant effect of acetone extract against *S. littoralis* was too strong. Furthermore, the main works dealing with insecticidal effects focus on diterpene alkaloids as potent insecticidal and antifeedant compounds (Reina and Gonzalez-Coloma, 2007). These findings suggest a possible role of a labdane diterpene, marrubiin. Phenolic content was also determined since several works have shown the effective role of these compounds on microorganisms. Tannins known to possess the ability to precipitate proteins can reduce the nutritional value of several tissues. They affect the insect digestion by binding to mucoproteines of their oral cavity. It has been reported that plant tannins content affect the consumption of plants by the *S. latifascia* larvae (Harborne, 1988).

**CONCLUSION**

The results obtained here reveal that *M. vulgare* is a promising alternative source of natural compounds for crop protection, however further studies are needed to assess safety and efficacy of this plant extracts.

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