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Full Length Research Paper

Antimicrobial and anti-inflammatory activities of the volatile oil compounds from *Tropaeolum majus* L. (Nasturtium)

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This study was carried out to evaluate the antimicrobial and anti-inflammatory activity of some chemical compounds of the volatile oil extracted from *Tropaeolum majus* L. The chemical compounds extracted from the flowers and leaves of *T. majus* L. have been identified through color reactions and gas-chromatographic analysis combined with mass spectrometry. Quantitative testing and the ascertaining of the minimum inhibitory concentration (MIC) have been done through the binary micro dilution method for liquid environments against the following microbial types and species. The qualitative evaluation of the sensitivity of microbial stems against these compounds has been done through methods that have been adapted from the diffusimetric method. Of the qualitative methods used for the control of the antimicrobial activity, the method of diffusion on filter paper discs proved to be the most efficient, the results correlating well with the MIC. Our studies have demonstrated the efficiency of the natural compounds' of *T. majus* L. in anti-inflammatory treatments in animals. The antimicrobial activity proved to be selective, depending on the pathogen. These results are in agreement with those of other studies. Our results supported the selection and utilization of these compounds' as antimicrobial agents in the treatment of infections with microorganisms resistant to existent antibiotics.

Key words: Chemical compounds, *Tropaeolum majus* L, antimicrobial activity, anti-inflammatory activity.

INTRODUCTION

The emergence of the resistance and multiresistance phenomena of the infectious agents to antimicrobial agents has generated the intensification of research efforts to find new antimicrobial agents and to develop new strategies to treat infectious disease. Antibiotics are used on a wide scale against negative aerobic Gram bacilli and positive, atypical and anaerobical Gram germs. The emergence of new antibiotics with improved pharmacokinetics and bioavailability and with a net superior tissular action and diffusion spectrum has allowed the enlargement of the clinical indications range. Unfortunately, excessive use of antibiotics has led to the ever more frequent occurrence of resistant stems, especially in the case of first generation antibiotics (Vancanneyt et al., 2004). This is why knowing the pharmacodynamic properties and the resistance spectre is necessary for the judicious use of all antibiotics, in general. Because pathogen resistance against antibiotics is so wide spread, treatment effectiveness in infectious pathology is more and more uncertain.

For this reason, the past years have seen massive efforts in the attempt to identify alternative treatments to the classical ways represented by antibiotic therapy.

A possible solution is using natural products, such as volatile oils extracted from various plants (Nair et al., 2006). Plants have been used in traditional treatments to cure various diseases for thousands of years. Numerous studies have demonstrated that volatile oils obtained from plants represent antibacterial, antifungic, antiviral, insecticide and antioxidant properties (Yoneyama et al., 2006). The advantages of these plants due to their second-

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secondary metabolites have been intensely debated despite the recognition of their biological activity. Both the terpenoids and the phenolic compounds are important in the plant's protection against insects and herbivorous animals; in strive to find space and resources against competing plants and against pathogen microorganisms (Trott et al., 2007).

Secondary metabolites like the alkaloids, isoprenoids and pheolic acids are eliminated as exudates by the leaves, on their surface. Volatile oils (also called essential oils) are regular aromatic compounds from different parts of the plant (flowers, buds, seeds, branches, bark, wood, fruit or roots) (Swarna et al., 2009). They can be obtained through fermentation or extraction, but the most commercially used method is hydro distillation (Silva et al., 2005). Volatile oils contain a mix of compounds that from a chemical point of view derive from terpenoids or their oxygenated compounds. Volatile oil compounds have been used in the therapy of certain forms of cancer, others in food preservation (active preservation), or as source of active biological compounds (Pattnaik et al., 1997). The largest use of volatile oils in the European Union is registered in the food industry (spices), cosmetics and pharmaceuticals. Their antibacterial properties and those of their subcomponents have been exploited as various commercial products, such as: concoctions for the obturation of dental root canals, antiseptics, or additives for animal feeds. Because of this, we expect to discover that within the chemical composition of the volatile oils coming from different species of plants there are substances with antimicrobial properties with specific or general actions against microorganisms. Although, the antimicrobial properties of volatile oils and their compounds have been researched in the past, the mechanism of their action has not been studied in detail (Guardabassi et al., 2008). Taking into account the large number of the different groups of chemical compounds present in the composition of volatile oils, their antimicrobial activity cannot be assigned to a sole specific mechanism. Their activity depends on the process through which the volatile oil is obtained, the target location and the synergies of the constituent substances. The chemical structure of the individual compounds of volatile oils affects their specific antibacterial action. A characteristic of volatile oils and their compounds is hydrophobia, which permits them to affect the lipid structure of the bacterial cellular membrane and increase its permeability (Dolganiuc et al., 1997).

