CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *Tagetes patula* L. (ASTERACEAE) COLLECTED FROM THE VENEZUELA ANDES

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ABSTRACT

The essential oil of the aerial parts of *Tagetes patula* L., was analyzed by GC/MS. The oil obtained by hydrodestillation yielded 0.17 %. Thirty components were identified by comparison of their mass spectra with Wiley library data. The major constituents were piperitone (33.77 %), *trans*-β-ocymene (14.83 %), terpinolene (13.87 %) and β-caryophyllene (9.56 %). The essential oil showed strong antibacterial activity against important human pathogenic Gram positive and Gram negative bacteria.

Key Words: *Tagetes patula*, Asteraceae, essential oil composition, antibacterial activity, piperitone, *trans*-β-ocymene, terpinolene and β-caryophyllene

INTRODUCTION

Tagetes genus belongs to the family Asteraceae; comprises about 55 species distributed around the world. In Venezuela, there are only 5 of these located in the Andes and mountain areas; *Tagetes patula* L. is an annual plant, 40 cm of height that grows at 1500-2500 m above the sea level (Badillo, 2001). It has been used in folk medicine to treat colics, diarrhoea, vomit, fiver, skin diseases and hepatic disorders (Ivancheva and Zdravkova, 1993). Phytochemical studies carried out to different species of *Tagetes* have revealed the presence of flavonoids and terpenes displaying pharmacological and insecticidal properties (Tereschuk *et al.*, 1997; Perich *et al.*, 1995). Larvicidal activity was also tested to the essential oil of *Tagetes patula* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefaciatus* showing a potent activity specially against *Aedes aegypti* (Dharmagadda *et al.*, 2005). The essential oil of *Tagetes terniflora* (Tereschuk *et al.*, 2003) and *Tagetes lucida* have reported antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* (Caceres *et al.*, 1991), *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, *Shigella dysenteriae* and *Shigella flexneri* (Caceres *et al.*, 1990). The antibacterial and antifungal activity of the essential oil of *T. minuta* and *T. tilifolia* has also been reported (Zygadlo *et al.*, 1994). The purpose of the present study was to investigate the chemical composition of the essential oil of *Tagetes patula* cultivated in Venezuela

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Andes. Antibacterial activity of this species was also evaluated against Gram positive and Gram negative bacteria using the disc diffusion assay.

RESULTS AND DISCUSSION

The essential oil from aerial parts of *Tagetes patula* yielded 0.17 %. GC/MS analyses showed the presence of 15 components, 12 of these were identified and are listed along with their percentages of the total oil in Table 1. The main compounds of the oil were piperitone (33.77%), *trans*-β-ocymene (14.83 %), terpinolene (13.87 %) and β-caryophyllene (9.56 %). Antibacterial activity of the essential oil was evaluated against Gram positive and Gram negative bacteria displaying MIC values for *Staphylococcus aureus* (30 μg/ml), *Enterococcus faecalis* (30μg/ml), *Escherichia coli* (60 μg/ml), *Klebsiella pneumoniae* (90 μg/ml) and *Pseudomonas aeruginosa* (130 μg/ml), (Table 2). These results revealed the potential use of the essential oil against important human pathogenic Gram positive and Gram negative bacteria. The low dose antibacterial activity observed suggested that the essential oil of *T. patula* could represent an alternative for further investigations in the Pharmaceutical Industry. The major compound observed, piperitone, is a terpene ketone found in most of *Mentha* species well known to possess carminative properties (Dewick, 1998). However, in recent investigation piperitone (4.2 %) along with other components showed antibacterial activity against *Bacillus dipsauri*, *Corynebacterium cystitidis* and *Corynebacterium flavescens* (Ozturk *et al.*, 2006).

Trans-β-ocymene, terpinolene and β-caryophyllene, observed as well as main components in this investigation, have been reported previously to possess antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae*, *Salmonella typhi*, *Salmonella choleraesuis*, *Bacillus subtilis*, *Bacillus cereus*, *Acinetobacter calcoaceticus*, *Clostridium sporogenes*, *Clostridium perfringens*, and *Yersinia enterocolitica* (Magwa *et al.*, 2006; Khamis *et al.*, 2005; Yong-Suk and Dong-Hwa, 2005).

