

ATR-FTIR Study of *Thyme* and *Rosemary* Oils Extracted by Supercritical Carbon Dioxide

CARMEN MIHAELA TOPALA*, LAVINIA DIANA TATARU

University of Pitesti, Faculty of Science, Department of Natural Sciences, Pitești, 1 Tg. Vale Str., 110040, Pitesti, Romania

Thyme and *rosemary* are the herb plants which essential oil has demonstrated antiseptic and antiviral properties. Plant-derived polyphenols receive considerable attention because of their potential antioxidant and antimicrobial properties. The essential oils obtained from *Saturenja hortensis* (the cultivated variety of thyme) and from *Rosmarinus officinalis* were studied using ATR-FTIR spectroscopy. Supercritical fluid extraction (SFE) with supercritical carbon dioxide (CO₂) has recently gained in importance as an alternative to the classical procedures (steam distillation and extraction with organic solvents) because of legal limits regarding solvent residues. In conventional methods high temperatures is used, which can cause chemical modifications in the oil components and often a loss of the most volatile molecules. The extraction processes with carbon dioxide were compared to extraction processes with *n*-hexane. The extracts with CO₂ were obtained by supercritical carbon dioxide at 40°C and pressures of 10 MPa. The vibrational spectra obtained for essential oil from the Supercritical fluid extraction (SFE) present characteristic key bands of the main individual volatile components (e.g. carvacrol, thymol, *p*-cymene, α -pinene, 1,8-cineole).

Keywords supercritical extraction, thymol, thyme oil, rosemary oil, Attenuated Total Reflexion

Essential oils are complex mixtures of various terpenoids, aromatic substances, aldehydes, ketones, alcohols and esters. The fragrance and flavour substances are frequently isolated by ecological methods such as supercritical carbon dioxide extraction from the dried or fresh plant material (e.g. leaves, seeds, fruits, stems, bark or wood). Most of the oils are used in perfume compositions as well as for flavouring of food-stuffs or mouth care products.

Thyme contains essential oils (Eos) that are phenolic monoterpenoids for example; thymol, carvacrol, *p*-cymene and γ -terpene [1]. The spicy-phenolic odour of the oil is mainly related to the volatile component thymol and carvacrol (fig. 1).

Thymol (2-isopropyl-5-methylphenol) is a phenolic monoterpenoid and one of the major constituents of thyme oil and the antimicrobial action of thymol has received much attention in current research. Though the primary mode of antimicrobial action of the thymol compound remains obscure, it is thought to disrupt both outer- and inner-membranes and interacts with membrane proteins and intracellular components. Essential oils (EOs) from different types of thyme have been shown to have numerous antimicrobial i.e., antiviral, antifungal, antibacterial, antioxidant, and insecticidal properties [2, 3]. The substances that have the ability to eliminate pathogens and are less toxic to host cells can be considered for emerging antimicrobial drugs.

Strong antibacterial activity of *Thymus* EOs is attributed especially to their high content of thymol and carvacrol. Those phenolic compounds are well known for their antioxidant properties against microbial agents [4-7]. The antibacterial effects is explained by their ability to bind to the amine and hydroxylamine groups of the bacterial membrane proteins increasing the permeability, reducing the polarization of the cytoplasmic membrane and impairing efflux pump activity [8]. *Thymus* EOs may be

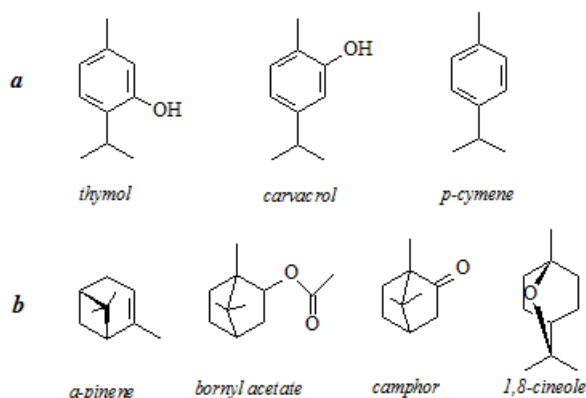


Fig. 1. Chemical structures of major thyme (a) and rosemary (b) oil compounds

used in combination with conventional antibiotics against multidrug-resistant bacteria [9].