The importance of the presence of the hydroxyls grouping within the phenolic compounds like carvacrol and thymol has been confirmed. The position of this grouping within the phenolic ring does not seem to significantly affect the degree of antibacterial activity (Tzakou et al., 2001). In Romania, the *Tropaeolum majus* is cultivated as a decorative plant. *T. majus* contains glucosynolates, sulphur glicosydes, an antibiotic (tromalyte), bensilglucosynolate, glucosinol, and glicotropeoline. The plant also contains flavonoids, vitamin C, iodide, spilantolic acid, oxalic acid and mirosine (enzymes).

Glicotropeopline frees a sulphurous compound added in water, has the properties of a disinfectant, but has also antibiotic and anti–tumoral effects (Vancanneyt et al., 2006). The volatile oil obtained from Nasturtium contains, in addition to flavonoids, acids from the group of the chlorogenic acid, carotenoids, cucurbitacins, proteins, aminoacids, sulphur, iron, manganese, phosphoric acid. The lipid part that covers the surface of the leaves is composed of a mix of aliphatic acids; the main substances are nonacosanol and nonacosanediol. The *T. majus* flowers are excellent sources of lutein, and the leaves contain both lutein and provitamin A and β–carotene (Luz et al., 2007).

MATERIALS AND METHODS

Materials

The aerial parts (leaves and flowers) of the species *T. majus* were harvested at blossom. Plants were identified and authenticated in the Botanical Laboratory of the U.S.A.M.V.B Timisoara. A labelled voucher has been kept in the laboratory herbary. The aerial parts of the *T. majus* plant were collected in May 2007 and dried at 18–22°C for two days. In order to minimize the loss of active components, the dehydration was done without thermic treatments. After drying, the material was kept at –20°C until it was used (Swarna et al., 2009).

The extraction method for volatile oils

For the extraction of volatile oil, we used a clevenger type hydro distillation device based on the recirculation of water, in order to eliminate losses of volatile oil through its solving in the water. The heating was done on direct fire, while the installation was permanently monitored in order to avoid overheating of the distillation container. The vegetal material (500 g) was introduced in the balloon of the device after it was weighed, adding 500 ml of water; the distillation lasted for 3–4 h. 50 g of finally ground plant were extracted with 50 ml of distilled water, methanol 100%, ethanol 80% and hexane for 72 h, under intermittent agitation. The extracts were than filtered.

Evaluating the anti–inflammatory action

Experiments were done on 65 white mice with corporal mass of 140–200 mg. The animals were distributed in 5 lots. The inflammation was induced in all the mice through the injection of 50 µl 4% formalin under aponeurosis of the back paw. The inflamemation degree was determined through the modification of the paw volume as could be observed on a graded column of water. The first lot of 15 mice was split as follows: 5 mice were administered, 5 days before the induction of the inflammation, intraperitoneally, 1 mg of antibiotic (based on chloramphenicol) as suspension; other 5 mice–3 days before; and the other 5–on the day of the induction of the inflammation.

The second lot of 15 mice was split as follows: 5 mice were administered, 5 days before the induction of the inflammation, intraperitoneally, 1 mg of antibiotic (based on penicillin) as suspension; 5 mice–3 days before; and the other 5–on the day of the inflammation. The third lot of 15 mice was split as follows: 5

mice were administered, 5 days before the induction of the inflamemation, intraperitoneally, 1 mg of antibiotic (based on cephalosporine) as suspension; 5 mice–3 days before; and the other 5–on the day of the inflammation. The fourth lot of 15 mice was split as follows: 5 mice were administered, 5 days before the induction of the inflammation, intraperitoneally, 1 mg of volatile oil obtained from Nasturtium, as suspension; 5 mice–3 days before; and the other 5–on the day of the inflammation. The fifth lot of 5 mice was the control lot in which we only induced the inflammation. Measurements were conducted 3, 24 and 48 h after the inflammation was induced (National Committee for Clinical Laboratory Standards, 2008).

Evaluating the antibacterial activity

The standard reference roots (*Staphylococcus aureus, Bacillus* sp., *Listeria monocytogenes, Escherichia coli, Salmonella* sp., *Pseudomonas aeruginosa* and *Candida albicans*) used in the evaluation of the *T. majus* volatile oil was provided by the Cantacuzino Institute Bucharest.