Table 1: Composition of the essential oil of *Tagetes patula**

Compound	RT (min)	Area (%)
Myrcene	6.25	0.53
Limonene	7.26	7.78
Cis-β-Ocymene	7.47	5.01
Trans-β-Ocymene	7.77	14.83
Terpinolene	8.96	13.87
Linalool	9.27	0.73
Epoxy-ocymene	10.57	2.27
Piperitone	14.23	33.77
β-Caryophyllene	19.43	9.56
β-Farnesene	20.53	1.48
Germacrene-D	21.31	1.45
Bicyclogermacrene	21.77	1.52

*The composition of the essential oil was determined by comparison of their mass spectra with Wiley GC/M library data and also based on the retention times (RT)

EXPERIMENTAL WORK

Plant Material

The aerial parts of *Tagetes patula* L., were colleted in January 2005 in Avenida Los Próceres, Albarregas, Municipio Libertador, Mérida Edo. Mérida, located at 1680 m over the sea level. Voucher specimen (MERF 709) has been deposited in the Herbarium of the Faculty of Pharmacy and Biomedical Sciences of the University of Los Andes.

Isolation of Essential Oils

Leaves (1450 g) were cut into small pieces and subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and stored at 4 oC.

Analysis of Essential Oil

GC-MS analysis was carried out using a Hewlett-Packard HP 6890 Series GC System

Table 2: Antimicrobial activity of essential oil of *Tagetes patula*

Microorganism	Inhibition zone (mm)						MIC
	Essential oil	Positive control					μg/ml
		SAM	VA	NET	AZT	CEF	
Staphylococcus aureus ATCC (25923)	22*	35*					30
Enterococcus faecalis ATCC (29212)	27*		21*				30
Escherichia coli ATCC (25992)	17*			23*			60
Klebsiella pneumoniae ATCC (23357)	15*				31*		90
Pseudomonas aeruginosa ATCC (27853)	12*					28*	130

SAM: Sulbactam -Ampicillin® (10μg/10 μg), VA: Vancomycin® (30 μg), NET: Netilmicin® (30 μg), AZT: Aztreonam® (30μg), CEF: Cefoperazone® (75 μg).
*Inhibition zone, diameter measured in mm, disc diameter 6 mm, average of two consecutive assays.
MIC: Minimal inhibitory concentration, concentration range 10-160 μg/ml.

and Hewlett-Packard HP 5973 Mass selective detector, equipped with a HP 5MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm). The initial temperature of the column was 60 °C reaching 260 °C at a rate of 4 °C/min. Carrier gas, He; split ratio 1:10.

Microbiological Analysis

Bacterial Strains

Staphylococcus aureus (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25992), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 23357) were used in this study.

Antimicrobial Method

The antimicrobial activity was carried out according to the disc diffusion assay described by Rondón *et al.* (2005). The strains were maintained in agar conservation at room temperature. 2.5 ml of every bacteria inoculum were incubated in Mueller-Hinton broth at 37°C for 18 hours. The bacterial inoculum was diluted in sterile 0,85% saline to obtain a turbidity visually comparable of a McFarland No 0.5 standard (10^{6-8} CFU/ml). Every inoculum was spread over plates containing Mueller-Hinton agar and a paper filter disc (6 mm) saturated with 10 μl of essential oil. The plates were left for 30 min at room temperature and then incubated at 37 °C for 24 h.

The inhibitory zone around the disc was measured and expressed in mm. A positive control was also assayed to check the sensitivity of the tested organisms using the following antibiotics: Sulbactam-Ampicilin® (10μg/10 μg), Vancomycin® (30 μg), Netilmicin® (30 μg), Cefoperazone® (75 μg) and Aztreonam® (30 μg) (table 2).

The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the essential oil in dimethylsulphoxide (DMSO) pipetting 10 μl of each dilution onto a filter paper disc. Dilutions of the oil within a concentration range of 10-160 μg/ml were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth (NCCLS, 2005).

A negative control was also included in the test using a filter paper disc saturated

with DMSO to check possible activity of this solvent against the bacteria assayed. The experiments were repeated at least twice.

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