academicJournals

Vol. 7(23), pp. 1736-1745, 17 June, 2013
DOI: 10.5897/JMPR2012.1203
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Study of the composition of *Thymus vulgaris* essential oil, developing of topic formulations and evaluation of antimicrobial efficacy

Gisele Mara Silva Gonçalves[1]*, Silvana Mariana Srebernich[2], Neura Bragagnolo[3], Elisângela Serenato Madalozzo[3], Vania Leandro Merhi[2] and Denise Cristina Pires[1]

[1]School of Pharmaceutical Science, Pontifícia Universidade Católica de Campinas, Brazil.
[2]School of Nutrition, Pontifícia Universidade Católica de Campinas, Brazil.
[3]School of Food Engineering, UNICAMP, Brazil.

Accepted 11 January, 2013

The essential oil of *Thymus vulgaris* has been the subject of extensive studies and developing formulations for the placement of essential oils is not an easy task due especially to it high volatility and low stability. The aims of this work were to study of the composition of *T. vulgaris* essential oil, development of topic formulations and evaluation of antimicrobial efficacy on *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa.* The composition of the oil was analyzed using gas chromatography/mass spectrometry. Microbiological disk diffusion and microdilution tests were carried out to determine the minimum inhibitory and minimum bactericidal concentrations. The substance was added to a cream gel formulation which underwent thermal stability, physical and chemical-physical tests (in addition to a drop test) to verify if the antimicrobial effects remained unchanged. The most abundant compounds were geraniol, thymol, gama-terpinene, para-cymene, citral, 3-octanone, and 3-octenol. The essential oil in formulation had similar antimicrobial effects in comparison to the essential oil itself; the formulation was partially stable during the study period. Formulations with *T. vulgaris* essential oil effectively inhibited microbial growth. The results show a reasonable stability of the formulation. The topical use of essential oil from thyme is a promising alternative for cosmetic and phytotherapeutic use.

Key words: Antimicrobial, drip, essential oil, phytocosmetic, *Thymus vulgaris*.

INTRODUCTION

Plants have been used for therapeutic purposes since the most remote of times. The extraordinary biodiversity of Brazil represents a promising source of substances for this purpose. In this sense, the popular use of plants for specific purposes may stimulate scientific studies to investigate substances, and consequently, establish their therapeutic use. The therapeutic use of plants may also help to raise awareness about preservation of the environment and the sustainable use of plants (Simões and Schenkel, 2002).

Like other plant-derived medications, the action of phytotherapeutic compounds comes from the mixture of many active substances that are highly effective (Isaac et al., 2008). Phytotherapeutic medications use derivatives of vegetable drugs, such as extracts, dyes, waxes, juices and/or essential oils (Brazil, 2004a).

*Corresponding author. E-mail: gmsg@puc-campinas.edu.br. Tel: +5501933436865. Fax: +550933436806.

Phyotherapeutic medications have been shown to have proven therapeutic effect and quality and have standardized compositions; also, medication safety can be ensured if ethnopharmacological, pharmacological and toxicological studies are carried out and if the raw materials and finished products are well controlled. The stability and effectiveness of the medications must also be evaluated (Brazil, 2004b).

Thymus vulgaris, popularly known as thyme, is a subshrub, woody plant; it is ramified at the base and small, with small oval and opposing leaves. Thyme is a native plant of the dry and hot soils of the Mediterranean. It is also cultivated in some European and American countries, such as Brazil. It is a very popular aromatic herb used as condiment in many dishes (Jakiemiu et al., 2010). The aromatic property of thyme is a result of a great variety of volatile substances, especially in the aerial parts of the thyme plant. Thyme is used for cooking and by popular medicine for the treatment of many maladies. Its use has been subjected to scientific investigation. Studies have found terpenoids in the plant, whose major components in the plant are p-cymene and thymol. The components are extracted by steam distillation and, when combined, constitute the essential thyme oil. The oil has been shown to have antimicrobial activity. These properties are associated with its popularity (Simões and Schenkel, 2002). The objective of the present study was to obtain *T. vulgaris* essential oil and evaluate its composition, antimicrobial activity, and vehicle on a formula prepared for topical treatment; the goal was to also evaluate its stability.

MATERIALS AND METHODS

Extraction of the essential oil of *T. vulgaris*

Samples of *T. vulgaris* were collected in August and September 2011, on a commercial plantation that provides thyme for cooking. This plantation is located in the city of Jundiai, Sao Paulo, Brazil. The plant was authenticated at the Pontifícia Universidade Católica de Campinas, Campinas, Brazil. Throughout the study, nine extractions were performed. Each extraction used approximately 200 g of the aerial parts of the plant (about 4 to 5 plants); the plant was previously washed in tap water and extracted by steam distillation, according to the Brazilian Pharmacopeia V (Brazil, 2010). The liquid-liquid partitioning of the hydrolate was carried out with chloroform (three 50 ml portions) in a separatory funnel (Silva, 2010). The organic fraction (chloroform and essential oil) was treated with anhydrous sodium sulfate in order to remove the remaining moisture. Next, the fraction was filtered with filter paper and the remaining contents were transferred to a round-bottom flask. Evaporation of the solvent was carried out in a rotary evaporator at 50°C and 40 rpm for 30 min.

Characterization and stability assessment of the essential oil

The essential oil was described in terms of volume, color, aspect, intensity and odor; the odor and pH of the oil were compared to those of the fresh plant (Brazil, 2004b, 2007). The oil was stored in an amber vial and frozen at -18°C for 120 days with weekly reassessments (Duarte et al., 2005).