The qualitative screening of the susceptibility spectra of different microbial strains to the tested compounds was performed by adapted diffusion techniques. We used filter paper discs impregnated with solutions of the tested substances and the repartition in spot of the tested substances on solid media seeded in canvass. The study of the antimicrobial properties by the adapted disk diffusion method–Petri dishes with Mueller Hinton (for bacterial strains)/YPG (Yeast Peptone Glucose) (for yeasts) medium were seeded with bacterial inoculum as for the classical antibiotic susceptibility testing disk diffusion method (CLSI); 5 mm diameter paper filter disks were placed on the seeded medium, at 30 mm distance. Subsequently, the disks were impregnated with 5 ml of compound solution; by method Clinical and Laboratory Standards Institute (2007).

The study of the antimicrobial properties by spotting the tested solution on the culture medium seeded with the microbial suspension, the antimicrobial activity being expressed as the absence of microbial growth on the spot area.

MIC assays

The quantitative assay of minimal inhibitory concentration (M.I.C., µg/ml) value was based on liquid medium serial micro dilutions. Serial binary dilutions of the tested compounds (ranging between 1024 and 8 mg/ml) were performed in a 200 ml volume of nutrient broth and each well was seeded with 50 ml of microbial inoculum (0.5 McFarland density). The plates were incubated for 24 h at 37°C, and MICs were read as the lowest concentration of compound which inhibited the microbial growth; by method Clinical and Laboratory Standards Institute (2006). The standard culture environments came from I. Cantacuzino. Every environment box comes with quality control certificates.

These certificates enumerate the testing organisms, including the ATCC cultures specified in the CLSI M22 standard Quality control for commercialized culture environment preparates. The time interval for detection was ≤72 h for each of the organisms enumerated in the quality control certificate of this environment; by method FDA. (2006).

The decisive test local application; day 20–22, control and treatment groups. The ribs of the animals from the control and treatment groups were shaved of hair. A compression soaked in the test substance (volatile oil of *T. majus*) is applied on a single rib of the mouse and another compression soaked only in the vehicle can also be applied on the other rib. The compressions are kept in contact with the skin through occlusive gauze for 24 h. Control and treatment groups. Approximately 21 h after the compression has been removes; the tested zone is cleaned and carefully shaved, if necessary.

Approximately 3 h later (so approximately 48 h from the beginning of the application) the cutaneous reaction is registered and observed according to the degrees showed. Approximately 24 h after this observation, we make a second observation (at 72 h) and another recording. We recommend both on the control and the treatment lots. The graded Magnusson and Kligman scale for the evaluation of reactions to the application of the compression: 0=no visible change; 1=small or irregular erythema; 2=moderate and confluent (adherent) erythema; 3=intense and swollen erythema (U.S. Department, 2007). The Institutional Animal Care approved all of the experimental protocols. The number of experimental animals was kept to a minimum.

Statistical analysis

Results were analyzed using Student's *t*–test and expressed as mean± SEM. Differences between the mean of treated animals and control groups were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The analysis of the volatile oil

The preliminary phytochemical investigation of the aqueous extracts of the *T. majus*, preliminary results showed that it contains volatile oils and terpenoids, saponins, tannins, glycosides, alkaloids, fatty acids and antibiotic–tromalyt. The vegetable material composed of a mix of flowers and leaves of *T. majus* underwent hydro distillation and extraction with inorganic and organic solvents respectively. For the qualitative phytochemical analysis, we obtained extracts and volatile oil that were then used for the evaluation of their anti-inflammatory and antimicrobial potential. The qualitative reactions highlighted the fatty acids, sterols, flavonoids, free sugars, organic acids, tannins, alkaloids, saponoids and polysaccharides (Table 1).

The natural compounds of the isolated volatile oil have been identified and determined quantitatively, through gas–chromatographic analysis coupled with mass spectrometry. For this, we separately evaluated the aerial parts of the plants. We determined 450±50 µg/g lutein in the yellow–colored flowers and 350±60 µg/g lutein in the orange–colored flowers. In the volatile oil obtained from the aerial parts, we also detected 136±18 µg/g lutein, 69±7 µg/g β–caroten, 74±23 µg/g violaxanthin, 24±3 µg/g hetero–transglicosilatin and 48±13 µg/g neoxanthin. Violaxanthin, antheraxanthin, zeaxanthin, zeinoxanthin, β–cryptoxanthin, α–caroten, and β–caroten were detected in small concentrations in all cases. Results indicated the fact that there are no significant differences among extracts obtained from leaves or flowers from the point of view of the types of compounds detected.

Therefore, the mixture of all the aerial parts of the plant was considered optimal for the purpose of this study. Saponins are present on average 2.153±0.07%. Chromatographic analysis shows that saponins from *T. majus*

Table 1. Results of phytochemical screening of *T. majus.*

S/N	Group	Phytochemical reaction
1	Saponins	present
2	Steriods	present
3	Tannins	present
4	Glycosides	present
5	Alkaloids	present
6	Fatty acids	present
7	Flavanoids	present
8	Polyzaharides	present
9	Ketones, hydroxyketones, diketones	present

represent the sum of ester–saponins, bisdesmosides and monodesmosides of hederagenol (Figure 1). Fatty acids, such as palmitic oleic, stearic or docosanoic acid, were present in a concentration of 9.5± 0.12%, sterols 6.6± 0.1%, and esther sterols 8.4± 0.01%.