It is known that thymol and carvacrol both have significant antiviral activity against some viruses, especially against *Herpes simplex* virus. These two phenolic monoterpenes showed also a significant potential in reduction the mosaic virus infection on cultivated plants making them recommended in the control of plant virus diseases [10]. *Thymus serpyllum* oils demonstrated a strong antiviral effect on *Nicotiana tabacum* plants mechanically inoculated with PVY (potato virus Y) and injected with those oils. The absorbance values obtained after testing (by DAS ELISA technique) the healthy and inoculated plants were significantly lower than the untreated and inoculated controls, the best results being obtained by using the 1/100 oil dilution [11].

Some similar studies were performed by combined therapies used for decreasing the potato viruses X and Y level on infected *Solanum Tuberosum* L. plantlets - meristem culture, chemotherapy and treatments with *Satureja hortensis* essential oil. The treatments with ribavirin

* email: carmen.topala@gmail.com; Tel.: 0745981621

and oseltamivire (40 mg/L) added to the tissue culture medium followed by treatments with *Satureja hortensis* essential oil suspension (1/1000) and H₂O₂ (1mM, 3mM pH = 5.6) of acclimatised plants revealed positive effects on PVX and PVY elimination from potato plant tissues showing a higher rate of virus eradication comparative to chemotherapy or electrotherapy [12].

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity. Rosemary essential oil is also used as an antibacterial, antifungal [13 -15] and anticancer agent [16]. Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes. The main compounds responsible for the antimicrobial activity of rosemary are α -pinene, bornyl acetate, camphor and 1,8-cineole [17- 19] and were presented in figure 1. Flamini et al. (2002) classified rosemary oil into two chemotypes: the α -pinene chemotype with the main compounds being α -pinene (20.6%) and 1,8-cineole (6.6%) and the 1,8-cineole chemotype with the major components being 1,8-cineole (40.2%) and α -pinene (13.2%).

Supercritical fluid extraction (SFE) is an environmentally benign and efficient extraction technique for solid materials and has been extensively studied for the separation of active compounds from herbs. In this work, we apply ATR-FTIR spectroscopy technique to investigate essential oils obtained from *Saturenja hortensis* and *Rosmarinus officinalis* by SFE extraction.

Experimental part

The essential oils analysed in this study were isolated from *Saturenja hortensis* and from *Rosmarinus officinalis*. Thyme leaves are local and rosemary leaves were commercially. Dried leaves were ground in a domestic mixer for 10 s.

Pure standard substance (thymol) was purchased from Sigma-Aldrich.

The ATR-FTIR spectra were recorder in a range between 4000-400 cm⁻¹ using a FTIR Jasco 6300 spectrometer, detector TGS, apodization Cosine. An ATR accessory equipped with a diamond crystal (Pike Technologies) was used for sampling. 5–10 mL of the essential oil were placed on the surface of the diamond ATR crystal. The spectral data were processed with JASCO SpectraManager II software. Samples were scanned at 4 cm⁻¹ resolution, accumulation: 100 scans.

Supercritical Fluid Extraction [SFE]

The Supercritical carbon dioxide extraction system and components were acquired from JASCO (Japan Spectroscopic Co.). Supercritical fluid extractor included the following: 250x 20 mm extraction vessel, column oven temperature (JASCO CO-2060), high-pressure pump (JASCO-PU-2080-CO₂), automated back pressure regulator (JASCO BP-2080). The experiments were carried out at 40°C and pressures of 10 MPa. Solvent mass flow rate was kept at 2 mL/min. At this flow rate it can be assumed that equilibrium concentration for the solvent and solute is achieved. Samples of 2.44 g of dried and chopped thyme leaves and 2.26 g rosemary respectively were placed in the extractor. The extracts were collected in one tube throughout the 180 min, and the yield was calculated.

Preparation of n-hexane extracts

5 g of powdered material were extracted using n-hexane during 8 h with agitation, at room temperature. The extract

was filtered and evaporated using a rotary evaporator a rotary evaporator (Rotavapor, Heidolph) and a vacuum pump (Buchi V-700) and used for analysis.