Determining compounds in the oil of *T. vulgaris*

Three samples of thyme essential oil obtained by hydrodistillation were analyzed using gas chromatography-mass spectrometry (GC-MS). Shimadzu GC-MS model QP 2010 Ultra equipped with the AOC 5000 autoinjector was used; the following chromatography conditions were used (Nguefack, 2012): fused-silica capillary (Rtx 5MS; 30 m × 0.25 mm × 0.25 µm); helium carrier gas at 1 ml/min; injection of 1 µl of essential oil of thyme and a split ratio of 1:20; the injector operated at 220°C, the interface at 280°C. The column temperature increased from 40 to 300°C at a rate of 7°C min^{-1}. The mass ratio analyzed was 33 to 500 m/z. The identification of compounds was based on retention times, retention index (Kovats retention index) of compounds (relative to C_6 to C_{25} series of n-alkanes). Volatile compounds were identified by comparison of Kovats Retention Index obtained in this study with the literature (Golmakani and Rezaei, 2008; Gavahian et al., 2012) and the similarity of mass spectra between the compounds and the record for mass spectra data (Wiley 9th edition). Standard deviation (SD) and relative standard deviation (RSD) of Kovats Retention Index were calculated. The analysis of the hydrocarbons series, for the calculation of Kovats Retention Index, was carried out with the same methodology used for analyzing the samples, that is, using set temperature and Rtx 5MS column. The calculation was made according to the IUPAC (Compendium of Chemical Terminology, 2nd ed. (The "Gold Book") compiled by AD McNaught and A. Wilkinson) Blackwell Scientific Publications, Oxford (1997).

Antimicrobial activity

Microorganisms commonly present on skin microbiota were selected for evaluation of essential oil antimicrobial activity. Initially, a test of microbial susceptibility was carried out by disk diffusion; next, a microdilution test was performed (Kalemba and Kunicka, 2003; Sawaia et al., 2002).

Inoculants

The microorganism strains used were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 13525. Inoculants were prepared using 24 h cultures of the microorganisms in BHI agar (Oxoid™), which were resuspended in sterile 0.85% saline solution mixed by a vortex mixer. Readings were carried out using a spectrophotometer with a wavelength of 635 nm to obtain the absorbance value between the interval of 0.08 and 0.1, which corresponds to the interval of 1 to 2×10^8 CFU/ml; this is equivalent to the 0.5 standard of the Mac Farland scale (Probac Brazil™) (Sawaia et al., 2002).

Disk diffusion test

The seeding was plated on Mueller-Hinton agar (Merck™), with a pH 7.4 and with the aid of a swab saturated with the inoculants. Next, a sterile disk of 6-mm diameter filter paper was transferred to the surface of the inoculated culture medium. The disk was impregnated with 10 µl of essential oil. This test was carried out twice. A positive control was also prepared: each strain was seeded on a plate without filter paper containing essential oil. The samples were incubated for 24 h at 35°C in a bacterial incubator. The reading of the plates and of the size of the inhibition halos around the disks were carried out (NCCLS, 2003; NCCLS, 2005).

Microdilution test

The essential oil was emulsified in polysorbate 80 and diluted in Mueller-Hinton broth (Oxoid™) at a pH of 7.3; the concentrations of essential oil varied from 0.5 to 5.0% (v/v). For the first ten orifices of the microdilution plate, 180 µl were transferred from each of the 0.5 to 4.5% concentrations; subsequently, then added 20 µl of the inoculants. The last two orifices were used for positive and negative controls. Controls were created using a benzathine benzylpenicillin standard at a concentration of 100 U/ml for test validation (Bezzera et al., 2006). After the 24 h incubation period at 35°C, a subculture of the contents was produced with the orifices of the plates containing half Mueller-Hinton agar (Merck™) (incubated for 24 h at 35°C); subsequently, the readings were carried out. Triphenyl tetrazolium was used for the reading of the microdilution plate. Triphenyl tetrazolium changes the color of the medium if there is bacterial growth, thus facilitating the reading. The minimum inhibitory concentration (MIC) corresponded to the lowest concentration of the oil that inhibited visible bacterial growth in the microdilution plate. The minimum bactericidal concentration (MBC) corresponded to the lowest concentration of the oil that inhibited visible growth in the subculture of the microorganism (NCCLS, 2003). These two tests were carried out three times.

Development and assessment of the formulations

Two cream gel formulations were prepared. The first base formulation (formulation A) consisted of hydroxyethylcellulose (2.0%), glycerin (5.0%), (commercial mixture) phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben (0.6%), glycerin (silicone microemulsion), diphenyl dimethicone, triethylhexanoin, water, polyglyceryl-10 myristate (3.0%) and distilled water (q.s.p. 100); the second base formulation (formulation B) consisted of glycerin (3.0%), phenoxyethanol (commercial mixture), methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben (0.6%), jellifying polymer (ammonium acryloyldimethyl taurate/vp copolymer) (1.5%) and distilled water (qsp 100). Fresh essential oil at a concentration of 4.5% was added to the formulations; then, stability studies were carried out.

Centrifugation test

The fresh formulations were centrifuged at 3000 rpm for 30 min to verify if phase separation occurred (Silva and Campos, 2000; Brazil, 2004b, 2007).

Stability tests

The pH, homogeneity, color, odor and viscosity were determined for the samples submitted to centrifugation. Samples were stored in PVC jars for creams, and in glass vials with glass caps. Samples were stored at the following temperatures: 4, 25 (room temperature), 40 and 60°C, for fifty days. The parameters were reevaluated weekly and every 21 days; thermal stability was also determined (Silva and Campos, 2000; Brazil, 2004b, 2007). On test days, all the formulations were kept under room temperature for a few hours to allow acclimation before the tests were performed.

pH

The pH of the formulation (diluted to 10% with distilled water) was determined using a pH meter (Silva and Campos, 2000; Brazil, 2004b, 2007).

Organoleptic and visual evaluation:

The formulations were evaluated to determine if their homogeneity remained stable and if there were changes in color and odor (Silva and Campos, 2000; Brazil, 2004b, 2007).

Assessment of physical stability by viscosity and rheologic behavior

The apparent viscosity of the formulations (stored in glass vials with glass caps) was determined using a Brookfield RVII™ viscometer fitted with spindle number 6. The speed was increased every minute (1, 2, 2.5, 4 and 5 rpm). Next, the ascending rheological curve was constructed. Subsequently, the speeds were decreased at every minute (5, 4, 2.5, 2 and 1 rpm) and the descending rheological curve was constructed (Isaac et al., 2008; Brazil, 2007).