The anti–inflammatory effect of volatile oil

Results presented in Table 2 shows that metabolites from volatile oil extracted through hydro distillation from *T. majus* prevents inflammation and influences its degree of manifestation, especially after 24 and 48 h. The degree of manifestation is more reduced in mice which were administered *T. majus* volatile oil, as compared to those having been treated with synthesised products. The anti-inflammatory effects of the volatile oil extracted through hydro distillation from *T. majus* are explained by the presence of tiosulphinates. These compounds have the capacity to inhibited synthesis and the release of some pro–inflammatory mediators. Comparative analysis between the anti-inflammatory potential of the *T. majus* volatile oil and other classical anti-inflammatory shows a strong anti-inflammatory activity in the case of the first, where we could observe a complete resorption within 48 h from administration (Table 3). This action seems to be caused mainly by the saponoids identified in the volatile oil (Figure 1), but also to the content of lecithin's, acetil-balcanolids and the asulenes from the volatile oil. Another category of natural compounds comprised in the volatile oil is that of lactones, sesquiterpens, polynesians and polyphenols, which have as effect the inhibition of the development of the induced edema (Table 3).

Testing of anti-irritant and anti-inflammatory effect of *T. majus* volatile oil

Sodium lauryl sulphate (SLS) is a well-known tension-active agents used as an irritation model. This substance determines important toxic effects at skin level; the intensity of the effect depends on the relationship dose-effect. Its primary effects are the production of erythema and edema. Concentrations above 10% are harmful for the skin, especially when it is applied as sole compound. The *T. majus* volatile oil has been used directly on the skin immediately after the irritation was induced with SLS. The experimental mice lot on which the anti-irritant effect was tested is presented under the methods section. Volatile oil containing natural substances from *T. majus* presented a remarkable activity of inflammation reduction of up to 85%. The results of the visual evaluation of the potential to reduce irritation with SLS are shown in Table 4. In order to evaluate the effects of volatile oil, the aspect of the skin was monitored visually at certain time intervals.

At the visual evaluation of the skin appearance (Table 5) after the application of the preparation containing *T. majus* volatile oil, compared to other classical anti-inflammatory preparations, we observed that the integrity of the hydric layer of the skin was significantly improved right after the application of the product (30 min). The phytochemical study demonstrated the presence in the *T. majus* volatile oil and extracts of some metabolites that present antimicrobial property (tannins and flavonoids).

Testing of antimicrobial action of *T. majus* volatile oil

The antimicrobial action is determined by the phenols and metil–ethers identified in the *T. Majus* extracts, but also by the tymol and carvacrol present in the volatile oil.

The volatile oils tested presented a wide range of action over both Gram-positive and Gram–negative species (Tables 6 and 7). Among the qualitative methods employed for the control of the antimicrobial activity, the method of diffusion on filter paper disk proved to be most efficient, the results correlating well with MIC (Table 6). In the case of the quantitative analysis of the antimicrobial activity of the compounds tested through the method of the micro dilutions in a liquid environment, the development of the microbial cells was stopped by all the tested compounds (Table 7). At smaller concentrations (256 µl essential oil) of natural compounds, the microbial culture