Results and discussions

Supercritical CO₂ extraction

The traditional extraction method for obtaining thyme oil is steam distillation (SD) of the aerial parts of the plant. The disadvantages of this method are the high energy consumption and the use of high temperatures which can induce the degradation of thermosensitive compounds of extracts. The content of thymol, one of the quality parameters of thyme extract, may be affected as this natural monoterpene phenol can suffer thermal degradation during SD.

Supercritical fluid extraction (SFE) is a viable option to extract valuable compounds from plants. It solves the problem of thermal degradation, it is environmentally safe, since avoid the use of solvent and therefore the presence of solvent traces in the extracts, and presents low operational cost [20, 21].

SFE using carbon dioxide (CO₂) is often proposed as an alternative extraction method for obtaining thyme extracts. CO₂ (Tc=31.2°C, Pc = 7.3MPa) has a polarity comparable to liquid pentane and thus it is compatible for dissolution of lipophilic constituents such as essential oils. [22, 23].

Thymus extracts with the highest thymol content were reported at 313 - 328 K and different pressures from 10 to 30 MPa, with a total extraction yield of 2-5%. Other terpenoids present in lower amounts in the extracts were: carvacrol, menthol, camphor and phytol [24, 25]. The kinetic behavior of time SFE was studied to set an adequate extraction time and an almost complete extraction was obtained after around 240 min of extraction [26]. For rosemary the highest global yield for SFE (3.52% d.b.) was obtained at 300 bar/50 °C [27].

In our study thyme and rosemary oils were obtained at 40°C and pressures of 10 MPa, after 180 min by SFE extraction and the yield was calculated (1% for thyme and 1.3% for rosemary).

FTIR analysis

The spectral analysis of the investigated oils is based on their vibrational spectra.

Terpenes are built from isoprenoid units with the general formula (C₅H₈)_n. According to the amount of isoprenoid units (n) they can be divided into several classes: hemiterpenes (C₅H₈)_n, monoterpenes (C₁₀H₁₆)_n, sesquiterpenes (C₁₅H₂₄)_n, diterpenes (C₂₀H₃₂)_n, triterpenes (C₃₀H₄₈)_n, tetraterpenes (C₄₀H₆₄)_n and polyterpenes (C₅H₈)_n. It is difficult to identify the individual spectral contribution of each of these groups; however some spectral features can be noticed [28]. The ATR-FTIR spectra of terpenoid compounds show some characteristic key bands, which can be used for discrimination between carvacrol, thymol, *p*-cymene, 1,8-cineole, camphor, α - and β -pinene. Isomeric compounds like thymol and carvacrol show significant differences in ATR-IR spectra. In the ATR-IR spectrum the most intense bands are seen at 804 cm⁻¹ (thymol) and 811 cm⁻¹ (carvacrol). These bands can be assigned to out-of-plane CH wagging vibrations, which are the most significant signals used in distinguishing different types of aromatic ring substitution [29]. Most of terpenoids show also characteristic signals in the ATR-IR spectrum due to wagging vibrations of CH and CH₂ groups e.g. at 813 cm⁻¹ for *p*-cymene, at 984 cm⁻¹ for 1,8-cineole, at 873 cm⁻¹ for β -pinene. Camphor cause only weak IR bands in this range, but reveal intensive band due to C-O stretching vibrations at 1739 cm⁻¹.

