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ANTIMICROBIAL EFFECTS OF THE ETHANOLIC EXTRACTS AND ESSENTIAL OILS OF *TANACETUM VULGARE L* FROM ROMANIA

Maria Lucia MUREŞAN[1]

Faculty of Farmacy, Lucian Blaga University of Sibiu, Romania

Abstract: This paper investigates the antimicrobial action of the extracts and essential oil of wildgrowing *Tanacetum vulgare L* on: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacilus subtilis,*using the diffusion disc method. The essential oils but also the ethanolic extracts tested exhibited moderate action on Staphilococcus aureus and Bacillus subtilis and low action on *E. coli* and *Pseudomonas aeruginosa*. The moderate antimicrobial activity is related to the amount of some chemical components of the essential oil of *T. vulgare flos*. Thus, this paper presents also the quantitative and qualitative analysis of the essential oils of T. vulgare harvested from two different habitats. The essential oils obtained by steam-distillation were analysed by gas-cromatography coupled with mass spectrometry (GC-MS).

Keywords: essential oil, extract, *Tanacetum vulgare*, antimicrobial effects

INTRODUCTION

From ancient times, the beneficial effects of plant extracts in healing or treating diseases were known (Tabata et al., 1988). The properties of these plants are based on their composition, rich in bioactive compounds, such as essential oil, polyphenols, flavonoids or tannins. The essential oils extracted from plants contain terpenes and its subclasses: monoterpenes and sesquiterpenes, which have antibacterial, anti-septic, anti-inflammatory properties (Wallace,2004; Bagci,2010; Georgescu and Mironescu, 2011; Benedec et al.,2013; Mureşan et al.,2014; Şandru 2015). The mechanism of the action of the terpenes is not fully known. There is only an assumption that the cell membrane is distroyed by the lipophilic compounds.
Scientific research has reported positive results on the antimicrobial activity of the essential oils of *Tanacetum vulgare* harvested outside Romania (Cowan

[1] Corresponding author. Mailing adress: Mureşan Maria Lucia, Faculty of Pharmacy, Lucian Blaga University of Sibiu, Romania, , Lucian Blaga street no 2A.E-Mail: nartea_maria@yahoo.de

1999; Jussi-Pekka et al. 1999; Salamcia et al., 2007; Nurhayat et al., 2007; Kaan et al. 2010). Therefore, this paper aims are, to analyse the antimicrobial effect on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis* of the essential oils, but also of the ethanolic extracts of *T. vulgare* harvested in Transylvania/ Romania. In order to explain the antimicrobial effect, another aim of this research is to evaluate the chemical composition of the essential oil.

MATERIALS AND METHODS

Plant material: headflowers of *T. vulgare* harvested from two different habitats of Transylvania, Alba and Sibiu.
Two essential oils , U1- essential oil of *T. vulgare* harvested from Sibiu, U2- essential oil of *T. vulgare* harvested from Alba (*Tanacetum aetheroleum*) and two ethanolic extracts, T1 and T2, obtained from the same material were used. For comparison the essential oils were obtained by steam distillation of the dried material in accordance with the Romanian Pharmacopoeia. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4° C until the analyses were carried out.
Essential oil analysis procedure: The three samples were diluted with cyclohexane in a 1:1 proportion and a volume of 0.1 µL was injected. An Agilent 6890 Series gas-chromatograph coupled with a Hewlett Packard 5973 mass spectrometer, and a capillary Permabond column SE-52, (60m x 450 FARMACIA, 2013, Vol. 61, 3 0.25mm, 0.25µm) were used. The injector and detector temperatures were kept at 250°C and 280°C, respectively. Helium was used as a carrier gas, with a flow rate of 0.6 mL/min; oven temperature programmed was 60- 240°C at a rate of 3°C/min. Mass spectrometry (MS) analysis was performed in the same conditions, with an ionization voltage of 70eV and mass range was from 35-700 u.i.m. Identification of individual compounds was performed by comparison of their mass spectra with those of the internal reference mass spectra libraries (Wiley 275 and NIST 98) (Mureşan, 2014; Oprean et al.1998).
To determinate the antimicrobial activity we used the disk diffusion method on culture Mueller-Hinton medium, agar poured uniformly on a thin layer of 4mm, pH 7.2 (Marian 2013; Şandru, 2015; Mironescu et al., 2010). As test microorganisms it was used *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853 *and Bacillus subtilis.* The inoculated plates were then incubated at 37°C, 10 minutes. Filter paper discs (6mm in diameter) were impregnated with 15µL essential oil and 15 µL ethanolic extract and placed on seeded plates. For comparison there were used 2 antibiotics, Gentamicine and Ciprofloxacine. In the concentration of 10

mg/disc The activity was determined after maintaining the samples for 24 hours at 37°C (Benedec et al., 2013; Marian et al., 2013; Moldovan et al., 2014)

RESULTS AND DISCUSSIONS

The essential oils' level (mL essential oil/100g natural product ± SD) for the two samples was determined by steam distillation: 0.64%±0.03 (sample 1, headflowers harvested from Sibiu in august 2013) and 0.55%±0.03 (sample 2, headflowers harvested from Alba in august 2013). Considering the quantity of essential oils, August was the optimal period for harvesting the natural product. In the literature, the reported levels of essential oil in *Tanacetum vulgare* headflowers are between 0.4% and 1.1% (Mureşan ML, 2014, Tetenyi et al. 1975).