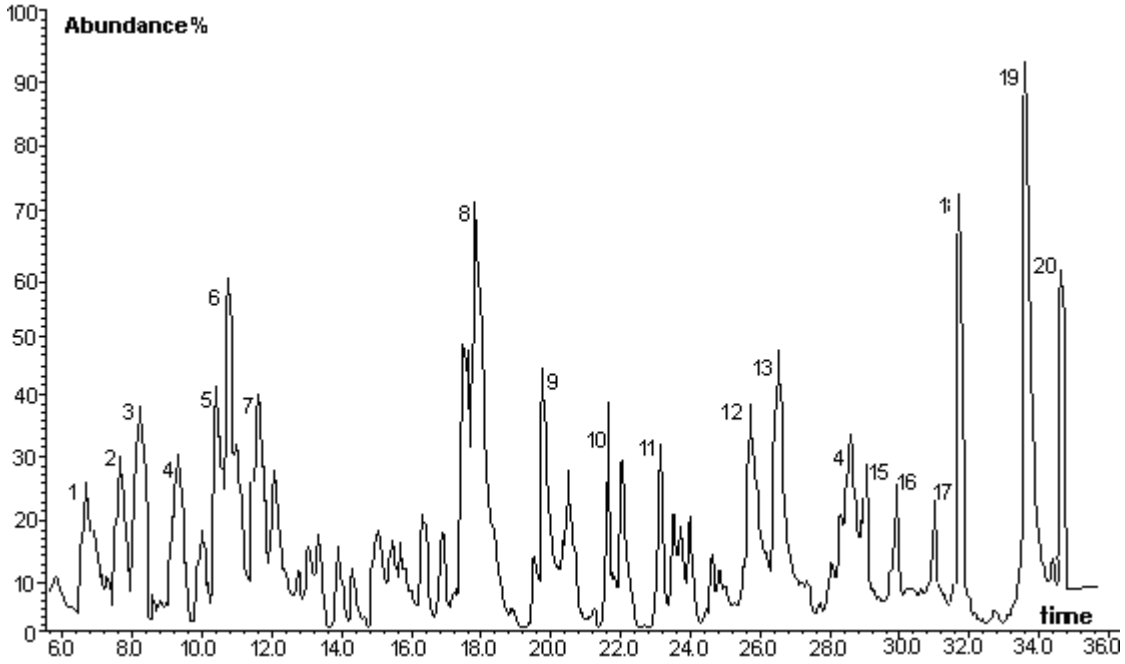

Figure 1. The Chromatogram of hydro–distilled extract from *Tropaeolum Majus* each peak represents one of the detected compounds: fatty acids (1–palmitic acid, 2–stearic acid, 3–oleic acid, 4–linoleic acid and 5–linolenic acid) 6–docosahexaenoic acid; 7–sterols, 8–sterol esters; carotenoid pigments (9– lutein; 10–beta carotene; 11–violaxanthin); 12–hetero–transglicosilating; 13– neoxanthin; 14–saponins; 15–bis–desmosides;16–mono–desmosides; 17–hederagenol; 18– flavonoids; 19–tromalyt; and 20–tannins.

Table 2. The evaluation of the efficiency of volatile oil compounds' on the evolution of the inflammatory process.

Preparation		Time of measurement		
		3 h	24 h	48 h
Preparation based on chloramphenicol	Administration 5 days before the inflammation was induced.	0.082 ml	0.075ml	0.045 ml
	Administration 3 days before the inflammation was induced.	0.086 ml	0.08 ml	0.046 ml
	Administration on the day when the inflammation was induced.	0.11 ml	0.09 ml	0.09 ml
T. Majus volatile oil	Administration 5 days before the inflammation was induced.	0.132 ml	0.96 ml	0.024 ml
	Administration 3 days before the inflammation was induced.	0.083 ml	0.080 ml	0.06 ml
	Administration on the day when the inflammation was induced.	0.1 ml	0.08 ml	0.00 ml
Preparation based on penicillin	Administration 5 days before the inflammation was induced.	0.12 ml	0.11ml	0.09 ml
	Administration 3 days before the inflammation was induced.	0.113 ml	0.1 ml	0.086 ml
	Administration on the day when the inflammation was induced.	0.11 ml	0.11 ml	0.09 ml
Preparation based on cephalosporine	Administration on the day when the inflammation was induced.	0.114 ml	0.1 ml	0.056 ml
Control		0.1 ml	0.11 ml	0.09 ml

Table 3. Percents of inhibition of the inflammatory edema for the studied substances.

Preparation	Dose mg / kg body mass	Edema inhibition after 2 h %	Edema inhibition after 4 h %	Edema inhibition after 24 h %
Distilled water	–	–	–	–
Preparation based on chloramphenicol	1.4	28.57	48.71	34.41
T. majus volatile oil	1.4	31.42	18.20	11.18
Preparation based on penicillin	1.4	24.28	24.35	21.42

Table 4. Visual evaluation of the effects of volatile oil in the reduction of irritation produced by SLS.

Preparation	*T. majus* volatile oil	Preparation based on chloramphenicol	Preparation based on penicillin	Witness
Erythema	0	0.5	1	0
Desquamation	0	0	0	0
Edema	–	–	–	–
% of mice with a positive response	85%	75%	25%	100%
Observations	15% of the subjects presented redness, which disappeared within an hour after the application of the preparation	25% of the tested subjects presented edema or diffuse redness or symptoms that disappeared several hours after the application of the preparation	80 % of the tested subjects presented very evident edema, skin irritation, erythema that persisted for 24 h	–

Sodium lauryl sulphate (SLS) is a well-known tensioactive agents used as an irritation model.

Table 5. Visual evaluation of the appearance of the irritated skin after the application of the *T. majus* volatile oil treatment.