Thermal stability assessment by differential scanning calorimetry (DSC)

Differential scanning calorimetry equipment Shimadzu™ was used (model DSC-60), at a constant heating rate of 10°C min^{-1} until a maximum temperature of 180°C to determine the thermal stability of the samples and their respective thermograms (Isaac et al., 2008; Silva et al., 2007).

Assessment of the antimicrobial activity of the formulations by the drop test

Formulations were prepared without preservatives in order to avoid affecting the results. The same inoculants were used. Seeding was done on the agar surface with a swab impregnated with the inoculants in suspension. The formulations were dripped with the aid of a disposable 5 ml syringe, and incubated at 35°C for 24 h. The readings included measuring the diameters of the inhibition halos around the drops of the formulation (Note: the drop test is similar to the disk diffusion test). A standardized amount of the formulation studied (0.2 g) was dripped over the inoculated culture medium, which was then incubated without the inversion of the plate so that the formulation remained where it was dripped. After the appropriate incubation, a slide caliper was used to determine the diameter of the microbial growth inhibition halo.

RESULTS AND DISCUSSION

The extraction process allowed roughly 0.5 ml from each 200 g sample of fresh plant. The amount of oil obtained was below (though it was approximate to) the minimum percentage of 1.0% of the volatile oils for thyme, in the form of dry plant (ISO 6754:1996). The lower results may be due to the differences in the conditions of the extraction, the degree of hydration and age of the plants, and the use of different solvents.

The color of the essential oil was a light yellow; it had a translucent aspect and was slightly viscous. The oil had the intense odor highly characteristic of the *T. vulgaris* plant. The pH was close to 6.2. After 90 days of storage, the pH was 7.2 and after 120 days of storage, the pH rose to 8.4. The yellow color became slightly more intense; this indicates that there was some loss of

stability. The other parameters did not change. It is necessary to carry out future studies on ways to prevent the change in pH; it is important to prevent changes in pH in order to ensure greater stability to the formulation containing the oil.

Sixty four volatile compounds were identified in the essential oil (Table 1 and Figure 4). The analyses for the identification of the compounds have been performed using only Rtx 5MS column, according to the literature to choose the best method for the determination of such compound recommended in the column (Jordán et al., 2006). The most abundant compounds were geraniol, thymol, gama-terpinene, para-cymene, citral, 3-octanone, and 3-octenol. The composition differed slightly from that reported by others (Tohidpour et al., 2010), who said that thymol was the major component in a sample that inhibited *S. aureus*. As thyme presents different chemotypes, according to the growing conditions, which results in different compositions of the essential oil and directly reflects on the effects (in inhibition of *S. aureus*), it does not reject the possibility of having used a mixture of collections, reflecting the addition of different chemotypes.

Microbiological tests were carried out to determine the minimum concentration of essential oil necessary for efficient antimicrobial effect. The only strain that presented resistance to the oil was *P. aeruginosa* (microbial susceptibility by diffusion in agar); the resistance was determined due to the inexistence of an inhibition halo. Consequently, the strain was removed from further testing. For the other strains, the diameters of the halos were 10 mm for *E. coli*, 14 mm for *S. aureus* and 16 mm for *S. epidermidis*. The results indicate that the strains were sensitive to the oil. Other researchers have dissolved the essential oil with methanol or ethanol for the disk diffusion test; in those studies halos with larger diameters were obtained. The presence of solvents facilitates diffusion, which explains the larger halos (Santurio et al., 2011).

After preliminary studies to determine the concentration range, microdilution tests were carried out to determine the MIC for the strains. In the second test, the essential oil inhibited the growth of *S. aureus* and *S. epidermidis* when the minimum concentration was 4.0%, and it inhibited the growth of *E. coli* when the minimum concentration was 4.5% (Table 2). The results show that the essential oil should be used at a concentration of at least 4.5% to produce effective antimicrobial activity against the three strains.

A review of recent research on essential oils used as antimicrobial and antifungal agents suggested methods of evaluation. The methods suggested included the agar diffusion test and the broth dilution test; for a sample of *T. vulgaris*, the MIC was 18.5 µg/ml for methicillin-resistant *S. aureus*. The concentration is well below that which was found effective in the present study (Lang and Buchbauer, 2011).

The essential oil was incorporated in the two formulations at a concentration of 4.5%. Two emulsions were needed. Emulsion was the pharmaceutical form of preference, because of the lipophilic nature of the essential oil. In the form of cream gel, emulsions can be applied to all skin types provided that the formulations pass all tests.

After the centrifugation test, only formulation B remained stable and was selected to undergo the stability studies. The addition of essential oil to formulation B did not change its pH (pH 5.5); however, this formulation became slightly yellowish, with an opaque aspect and odor of moderate intensity, characteristic of the essential oil. The rheological profile changed slightly (Figure 1). The apparent viscosity of the formulation decreased; however, the formulation continued to present a pseudoplastic profile and stable rheological behavior. The viscosity of the base formulation decreased after the essential oil was added (Figure 1). This was expected because the essential oil has low viscosity.

After a storage period of seven days at 60°C, the formulation lost stability, darkened and became less viscous. Essential oils, in general, are not stable at high temperatures. The darkening of the oil indicates the components of the formulation; in this case, the oil may have oxidized. The base formulation (control) did not present any changes in aspect when stored for the same period and under the same temperature. No further tests were carried out using this temperature.

The samples were stored at room temperature for acclimation before the tests in order to avoid interference caused by temperature differences.

The sample stored at 4°C for 50 days remained unchanged, except for its odor, which became slightly milder when compared with the characteristics of the base formulation right after the essential oil was added. The yellow coloration of the sample stored at 25°C became slightly more intense, but the other parameters remained unchanged. The sample stored at 40°C darkened; it turned a light brown color and the characteristic odor became stronger, but the other parameters remained unchanged (Table 3). These results indicate a small loss of stability, especially when it comes to an essential oil.