The main compounds of the essential oils of *T. vulgare flos* and their levels are presented in Table 1 (percentage value represents the mean of three determinations ± SD). The isolated oils were analysed by GC-MS and there were identified between 32(in the oil of headflowers of *T. vulgare* collected from Alba) and 42 compounds (in the oil of the headflowers collected from Sibiu). The constituents given in Table 1 made up 54.01% - 84.98 % of each oil content.

In the essential oils obtained from sample 1, 14 compounds were identified, representing 87.24%, whilst in sample 2, 16 compounds, representing 89.07% of the essential oil. The basic components were represented by monoterpenes and sesquiterpenes. Regarding the antimicrobial activity, the main components responsible for this activity, also reported in other scientific research, are camphor, borneol, thymol. The results showed a higher concentration of this components in the sample harvested from Alba. The antimicrobial effect of these samples was tested. The diameter of the inhibition zones in the Petri dishes are presented in Table 2.

Table 1. Chemical composition of the essential oils of wild *T. vulgare* from Sibiu (sample 1) and Alba (sample 2).

Compound	t_R	I	Sample 1, %	Sample 2, %
1,8-Cineol (MT)	8.18	1030	3.50	3.27
Artemisia ketone (MT)	9.20	1060	2.19	7.88
Artemesia alcohol (MT)	10.00	1083	-	0.70
alpha-Thujone (MT)	10.92	1108	24.81	13.21
beta-Thujone (MT)	11.25	1116	14.41	12.79
Camphor (MT)	12.35	1144	1.16	10.17
Borneol L (MT)	13.26	1162	0.93	9.34
4-Terpineol (MT)	13.69	1177	-	0.75
Myrtenol (MT)	14.45	1197	-	2.99

Crysanthenyl acetat (ST)	16.25	1239	25.84	20.06
Piperitone (MT)	16.89	1254	-	0.88
Carveol (MT)	18.31	1288	-	2.89
Thymol (MT)	18.50	1293	0.60	1.55
Azulene (MT)	18.79	1300	0.57	1.16
p-Cymene (MT)	19.88	1326	0.56	-
Spathulenol (ST)	29.93	1575	0.76	-
(-)-Caryophyllene oxide (ST)	30.14	1580	3.07	-
Cubenol (ST)	31.91	1627	3.31	0.49
(+,-)-.beta.-Himachalene (ST)	32.95	1655	5.53	0.94
Total			**87.24**	**89.07**

MT- Monoterpene, ST- Sesquiterpene

Table 2. Antimicrobial activity of the essential oils and ethanolic extracts of *T. vulgare* harvested from Tranylvania (Alba and Sibiu) measured in mm after 24h.

Extract/control product	Diameter of the inhibition zones			
	S. aureus ATCC 25923	*B. subtilis*	*E.coli* ATCC 25922	*Ps. aeruginosa* ATCC 27853
U1	12	11	7	7
U2	16	12	8	6
T1	11	10	6	6
T2	12	11	7	5
Gentamicine	28	23	23	21
Ciprofloxacine	30	23	36	20

Both, essential oils and ethanolic extracts (70%), have moderate activity on *Staphylococcus aureus* (U1/U2=16 mm/12mm, T1/T2=12mm/11mm) and *Bacillus subtilis (*U1/U2=12 mm/11mm, T1/T2= 11/10*)* and low activity on *E. coli* (U1/U2=8mm/7mm, T1/T2= 7mm/6mm) and *Pseudomonas aeruginosa* (U1/U2= 6mm/7mm, T1/T2= 5mm/6mm). In comparison to the plant material, the two antibiotics, Gentamicin and Ciprofloxacin have proved to have significantly higher antimicrobial activity.

These results are in accordance with other similar researches on volatile oils extracted from *T. vulgare* harvested from outside of Romania.

The modest results are explained by the presence in certain concentration of terpenes, camphor, borneol and 1,8-cineol, in both samples. The best antimicrobial result was determined by the sample harvested from Alba, activity reflected also by the higher concentration of the chemical constituents.

CONCLUSIONS

From the obtained results it can be concluded that the selected essential oils, but also the ethanolic extracts feature antimicrobial activity. All 4 samples exhibited moderate activity on *Staphylococcus aureus* and *B. subtilis* and low activity on *E. coli* and *Pseudomonas aeruginosa.* The antimicrobial activity of *T. vulgare* harvested from two different habitats, Sibiu and Alba, can be explained by the moderate presence of the three terpenes, camphor, 1,8-cineol and borneol. Thus, the sample harvested from Alba showed better results concerning the chemical composition and also the antimicrobial activity. The majority of the chemical components of both samples was represented by monoterpenes (48,73% for sample 1 and 67,58% for sample 2), and sesquiterpenes (38,51% for sample 1 and 21,49% for sample 2).

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