Preparation	after 30min	after 1h	after 3h	after 5h	Observation
T. majus volatile oil	+60% (9 out of 15)	+50% (3 out of 6)	+66.6% (2 out of 3)	0% (1 out of 1)	The integrity of the hydric layer was improved right after the application of the product; the effect persists more than 3 h
Preparation based on chloramphenicol	13,3 (2 out of 15)	+15.38% (2 out of 13)	+45.45% (5 out 11)	Does not vary	This preparation changes significantly the hydric barrier functions of the skin
Preparation based on penicillin	–35% (4 out of 15)	–70% (2 out of 11)	–15% (3 out of 9)	Does not vary	We observe a hydric degrading, correlated to an even greater evaporation of water in the first hour after the application of the product

Initial number of mice: 15. n = number of mice.

becomes visible. In the case of smaller concentrations than 256 µl, the microbial cultures grow. Regarding the qualitative analysis of the CMI values, the most active compounds from the first series (Table 6) proved to be those from the mix containing 1024 µl, respectively 512 µl volatile oil, which presented an antimicrobial action against all tested species, with a constant value of CMI of 256 micrograms / ml. The followers are the mixes of the

Table 6. Antimicrobial susceptibility pattern of essential (10 µl/disc) oil, aqueous solution, methanol extract, ethanol extract and n–hexane extract (300 µg/disc) of *T. majus*.

Microorganism	Inhibition zone diameter (mm)					Synergistic activity of mixture in relation to 1:1:1:1:1(µg/ml)
	Essential oil	Aqueous Extract(Aq)	Methanol extract(Me)	Ethanol extract(Et)	Hexane extract(He)	
Gram (–)						
E. coli	28 ± 0.5	13 ± 0.5	18 ± 0.3	16 ± 0.4	17 ± 0.3	36 ± 0.5
Salmonella sp.	24 ± 0.5	11 ± 0.5	19 ± 0.5	20 ± 0.5	22 ± 0.5	34 ± 0.5
P. aeruginosa	29 ± 0.5	12 ± 0.3	16 ± 0.5	23 ± 0.5	19 ± 0.5	32 ± 0.5
Gram (+)						
L. monocytogenes	34 ± 0.5	19 ± 0.5	21 ± 0.2	25 ± 0.5	21 ± 0.3	35 ± 0.7
S. aureus	29 ± 0.5	10 ± 0.3	18 ± 0.3	19 ± 0.4	23 ± 0.5	35 ± 0.7
B. subtilis	27 ± 0.4	19 ± 0.3	17 ± 0.4	16 ± 0.3	22 ± 0.3	31 ± 0.5
Diploid fungus (a form of yeast)						
C. albicans	35 ± 0.5	23 ± 0.5	19 ± 0.5	22 ± 0.5	12 ± 0.3	42 ± 0.7

Average value ± SD, n=3 (the zone of inhibition in millimetre including disc of 6 mm in diameter). Solvent was negative.

second series, the 512 µl volatile oil mix with the 512 µl methanolic extract of *T. majus*; the 512 µl volatile oil with the 512 µl ethanolic extract of *T. majus*; and the 256 µl volatile oil and 256 µl hexanolic extract of *T. majus.* All these present a good antimicrobial action against the majority of the tested species, except for *E. coli* and *S. aureus* (CMI varying between 64–256 micrograms/ml). The other mixes tested presented variable levels and ranges of antimicrobial action. The microorganism that is most sensitive to the action of the tested natural compounds of *T. majus* proved to be *P. aeruginosa* and *C. albicans*, followed by *Salmonella* sp. and *Bacillus* sp, while the most resistant is the *E. coli* stem.

The activity of these oils over the Gram–positive bacteria included in the test was superior or similar to the action of standard antibiotics ampicillin and chloramphenicol. All the tested oils presented an antifungical action similar or better than the action of the standard benchmarks carvacrol and tymol. Volatile oil seems to be active in the 1024 and 512 µl concentrations employed. The aqueous extract of *T. majus* presented a weak action, whereas the alcoholic and hexane extracts of the same plant proved to be active. We observed an antilevuric action of the same concentrations, but also of the mixes of volatile oil and extracts, both alcoholic and hexanic, except the mix of volatile oil and extract aqueous of *T. majus.* The volatile oil obtained as descrybed in the previous paragraphs is applied on the mouse model with cutaneous irritation and the evolution of the area is observed in time. After a few applications, we can observe a visible improvement.