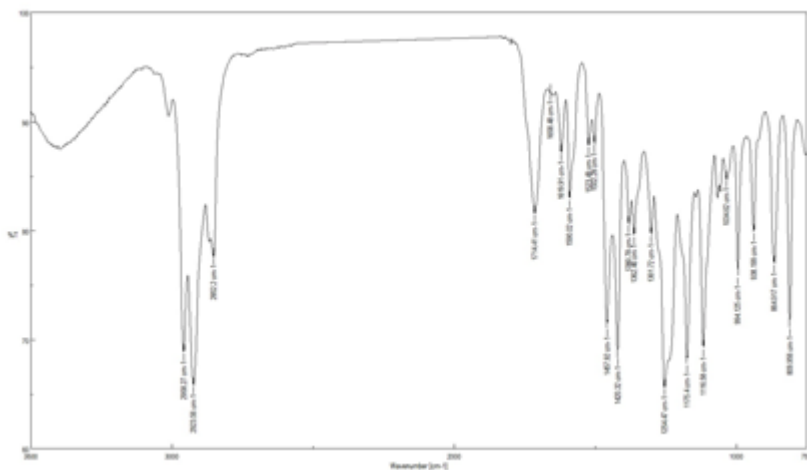


Fig. 2. FTIR spectrum for thyme oil extracted by using supercritical CO₂

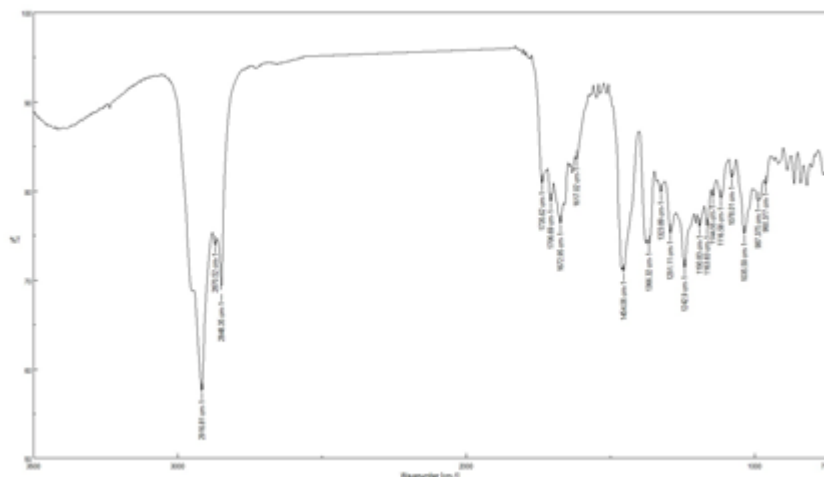


Fig. 3. FTIR spectrum for rosemary oil extracted by using supercritical CO₂

FTIR spectra obtained for thyme and rosemary oils by SFE extraction are shown in figures 2 and 3.

The FTIR spectra (4000-400 cm⁻¹) of CO₂ extracts of each plant were registered and the specific wavenumbers

and intensities were considered. Table 1 presents the FT-IR absorption bands for oil from plant extracts. The vibrational assignments for plant extracts were compared with literature data [30]. The functional groups identification

Wavenumbers [cm ⁻¹] of measured peaks for		Assignment
Thyme oil	Rosemary oil	
1	2	
2958.27 2923.56 2852.20	2950.55 2916.81 2870.52 2848.35	C-H stretching vibrations specific to CH ₃ and CH ₂
1714.41 1619.91	1672.95 1659.45 1641.13 1617.02	C=O stretching vibration, C-N stretching, COO ⁻ antisymmetric stretching
1590.02 1502.28	1572.66	aromatic domain and N-H bending
1457.92 1420.32 1380.78 1362.46 1301.72	1454.06 1376.93 1366.32	C-O stretching vibrations (amide) and C-C stretching from phenyl groups, COO ⁻ symmetric stretching, CH ₂ bending
1254.47	1291.11 1242.90 1203.36	C-O stretching
1175.40 1116.58 1066.44 1055.84 1034.62	1190.83 1163.83 1144.85 1116.58 1078.01 1035.59	stretching vibrations C-O of mono-, oligo- and carbohydrates
994.12 938.19 864.91 809.56	987.35 960.37 917.95 886.13 787.77	C-H out-of-plane bending vibrations from isoprenoids

Table 1
VIBRATION
ASSIGNMENTS FOR OIL
FROM PLANT
EXTRACTS

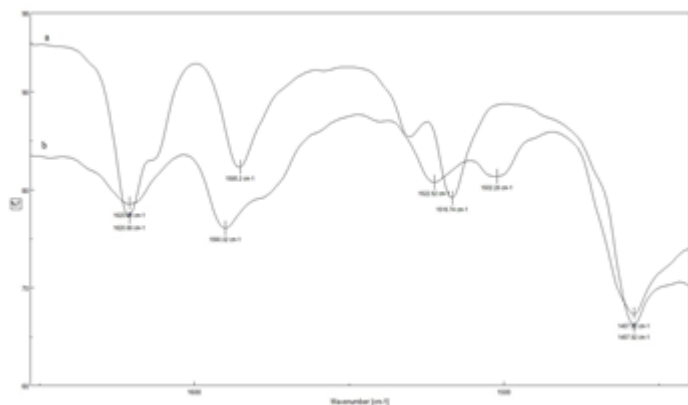


Fig. 4. ATR-FTIR Spectra of the phenolic rings at wavenumbers ranging 1620-1457 cm^{-1} of thymol (a) and thyme oil (b)