During the 50-day storage period, all the formulations became less viscous. The formulation stored at 40°C lost more viscosity than the other formulations, thus indicating that this is not an optimal temperature for storage (Figure 2).

The profiles of the curves in rheograms b, c and d after the storage period remained similar to that of the initial formulation. Thus, the cream gel has a pseudoplastic rheological behavior. The formulation appeared to be rheologically stable; only minor changes occurred when the formulation was stored at room temperature (25°C), the most common storage temperature. However, given the lower reduction in viscosity presented by the formu-

Table 1. Volatile compounds in essential oil from thyme.

Peak[a]	Compound	RT (min)	Kovats retention index	SD	RSD (%)	Peak identification
1	3-hexenol	5.213	854.62	0.05	0.006	RI + MS
2	tri-cyclen	6.565	924.80	0.03	0.003	RI + MS
3	alpha-thujene	6.638	928.27	0.05	0.005	RI + MS
4	alpha-pinene	6.803	936.13	0.05	0.005	RI + MS
5	camphene	7.132	951.83	0.03	0.003	RI + MS
6	sabinene	7.642	976.12	0.05	0.005	RI + MS
7	3-octenol	7.707	979.04	0.24	0.025	RI + MS
8	3-octanone	7.872	986.96	0.19	0.020	RI + MS
9	beta-myrcene	7.968	991.58	0.12	0.012	RI + MS
10	3-octanol	8.046	995.30	0.15	0.015	RI + MS
11	alpha-phellandrene	8.305	1007.42	0.09	0.009	RI + MS
12	delta-3-carene	8.442	1013.74	0.07	0.007	RI + MS
13	alpha-terpinene	8.570	1019.61	0.10	0.009	RI + MS
14	para-cymene	8.752	1027.84	0.24	0.023	RI + MS
15	sylvestrene	8.841	1032.09	0.10	0.009	RI + MS
16	1,8-cineole	8.912	1035.39	0.07	0.007	RI + MS
17	cis-ocimene	8.976	1038.34	0.07	0.007	RI + MS
18	beta-ocimene	9.210	1049.12	0.07	0.007	RI + MS
19	gamma-terpinene	9.506	1062.52	0.28	0.026	RI + MS
20	*cis*-sabinene hydrate	9.684	1071.00	0.03	0.002	RI + MS
21	3-nonenol	9.878	1079.93	0.03	0.002	RI + MS
22	3-nonene	10.042	1087.50	0.03	0.002	RI + MS
23	alpha terpinolene	10.146	1092.25	0.05	0.005	RI + MS
24	linalool	10.331	1100.72	0.12	0.011	RI + MS
25	nonanal	10.426	1105.26	0.07	0.007	RI + MS
26	terpineol	10.876	1126.55	0.05	0.004	RI + MS
27	carene	11.266	1144.95	0.05	0.005	MS
28	E-citral	11.310	1147.03	0.05	0.005	RI + MS
29	3,4-octadienal	11.487	1155.41	0.05	0.004	MS
30	verbenol	11.721	1166.45	0.05	0.005	RI + MS
31	endo-borneol	11.872	1173.50	0.12	0.011	RI + MS
32	3-nonenol	12.021	1180.60	0.07	0.006	MS
33	4-terpineol	12.090	1183.85	0.08	0.007	RI* + MS
34	alpha-terpineol	12.355	1196.38	0.07	0.006	RI + MS
35	dihydrocarvone	12.499	1203.39	0.03	0.002	RI + MS
36	decanal	12.567	1206.73	0.08	0.006	RI + MS
37	9-para menthenol	12.682	1212.44	0.06	0.005	RI + MS
38	nerol (2,6-octadienol)	13.078	1231.96	0.17	0.014	RI + MS
39	thymol methyl ether	13.207	1238.49	0.06	0.005	RI + MS
40	Z-citral (2,6-octadienal)	13.364	1246.09	0.23	0.018	RI + MS
41	anisole	13.413	1248.57	0.19	0.015	MS
42	geraniol	13.683	1261.37	0.70	0.056	RI + MS
43	citral	13.966	1275.81	0.36	0.028	RI + MS
44	thymol	14.402	1297.27	0.52	0.040	RI + MS
45	carvacrol	14.559	1305.65	0.29	0.022	RI + MS
46	octadienoic acid	14.951	1326.36	0.15	0.011	MS
47	geranic acid	15.436	1351.93	0.16	0.012	MS
48	thymol acetate	15.570	1358.87	0.11	0.008	RI + MS
49	geraniol acetate	16.058	1384.46	0.11	0.008	RI + MS
50	beta bourbonene	16.301	1397.27	0.05	0.004	RI + MS

Table 1. Contd.

51	trans-caryophyllene	16.971	1434.22	0.10	0.007	RI + MS
52	alpha-humulene	17.593	1468.77	0.06	0.004	RI* + MS
53	geranyl propionate	17.715	1475.49	0.10	0.007	RI + MS
54	Germacrene D	18.076	1495.53	0.08	0.006	RI + MS
55	lepidozene	18.358	1511.92	0.10	0.007	MS
56	beta bisabolene	18.430	1516.19	0.10	0.007	RI + MS
57	beta- sesquiphellandrene	18.712	1532.94	0.09	0.006	RI + MS
58	delta cadinene	18.758	1535.71	0.06	0.004	RI + MS
59	butanoic acid	19.212	1562.61	0.07	0.004	MS
60	spathulenol	19.775	1595.97	0.10	0.006	RI** + MS
61	caryophyllene oxide	19.899	1603.53	0.07	0.004	RI + MS
62	geranyl propionate	19.932	1605.60	0.06	0.004	MS
63	alloaromadendrene	20.761	1657.06	0.16	0.010	MS
64	T-cadinol	21.009	1672.66	0.04	0.002	RI + MS

[a]Numbered according to Figure 4. [b]The values correspond to the mean of three samples. RT: Retention time; SD: Standard deviation; RSD: Relative standard deviation, RI + MS: Kovats Retention Index according to www.pherobase.com and mass spectrometry according to Willey9 library; RI* + S: Kovats Retention Index according to Golmakani and Rezaei (2008) and mass spectrometry according to Willey9 library; RI** + MS: Kovats Retention Index according to Gavahian et al (2012) and mass spectrometry according to Willey9 library; MS: Mass spectrometry according to Willey9 library.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oil of *Thymus vulgaris* for the different microbial strains. Each test was carried out three times.