Table 8 compares the treatment with *T. majus* volatile oil with the use of other pharmaceutical products that contain zinc compounds (such as zinc oxide and zinc sulphate) used as antimicrobial. This behaviour is explained through the complexation of proteins by the tannins contained by the *T. majus* volatile oil, which leads the tissue to become denser, to contract, so that the surface of the lesion concentrates and the capillaries' are obturated, which leads to local homeostasis. At the surface of the skin, a film of protein–tannins gets formed, impermeable and unbootable, which determines a diminution of secretions and, therefore, the local irritation is reduced.

The same film protects the nervous terminations by the action of some irritant stimuli, which explains the slightly local aesthetic effect. In the case of the product based on the *T. majus* volatile oil, it can be seen in the table pointing out the cutaneous reactions of erythema and escares: the result is 0, like for the formation of edema.

The growth of the number of types of bacteria resistant to classical chemotherapeutics and antibiotics (including penicillin) has imposed the necessity to investigate alternative ways in antibiotic therapy. The discovery of plants with potentially antimicrobial properties opened a new horizon in this field.

The tests done to evidence the antimicrobial action showed that the majority of the used solutions derived from the initial extract from *T. majus* volatile oil present low CMI values (mg/ml), which demonstrates an efficient antimicrobial and anti-inflammatory action. Based on these results, it was possible to demonstrate that the presence of cardiotonic glycosides in the *T. majus* volatile oil renders the volatile oil and it's aqueous, alcoholic and hexanes' extracts an anti-inflammatory and antimicrobial potential, our results being in line with specialty literature (Directive, 2000). The positive results are connected to the presence of tannins and saponins in the *T. majus* volatile oil. On local application, the tannins act as astringents, healing, antiexudative, anti–irritative, anti-inflammatory,

Table 7. Minimal inhibitory concentration (MIC) of the natural compounds obtained from *T. majus* on the various microbial stems, using the micro dilution method.

Essential oil volume used and mixture		Microbial stem						
		E. coli	*Salmonella* sp.	*P. aeruginosa*	*L. monocytogenes*	*S. aureus*	*Bacillus* sp.	*Candida* sp.
Solvent	**µl oil**	**Inhibitory potential MIC/MBC (µg/ml) series I**						
DMSO	1024	256±0.12	256±0.37	256±0.11	256±0.14	256±0.05	256±0.24	256±0.15
DMSO	512	256±0.08	256±0.21	256±0.5	256±0.24	256±0.17	256±0.12	256±0.23
DMSO	256	512±0.15	>1024	256±0.11	1024	512	>1024	1024
DMSO	128	512±0.43	>1024	>1024	>1024	>1024	>1024	>1024
DMSO	64	1024	>1024	>1024	>1024	>1024	>1024	>1024
DMSO	32	1024	1024	1024	>1024	1024	256±0.18	>1024
DMSO	16	1024	1024	1024	>1024	1024	256±0.21	512
DMSO	8	>1024	>1024	>1024	>1024	>1024	256±0.20	>1024
Extract	**(µl)**	**Inhibitory potential MIC/MBC (µg/ml) series II**						
Hexane extract	1024	256±0.17	256±0.12	32±0.12	>1024	32±0.24	>1024	64±0.18
Methanol extract	1024	128±0.02	256±0.17	32±0.20	>1024	>1024	>1024	128±0.40
Ethanol extract	1024	1024	256±0.14	32±0.02	>1024	32±0.23	>1024	128±0.40
Aqueous extract	1024	512	512	32±0.01	1024	32±0.08	>1024	1024
Aqueous extract	512	>1024	>1024	256±0.06	>1024	>1024	>1024	256±0.35
Aqueous extract	512	>1024	>1024	256±0.03	>1024	>1024	>1024	256±0.14
Methanol extract	512	>1024	256±0.03	256±0.21	256±0.22	>1024	256±0.13	128±0.09
Ethanol extract	512	>1024	256±0.04	256±0.04	256±0.15	>1024	128±0.15	64±0.40
Hexane extract	256	>1024	256±0.05	256±0.05	256±0.20	>1024	128±0.23	64±0.25
Methanol extract	256	>1024	>1024	256±0.17	128±0.05	>1024	>1024	128±0.03
Ethanol extract	256	>1024	>1024	>1024	>1024	>1024	>1024	256±0.15
Aqueous extract	256	>1024	>1024	>1024	>1024	>1024	>1024	>1024
DMSO		256	256	256	256	256	256	256

n-3 MIC = minimal inhibitory concentration; MBC - minimal bacterial concentration

Table 8. Comparison between the treatment with *T. majus* volatile oil and other pharmaceutical compounds.