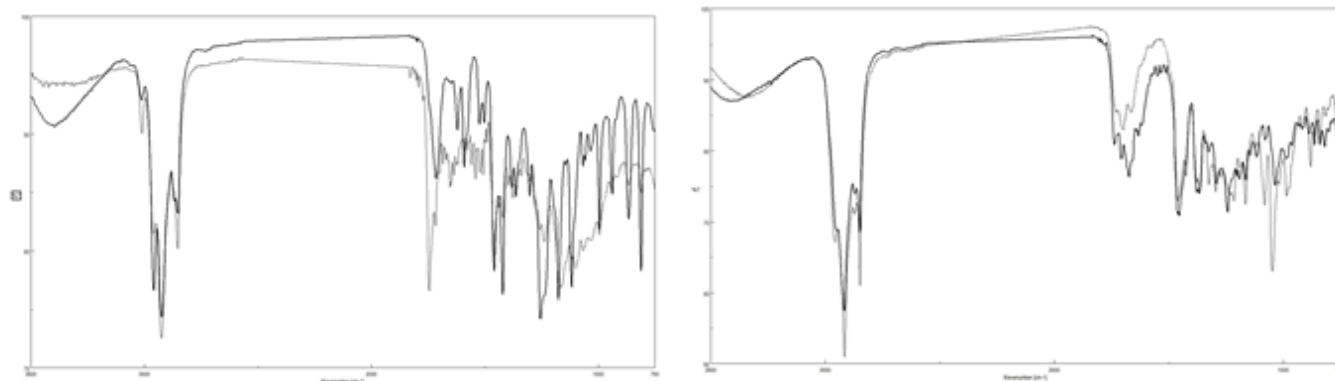


Fig.5. Comparison of CO_2 extract (—) and n-hexane extract (---) for thyme (a) and rosemary (b)

was based on the FTIR peaks attributed to stretching and bending vibrations.

The four peaks specific to the phenolic ring appear at wavenumbers ranging from 1620 cm^{-1} to 1457 cm^{-1} for thymol/ carvacrol [31] (fig. 4).

The ATR - FTIR spectrum of thyme oil (fig. 2), which shows an intense band at 809.95 cm^{-1} , reflects the complex composition of this essential oil: thymol and carvacrol. This band arises from the overlapping of thymol and carvacrol bands (803.206 and 811 cm^{-1}). The band can be attributed to out-of-plane CH wagging vibrations, which are the most important signals used in distinguishing different types of aromatic ring substitution [29].

From ATR-FTIR spectra (fig. 2 and table 1) of thyme oil, the strong signals at 809.86, 864.91, 1116.58, 1174.44, 1252.54, 1420.32 reflects the complex composition of this essential oil in which carvacrol is main component.

In the ATR-FTIR spectrum of rosemary essential oil (fig. 3) the following key bands are present: 1735.62; 1672.95; 1454.06; 1366.32; 1242.9; 1078.01; 987.37; 886.13; 839.84 and 787.79 cm^{-1} . These bands indicate that α - pinene and 1,8-cineole are main components of the rosemary oil [1].

Comparison of the two IR spectra of the CO_2 extract and the n-hexane extract for each oil (fig. 5a and b) reveals that the peaks positions, shapes and intensities of the main specific bands in the spectra are quite similar to each other since CO_2 and hexane used are nonpolar solvents.

Conclusions

In this paper an application of ATR-FTIR techniques for the characterisation of thyme and rosemary oils obtained by SFC extraction from *Saturenja hortensis* and *Rosmarinus officinalis* is presented. Supercritical fluid extraction has been extensively studied for the separation of active compounds from herbs. More detailed analysis of the essential oil composition can be based on the characteristic key bands of the individual volatile substances observed in their vibrational spectra.

Summing up, the increased demand to solve complex problems of plant biochemistry requires a multidisciplinary approach, in which FTIR spectroscopy can play a prominent role.

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