# Antimicrobial Activity of the Essential Oils of *Dittrichia viscosa* subsp. *viscosa* on *Helicobacter pylori*

D. Silva, E. Denham, L. Faleiro and G. Miguel
Faculdade de Engenharia de Recursos Naturais
Universidade do Algarve
Campus de Gambelas
8000-117 Faro
Portugal

C. Cavaleiro and L. Salgueiro
Centro de Estudos Farmacêuticos
Laboratório de Farmacognosia
Faculdade de Farmácia
Universidade de Coimbra
3000-295, Coimbra
Portugal

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## Abstract

***Dittrichia viscosa* subsp. *viscosa* (*Compositae*) is found on edges, wood clearings and in waste places of the Iberian Peninsula. Aerial parts of *D. viscosa* were collected at flowering phase in September-October 2001 around Lisbon, Portugal and the essential oils isolated by hydro-distillation for 4 h using a Clevenger-type apparatus. The oils were analyzed by gas chromatography and gas chromatography-mass spectrometry. Preliminary examination of the essential oils allowed the identification of 32 components. Only four components reached percentages over 5%: fokienol (11.8%), T-muurorol (7.9%), *(E)*-nerolidol (5.5%) and δ-cadinene (5.0%). The essential oils were tested against *Helicobacter pylori* and *Listeria monocytogenes*. Essential oils did not have antimicrobial activity against *L. monocytogenes*. The essential oil at 0.88 to 22.22 μg.ml$^{-1}$ did not inhibit the growth of *H. pylori*, affected the growth slightly at 44.40 μg.ml$^{-1}$, and completely inhibited the growth at 88.80 to 133.20 μg.ml$^{-1}$. Results show that use of *D. viscosa* essential oil in the treatment of gastric disorders caused by *H. pylori* can be effective.**

## INTRODUCTION

False yellow head (*Dittrichia viscosa* subsp. *viscosa*) (*Compositae*) is an herbaceous perennial found on edges, wood clearings and in waste places in Iberian Peninsula (Font Quer, 1978). Several compounds have been isolated from this plant, such as flavonoids, triterpenoids, sesquiterpene lactones, sesquiterpene acids structurally related to ilicic acid and essential oils (Grande et al., 1985; Simões and Nascimento, 1990; Grande et al., 1992; Grande and Bellido, 1992; Pérez-Alonso et al., 1996; Camacho et al., 2000). Decoctions of the plant are used in Spanish folk medicine to treat injuries, sprains, and bruises and combat gastro duodenal diseases (Font Quer, 1978; Camacho et al., 2000). Studies have demonstrated that *D. viscosa* possesses an antiulcerogenic effect due to the flavonoid fraction (Martin et al., 1988), which could be partly explained through non-prostaglandin-dependent mechanisms (Alarcón de la Lastra et al., 1993).

Studies of the essential oils isolated from *D. viscosa* (Pérez-Alonso et al., 1996; Camacho et al., 2000) demonstrated that the plants collected in Turkey and Spain varied in chemical composition. The antimicrobial activity of *D. viscosa* has been subjected to few studies (Müller-Riebau et al., 1995). In the present work a preliminary study regarding the chemical composition of the oils of *D. viscosa* collected in Portugal as well as their antimicrobial activity against *Helicobacter pylori* were tested.

*H. pylori* is a Gram negative bacteria that colonizes the human gastric mucosa causing several gastric malignancies such as chronic gastritis and ulcers which can progress to gastric carcinoma and MALT lymphoma (Parsonnet et al., 1991; Blaser, 1992; Parsonnet et al., 1994). *Listeria monocytogenes* is a gram-positive bacteria, which is the causative agent of human listeriosis with mortality rates of up to 30% being reported and food as a considerable vehicle of transmission (Rocourt, 1991). The use of essential oils can be an effective tool against infection considering the development of bacterial multi-resistance to antibiotics.

## MATERIALS AND METHODS

### Plant Material and Isolation Procedure

Aerial parts of *D. viscosa* subsp. *viscosa* were collected in September-October 2001, around Lisbon (Portugal). The oils were obtained by hydrodistillation for 4 h with a Clevenger-type apparatus, according to the European Pharmacopoeia (1997).

### Essential Oil Analysis

Volatile oil was analyzed by GC and GC-MS using fused silica capillary columns with two stationary phases (SPB-1 and SupelcoWax 10, 30 m × 0.20 mm i.d, film thickness 0.2 μm) (Cavaleiro et al., 2001). Constituents of the essential oil were identified on the basis of their retention indices (RI) determined by linear interpolation relative to retention times of a series of *n*-alkanes, and by matching their 70 eV mass spectra with our own data and reference libraries. Relative amounts of individual components were calculated based on peak areas without FID response factor correction.

### Antimicrobial Activity

The microorganisms used in this study were: *H. pylori* strain 200, a clinical strain isolated from a gastric biopsy at Faro Hospital (Portugal) from the Microbiology Laboratory of Faculty of Natural Resources Engineering, University of Algarve; *L. monocytogenes* strain NCTC 7973, a clinical strain isolated from Guinea pig, lymph nodes and obtained from the National Collection of Type Cultures (Colindale, England), and *L. monocytogenes* strain C882, a food isolate from the collection of Microbiology Laboratory of Instituto Nacional Engenharia e Tecnologia Industrial - Departamento de Tecnologia das Indústrias Alimentares (Lisbon, Portugal).