Microorganism	MIC (% v/v)	MBC (% v/v)
S. aureus	4.0	4.0
S. epidermidis	4.0	4.0
E. coli	4.5	4.5

Table 3. Stability of the formulations containing essential oil of *Thymus vulgaris* stored at temperatures of 4, 25 and 40°C for 50 days.

Storage temperature (°C)	Parameter	Time (days)						
		7	14	21	28	35	42	50
4	pH	5.6	5.7	5.8	5.5	5.4	5.6	5.5
	Color	LY	LY	LY	LY	LY	LY	LY
	Odor	CM	CM	CM	CM	CM	CM	CM
	Aspect	HM	HM	HM	HM	HM	HM	HM
25	pH	5.7	5.6	5.5	5.3	5.4	5.5	5.6
	Color	LY	LY	LY	LY	SDY	SDY	SDY
	Odor	CM	CM	CM	CM	CM	CM	CM
	Aspect	HM	HM	HM	HM	HM	HM	HM
40	pH	5.4	5.3	5.4	5.3	5.4	5.6	5.5
	Color	SDY	SDY	SDY	DY	LB	LB	LB
	Odor	CM	CM	CM	CI	CI	CI	CI
	Aspect	HM	HM	HM	HM	HM	HM	HM

LY: Light yellow, SDY: Slightly darkened yellow, DY: Dark yellow, LB: Light brown, CM: Characteristic and moderate, CL: Characteristic and mild, CI: Characteristic and intense, HM: Homogeneous and monophasic.

Table 4. Mean melting point (°C) of the formulations containing essential oil of *Thymus vulgaris* stored at 4, 25 and 40°C for 50 days.

Storage temperature (°C)	Mean melting point (°C)	
	21 days in storage	42 days in storage
4	80.01	84.95
25	69.43	49.61
40	77.81	74.99

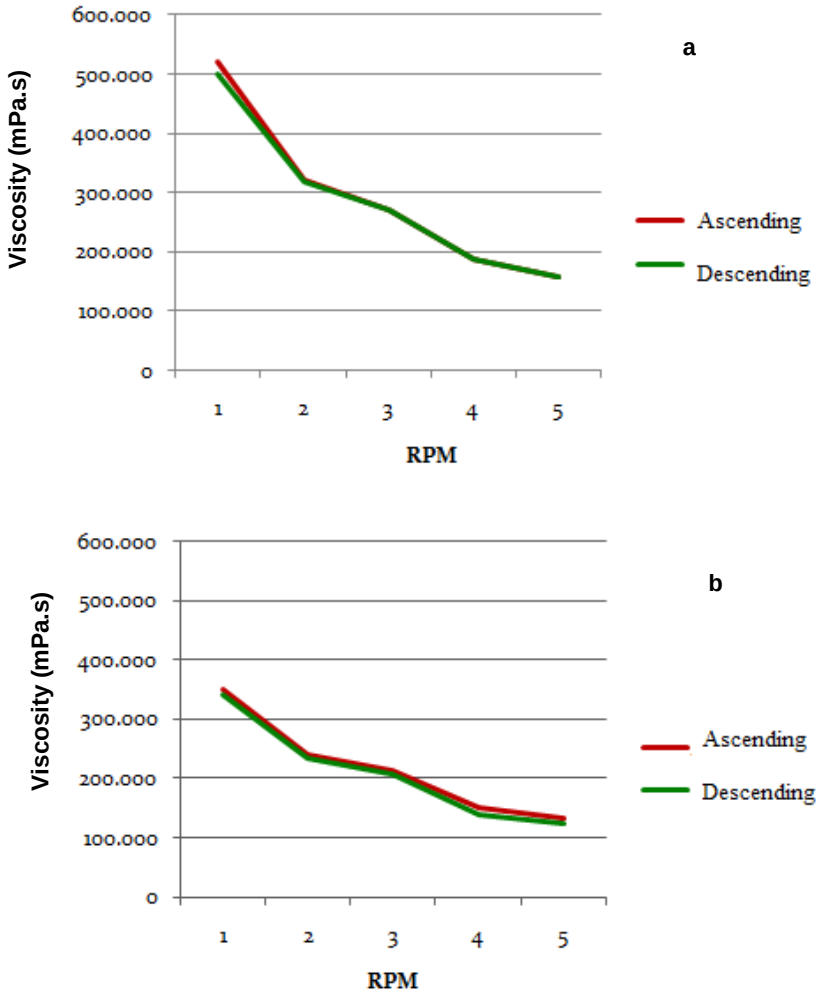

Figure 1. Rheograms of the base formulation without essential oil (a) and after the addition of the essential oil (b).

lation stored at 4°C, cooler temperatures might be more optimal for storage of the product.

The thermal behavior of formulation B changed after addition of the essential oil (Figure 3). The mean melting temperature of the formulation decreased from 80.29 to 65.04°C; this may be a result of the high volatility of the oil.

The thermal profile of all samples changed during the storage period (Table 4), therefore indicating changes in the physical-chemical properties and structure of the components of the formulation. The mean melting point of a formulation is one of the most important parameters. Given the considerable reduction of the mean melting point from 69.43 to 49.63°C (presented by the formulation stored at room temperature), reduction in stability probably followed. Therefore, the composition requires improvement and adjustments.