Edema formation	Number of day				
	Serious edema	Moderate edema	Light edema	Very light edema	No edema
Product used	**day**	**day**	**day**	**day**	**day**
Product A	0	3	5	6	7	10
Product B	0	4	5	5	7	8
T. majus volatile oil	0	2	3	4	5	7

Results

Product A	2 – 3
Product B	2
T. majus volatile oil	0

antiseptic, anesthesic and antioxidant. Tannins form complexes with the proteins from the superficial layers of the skin, leading to the formation of a protective layer of protein-tannins. Moreover, the tannins act as antiseptics through the precipitation of the proteins from the membrane of the microorganisms and anti-inflammatory through the inhibition of the synthesis of prostaglandins and the freeing of the plachetary activating factor (PAF) (Mahesh et al., 2008). Saponins, which from a structural point of view are glycosides, have an antiseptic and antimicrobial action, in a non-harmful way for the neighboring cellular tissues. The specialty literature draws attention to

the presence of flavonoids in the leaves of *T. majus.* The compounds form the volatile oil can have various reactions depending on the concentration of these metabolites, which in turn depends on the extraction conditions, the place in which the plant was cultivated or the timeframe in which it was cropped (Akinmoladun et al., 2007). Flavonoic derivates develop anti-inflammatory effects (mainly by inhibiting the freeing of lisosomal enzymes and reducing the level of oxygen–reactive species) (Casadevall et al., 2001), anti–allergic effects (by inhibiting the classical way the seric complement is activated), anti-microbial, capillary-protective and anti-oxidant effects (FDA, 2006). Although, the antimicrobial properties of the volatile oils and their components have been studied in the past, the mechanism of their action has never been studied in detail (Arai et al., 2005). Taking into account the large number of the different groups of chemical compounds present in the composition of the volatile oils, it is very possible that their antimicrobial activity cannot be assigned to a sole mechanism (Flayhart, 2007), but to the existence of a large number of target locations in the cell. Not all these mechanisms represent separate targets; some are consequences of other target mechanisms (Chatterjee et al., 2010). The chemical structure of the individual compounds of volatile oils affects in their specific ways the antibacterial action.

Thus, the presence and the position of the hydroxyl group among the phenolic acids like carvarol and thymol affects to a wide extent the degree of the antibacterial action.

The anti-infectious/antimicrobial action of the *T. majus* volatile oil is to a great extent given by the esthers of the phenolic acids with aromatic alcohols (Harris et al., 2010).

The cetones, other natural compounds of the *T. majus* volatile oil, have important actions over organisms (Nam and Jin, 2010), such as anti–infectious action (antibacterial, antiviral, antifungic, antiparasitary) (Paus et al., 2006), an action based on the biological nucleophilia of cetones towards the amino and tyol groupings, more exactly towards some active situses of some enzymes essential to their lives, inducing irreversible alchilations which lead to the interruption of the multiplication of the infectious agents (Peters et al., 2005).

Phenolic heterosides, contained in the *T. majus* volatile oil, (Jentschel et al., 2007) have the role of solubilising lipophilic metabolites (Ribeiro et al., 2007) so that these can then diffuse from the plasma to the vacuoles or through the cellular membranes, reaching the reserve organs (Matallana et al., 2006)

Conclusions

We have approached several methods to obtain natural compounds out of *T. majus* plants in order to determine the extract with the best biological action. Results indicated the fact that there are no significant differences between the extracts obtained from leaves or flowers regarding the detected compounds, so the mix of the aerial parts of the plant was considered optimal for ulterior analysis.

Following the investigations on the volatile oil obtained from the aerial parts of the *T. majus* species, we highlighted the characteristic compounds with antimicrobial and anti-infectious properties. We obtained and characterised through chromatographic methods the following series of compounds: Fatty acids, docosahexaenoic acid, sterols, sterol esters, carotenoid pigments, hetero–transglicosilating; neoxanthin; saponins; bis–desmosides; mono–desmosides; hederagenol; flavonoids; tromalyt and tannins. From the qualitative methods used to control the antimicrobial activity, the method of diffusion on filter paper disc proved most efficient, with results correlating well with the MIC. In the case of the quantitative analysis of the antimicrobial analysis of the compounds tested through the micro dilution in liquid environment method, the development of the microbial cells was stopped by all the tested compounds. At lower concentrations (256 µl essential oil) of natural compounds, the microbial culture becomes visible. The studied effects show that the majority of the used solutions derive from the initial extract of *T. majus* volatile oil present low CMI values (mg/ml), which demonstrated an efficient antimicrobial action. Thiosulphinates act as antimicrobials' and anti-inflammatory. The antimicrobial activity of the thiosulphinates has been explained through their possibility to react with the –SH groupings of the bacterial proteins, the result being the perturbation of the bacterial metabolism and, finally, the death of the bacterial cell. The anti-inflammatory effects of the thiosulphinates originate in their capacity to inhibit the synthesis and the freeing of pro-inflammatory mediators.

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