The antimicrobial activity of essential oils was tested by disc agar diffusion method and 0.1 mL of the culture was used to inoculate Columbia agar (Oxoid, CM 331, Madrid, Spain) supplemented with 10% human blood. Sterile filter paper discs of 6 mm were distributed on the agar surface containing 3 μL of the essential oil. In each plate a disc containing 3 μL of sterile distilled water and a disc containing the antibiotic tetracycline were included as control. Different concentrations of the essential oil were tested by incorporation in the same medium. Concentrations tested were (in $\mu g.mL^{-1}$) 0.88, 1.78, 2.66, 3.55, 4.44, 22.22, 44.40, 66.60, 88.80, and 133.20. The essential oil was eluted in 2-propanol (10%, v/v) and all concentrations were from the same stock solution of the eluted essential oil. The 2-propanol was previously tested for antimicrobial activity and at the concentration used and no effect on bacterial viability was registered. Bacterial viability was determined by Miles and Misra method (Barbosa et al., 1995). All the treatments were repeated three times.

## RESULTS AND DISCUSSION

Preliminary examination of the essential oils allowed the identification of 32 compounds. The major compounds were sesquiterpenes. Only four components reached percentages over 5%: fokienol (11.8%), T-muurorol (7.9%), *(E)*-nerolidol (5.5%) and δ-cadinene (5.0%). Some other important constituents identified were T-cadinol (4.8%), 1,8-cineole (3.7%), torreyol (1.8%), *p*-cymene-8-ol (1.8%), 4-terpineol (1.7%), γ-cadinene (1.4%) and α-muurolene (1.2%). The composition of the sample tested is closer to that previously reported for the oil obtained from plants from the Province of Jaénz (Spain) (Camacho et al., 2000), where fokienol reached 38.8% and *(E)*-nerolidol 5.5%. On the other hand, the composition of the sample tested is markedly different from the oil from Turkey (Pérez-Alonso et al., 1996), in which borneol (25.2%), isobornyl acetate (22.5%) and bornyl acetate (19.5%) were the main components. These compounds were not identified in the Portuguese oil.

Lower concentrations of the essential oil (0.88 to 44.4 $\mu g.mL^{-1}$) did not have antibacterial activity (Table 1), while 66.60 $\mu g.mL^{-1}$ reduced the population of *H. pylori* in 2 log. The initial population was 7.4 $\log_{10}$ of viable $cells.mL^{-1}$ and at concentration 66.6

$\mu g.mL^{-1}$ the population was reduced to 5.3 $\log_{10}$ of viable cells.$mL^{-1}$. The concentrations 88.8 and 133.2 $\mu g.mL^{-1}$ eliminated growth below the sensitivity of the viability determination test. These results indicate that the MIC concentration (Minimum Inhibitory Concentration) required 66.6 $\mu g.mL^{-1}$ of essential oil.

The antimicrobial activity of *D. viscosa* was also tested against *L. monocytogenes* strains using disc agar diffusion method. The *L. monocytogenes* strains used in the study were resistant to *D. viscosa* essential oil with no inhibition zone observed. The disc containing tetracycline had produced an inhibition zone of 28 mm (including the 6 mm diameter of the disc) for strain NCTC 7973 and 27 mm for strain C 882 (Table 1).

The in vitro antibacterial activity test using *D. viscosa* essential oil against *H. pylori* strain 200 is promising. A small amount of essential oil (MIC value of 66.6 $\mu g\ ml^{-1}$) can drastically reduce the growth of *H. pylori*. Surprisingly *L. monocytogenes* strains were resistant to this essential oil, indicating a specific *H. pylori* target. Other essential oils such as the rose oil (Boyanova and Neshev, 1999) and *Nepeta* oils (Kalpoutzakis et al., 2001) have been reported to demonstrate in vitro antimicrobial activity against *H. pylori* strains.

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## Tables

Table 1. Antimicrobial activity of essential oil of *Dittrichia viscosa* on *Helicobacter pylori* and *Listeria monocytognes*. *H. pylori* cell viability is expressed as $\log_{10}$ cfu.mL$^{-1}$ ± standard deviation. Antimicrobial activity against *L. monocytogenes* is expressed by diameter of inhibition zone (including the disc diameter-6 mm). Each disc had 3 μL of the essential oil. The values are the mean of three replicates ± the standard deviation.

| **Microorganism** | **Essential oil concentration (μg.mL$^{-1}$)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.88 | 1.78 | 2.66 | 3.55 | 4.44 | 22.22 | 44.4 | 66.60 | 88.80 | 133.20 |
| *H. pylori* 200 | 7.40±0.12 | 7.20±0.22 | 7.33±0.3 | 7.37±0.2 | 7.5±0.1 | 7.35±0.15 | 7.58±0.31 | 7.18±0.72 | 5.32±0.41 | NG | NG |
| | **Diameter of zone inhibition (mm)** | | | | | | | | | | |
| | **Tetracycline** | | | | | | **Essential oil** | | | | |
| *L. monocytogenes* NCTC 7973 | 28.0±4.2 | | | | | | NIZ | | | | |
| C882 | 27.0±3.24 | | | | | | NIZ | | | | |

NG - No growth
NIZ - No inhibition zone