Almeida et al. (2010) assessed and compared the

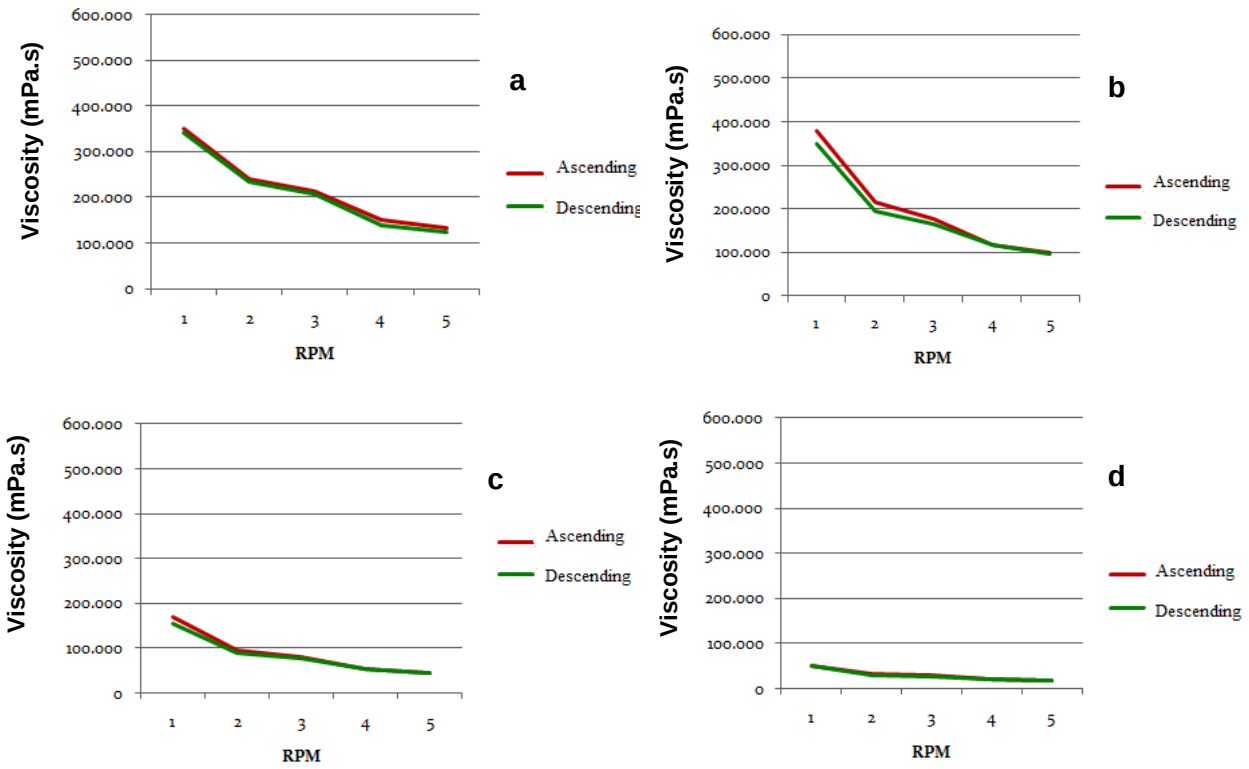

Figure 2. Rheograms of the formulation with essential oil: (a) initial, (b) after a 50-day storage period at 4°C, (c) after a 50-day storage period at 25°C, (d) after a 50-day storage period at 40°C.

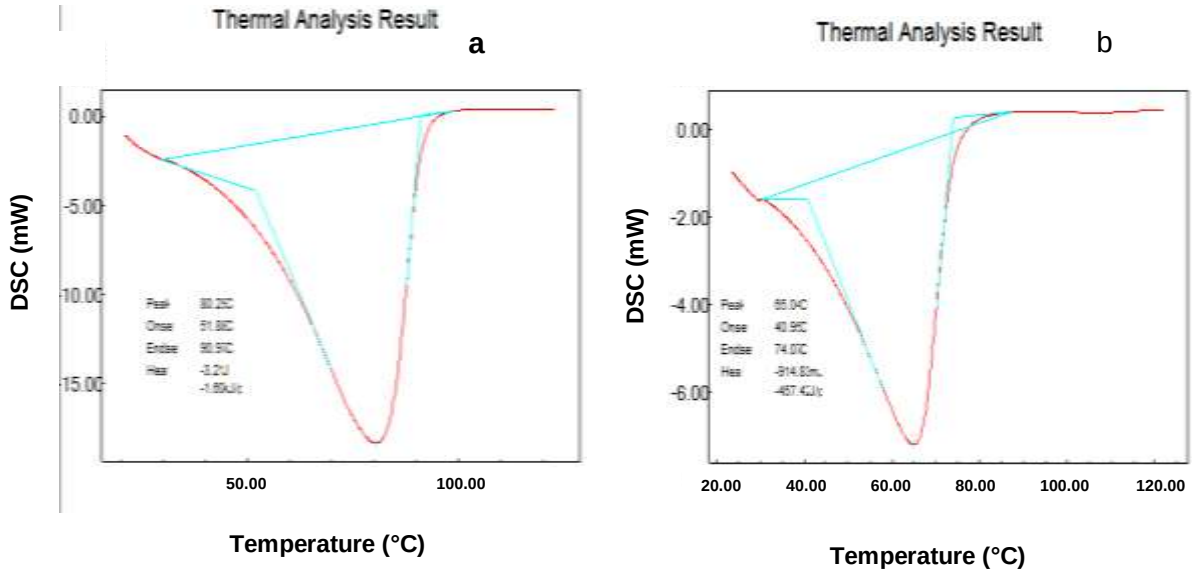

Figure 3. Thermograms (DSC curve) of the base formulation without the addition of the essential oil (a) and after the addition of the essential oil (b) obtained at a heating rate of 10°C min^{-1}.

thermal behavior of the active substances tocopheryl acetate and ascorbyl tetraisopalmitate. The substances were evaluated alone and in a base cream by DSC. The authors found that these vitamins remained stable in temperatures as high as 250°C. Thermal analysis proved to be an excellent tool for vitamin characterization. The base cream can be used in routine analysis for controlling the quality of this type of cosmetic formulation.

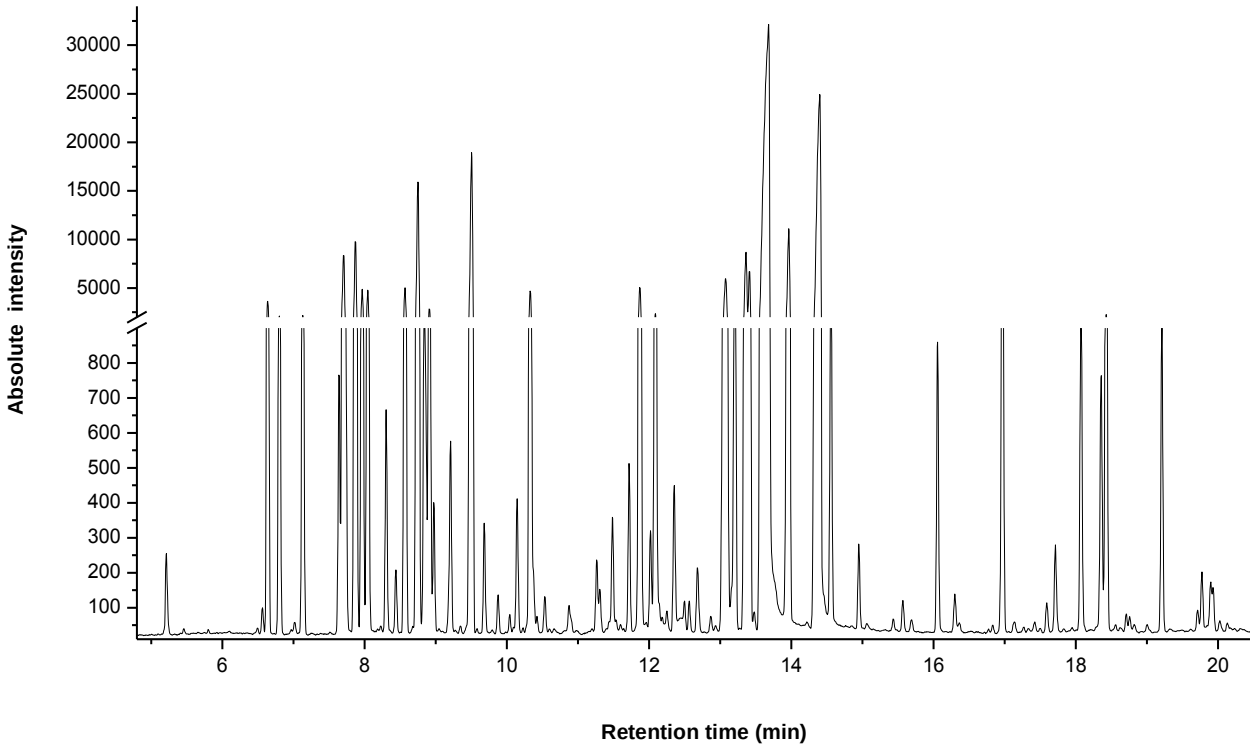

Figure 4. Chromatogram of thyme oil.

Comparison of the results with earlier results shows that changes took place. There were color changes and pH changes. Changes in the thermograms were not unexpected. It is not possible to state that the formulations are absolutely stable.

All the investigated parameters of the base formulations without essential oil remained stable. Therefore, the base components did not have a negative impact on the results of the stability tests.

The drop test evaluated if the essential oil of thyme kept its antibiotic properties when added to the formulations. The antimicrobial effectiveness remained unchanged, as shown by the inhibition halos of the bacterial strains. The antimicrobial action was not affected by of the components of the formulation. However, the halos were slightly larger than those observed in the disk diffusion test; the difference may have been caused by the emulsification of the oil.

From the standpoint of antimicrobial activity, the use of essential oil of *T. vulgaris* in formulations, using the concentration studied, seems to be a viable option. The method allows for the evaluation of the efficacy of the formulation. However, the test requires the removal of the preservatives from the preparation to prevent interference. The halos were observed only in the presence of the essential oil; the plates with the base formulation did not inhibit microbial growth. The results show that formulation B, with 4.5% of essential oil of thyme, has antimicrobial activity and that it can be used for the prevention and treatment of topical infections (after adjustments to improve stability).

According to Kapoor and Saraf (2011), topical herbal therapies may serve as additional treatment of acne. The authors mentioned the use of essential oil of thyme as one of the viable alternatives. The availability of the essential oil in the form of gel, as in the present study, is compatible with acne treatment and may assist in its treatment. Kunicka-Styczynska et al. (2011), with the goal of proposing the use of essential oils as preservatives of cosmetic products, evaluated lavender, tea tree, and lemon oils. The authors concluded that it is possible to use essential oils as components of preservative systems. But the stability of essential oils may be compromised by the complex composition of cosmetic products and the storage of these products in inadequate, adverse temperatures. Turek and Stintzing (2012) evaluated the impact of light and temperature on the physical-chemical properties as well as on the chemical composition of the four oils, including thyme. They showed that essential oil from thyme was resistant to the storage conditions evaluated. The essential oil from thyme can be considered comparably resistant to degradation of extended storage; they also suggested that the stability of the oil is due to the high amount of phenolic compounds such as thymol and carvacrol. These compounds scavenge free radicals generated during oxidation. In the present study, these substances were also found in the essential oil. Our results show that

topical use of essential oil from thyme may be a promising alternative for cosmetic and phytotherapeutic use.

Conclusion

The essential oil obtained presented as majority components geraniol, thymol, gama-terpinene, para-cymene, citral, 3-octanone, and 3-octenol. Thus, the essential oil should be used in formulations at a concentration of at least 4.5% to produce effective antimicrobial activity against the three strains. The cream gel formulation containing essential oil of *T. vulgaris* is a promising alternative for cosmetic and phytotherapeutic use. It is not possible to state that the formulations are absolutely stable. Therefore, after some adjustments to improve stability, the formulation could be used as an ally in the fight against topical infections. However, like all antimicrobial agents, it must be used with care to avoid increasing the number of strains resistant to therapeutic agents.

ACKNOWLEDGEMENTS

The authors wish to thank CNPq and the Pontifical Catholic University of Campinas for funding the study.

REFERENCES

Almeida MM, Lima CRRC, Quenca-Guillen HS, Moscardini Filho E, Mercuri LP, Santoro MIRM (2010). Stability evaluation of tocopheryl acetate and ascorbyl tetraisopalmitate in isolation and incorporated in cosmetic formulations using thermal analysis. Braz. J. Pharm. Sci. 46(1):129-134.

Brazil (National Health Surveillance Agency (ANVISA)) (2007). Guia de Controle de Qualidade de Produtos Cosméticos. Available at (http://www.anvisa.gov.br/cosmeticos/material/guia_cosmetico.pdf).

Brazil (National Health Surveillance Agency (ANVISA)) (2004a). Aprova o regulamento técnico de medicamentos fitoterápicos junto ao Sistema Nacional de Vigilância Sanitária. RDC 48, de 16 de março de 2004. Diário Oficial da União, Poder Executivo, DF, Brasília.

Brazil (National Health Surveillance Agency (ANVISA)) (2004b). Guia de Estabilidade de Produtos Cosméticos: Séries Temáticas. Available at (http://www.anvisa.gov.br/divulga/public/series/cosmeticos.pdf).

Bezerra JL, Costa GC, Lopes TC, Carvalho ICDS, Patrício FJ, Sousa SM, Amaral FMM, Rebelo JMM, Guerra RNM, Ribeiro MNS, Nascimento FRF (2006). Avaliação da atividade leishmanicida *in vitro* de plantas medicinais. Braz. J. Pharmacogn. 16:631-637.

Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C (2005). Anti-*Candida* activity of Brasilian medicinal plants. J. Ethnopharmacol. 97:305-311.

Brazilian Pharmacopeia V (2010). Farmacopéia Brasileira. 5. ed. Available at (http://www.anvisa.gov.br/hotsite/cd_farmacopeia/index.htm).

Gavahian M, Farahnaky A, Javidnia K, Majzoobi M (2012). Comparison of ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from *Thymus vulgaris* L. Innov Food Sci Emerg. 14: 85-91.

Golmakani MT, Rezaei K (2008). Comparison of microwave-assisted hydrodistillation whit traditional hydrodistillation method in the extraction of essential oils from *Thymus vulgaris* L. Food Chem. 109: 925-930.

Isaac VLB, Cefali IC, Chiari BG, Oliveira CCLG, Salgado HRN, Corrêa MA (2008). Protocolo para ensaios físico-químicos de estabilidade de fitocosméticos. Rev. Cienc. Farm. Basica Apl. 29(1):81-96.

Jakiemiu EAR, Scheer AP, Oliveira JS, Côcco LC, Yamamoto CI, Deschamps C (2010). Estudo da composição e do rendimento do óleo essencial de tomilho (*Thymus vulgaris* L.) Semina-Ciencias Agr., 31(3):683-88.

Jordán MJ, Martínez RM, Goodner KL, Baldwin EA, sotomayor JA (2006). Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oils composition. Ind Crop Prod. 24: 253-263.

Kalemba D, Kunicka A (2003). Antibacterial and antifungal properties of essential oils. Curr. Med. Chem.10:813-29.

Kapoor S, Saraf S (2011). Topical Herbal Therapies an Alternative and Complementary Choice to Combat Acne. Res. J. Med. Plant. 5(6): 650-669.

Kunicka-Styczyńska S, Sikora M, Kalemba D (2011). Lavender, tea tree and lemon oils as antimicrobials in washing liquids and soft body balms. Int. J. Cosmetic Sci. 33:53–61

Lang G, Buchbauer G (2012). A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. Flavour Frag. J. 27:13–39

NCCLS (2003) NCCLS document M7-A6. Wayne: Pennsylvania 19087-1898 USA. Available at (http://www.anvisa.gov.br/reblas/reblas_public_disco_difusao.pdf).

NCCLS (2005) Clinical and Laboratory Standards Institute. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretive Breakpoints; Informational Supplement - Second Edition. M27-S2. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Ngueface J, Tamgue O, Dongmo JBL, Dakole CD, Leth V, Vismer HF, Zollo PHA, Nkengfack AE (2012). Synergistic action between fractions of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against *Penicillium expansum*. Food Control. 23:377-383.

Santurio DF, Costa MM, Maboni G, Cavalheiro CP, Sá MF, Pozzo MD, Alves SH, Fries LLM (2011). Atividade antimicrobiana de óleos essenciais de condimentos frente a amostras de *Escherichia coli* isoladas de aves e bovinos. Cienc. Rur. on line. Available at (http://www.scielo.br/pdf/cr/2011nahead/a0511cr4430.pdf).

Sawaya AC, Palma AM, Caetano FM, Marcucci MC, da Silva Cunha IB, Araujo CE, Shimizu MT (2002). Comparative study of in vitro methods used to analyze the activity of propolis extracts with different compositions against species of Candida. Lett. Appl. Microbiol. 35:107-203.

Silva DM (2010). *Estudo químico e de atividades biológicas da Mansoa hirsuta D. C. (Bignoneaceae)*. UFAL. Available at (http://bdtd.ufal.br/tde_busca/arquivo.php?codArquivo=126).

Silva EC, Paola MVRV, Matos JR (2007). Análise térmica aplicada à cosmetologia. Braz. J. Pharm. Sci. 43(3): 34-56.

Silva GM, Maia Campos PMBG (2000). Ascorbic acid and its derivatives in cosmetic formulations. Cosmet Toiletries. 115(6):59-62.

Simões CMO, Schenkel EP (2002). A pesquisa e a produção brasileira de medicamentos a partir de plantas medicinais: a necessária interação da indústria com a academia. Rev. Bras. Farmacogn. 12(1):35-40.

Tohidpour A, Sattari A, Omidbaigi R, Yadegar A, Nazemi J (2010). Anttibacterial effect of essential oils from two medicinal plants against Methicillin-resistant *Staphylococcus aureus* (MRSA). Phytomedicine. 17:142–145.

Turek C, Stintzing FC (2012). Impact of different storage conditions on the quality of selected essential oils. Food Res. Int. 46:341–353.