Isolation, purification, characterization and functionalization of thymol from thyme

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Introduction

The essential oil (EO) of thyme (*Thymus vulgaris*) is a mixture of different components. The goal of this project was the separation and purification of thymol (see figure 1), being the main component, out of the EO.

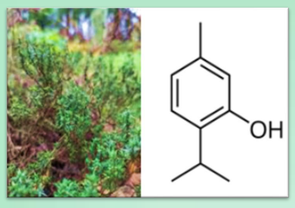

Figure 1: Thyme plant (left) and chemical structure of thymol (right)

Thymol and its derivatives have many applications, including potential pharmaceuticals with antibacterial, antifungal, anti-inflammatory, anticancer activity and many more.[1]

Materials and methods

- Thyme was purchased from Desmecht - Natural Solutions and the thymol reference was purchased from Acros.
- Hydrodistillation (HD), Extraction (E) and Soxhlet-extraction (SE) were the methods used to obtain the EOs from thyme, as presented in figure 2. Ethanol was the solvent used for the latter two techniques.

Figure 2: Experimental set-up for hydrodistillation (left), soxhlet-extraction (middle) and extraction (right)

- Column chromatography was used to purify the EOs. Silica and a mixture of heptane/ethanol (55:45) (l/l) is used as stationary phase and mobile phase, respectively. The separation of the thymol and the functionalization reactions were checked by thin-layer chromatography (TLC).[2]
- Thymol dissolved in ether $(C_2H_5)_2O$ was oxidized to thymoquinone using potassium permanganate ($KMnO_4$) dissolved in an aqueous acidic environment. Sodium dodecyl sulphate ($C_{12}H_{25}NaSO_4$) was used to emulsify the two solvents. The color indicator thymolphthalein was synthesized from thymol and phthalic anhydride in an aqueous acid medium.
- Verification of the isolation and functionalizations were done by infrared (IR) spectroscopy and nuclear magnetic resonance spectroscopy (^{1}H-NMR). A PerkinElmer FT-IR spectrometer and a Spinsolve Education 43 MHz NMR-Spectrometer were used.[3]

Results and discussion

Table 1 presents a summary of the results from the isolation of EOs out of thyme. The table shows that HD was the most efficient method. A very viscous oil was obtained via E and SE. Via HD, however, the oil was much more fluid, indicating a different composition of the oil.

Table 1: Summary of EO obtained by HD, E and SE

	HD1	HD2	HD3*	E	SE
Time (min)	240	240	240	210	210
Solvent	water	water	water	ethanol	ethanol
m_{thyme} (g)	120	75	120	100,23	30,2
Recovery weight (%)	0,50	0,52	0,73	0,12	0,33

* grinded thyme

Table 2 presents the thymol obtained after column chromatography of the EO.

Table 2: Amounts of purified thymol by column chromatography

	C1,HD1	C2,HD3	C3,E	C4,SE
m_{oil} (g)	0,5957	0,8751	1,386	0,9984
Recovery weight (%)	53,52	56,88	30,36	45,01

To verify the isolation and purification, the IR-spectrum of thymol of C1,HD1 was compared with the reference (see figure 3). The broad IR absorption peak, centred around 3174 cm^{-1} in the spectrum of the thymol reference and representing the hydroxyl group, is visible in the thymol after purification spectrum although shifted slightly. Furthermore, the ^{1}H-NMR spectrum of the thymol after purification resembles the thymol reference. Except for the fact that traces of solvent are still present.

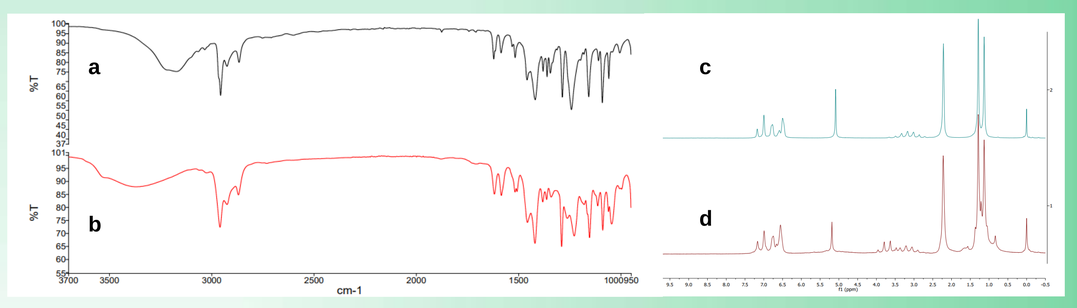

Figure 3: IR-spectrum of thymol reference (a), thymol after purification (b) and ^{1}H-NMR-spectrum of thymol reference (c), thymol after purification (d)

Figure 4.a/b presents the reactionscheme for the synthesis of the color indicator thymolphtaleine and the anti-inflammatory agent thymoquinone. To both further confirm the isolation of the thymol from C1,HD1 and to check the synthesis of the indicator, the pH activity of the indicator obtained from the isolated thymol was tested and compared with that from the reference (see figure 4.c/d).

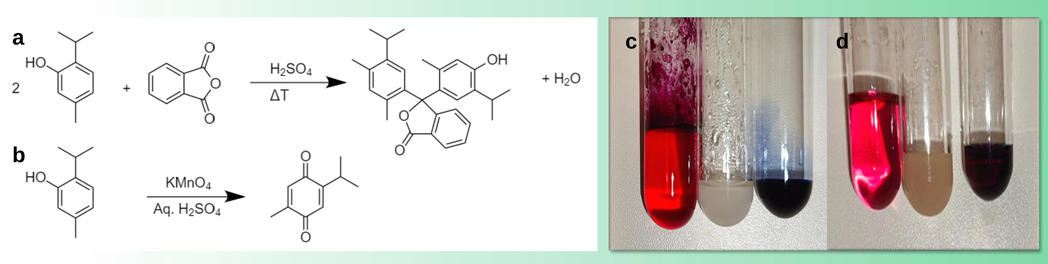

Figure 4: Reactionscheme of thymolphtalein (a) and thymoquinone (b). Testing the pH activity of the color indicator showing a red, white and dark blue color under respectively strong acid, intermediate and strong basic conditions: thymolphtalein synthezised from reference thymol (c) and isolated thymol (d)

Regarding the synthesis of thymoquinone, it can be concluded that the product was formed, as can be noticed in the IR spectrum after purification (figure 5.c) via the peak around 1700 cm^{-1}, representing the carbonyl groups. Furthermore, the NMR-signal at 5,2 ppm, standing for the ν_{OH} vibration of thymol has disappeared in the spectrum of the product (compare figure 3.c and 3.d with figure 5.d). However, the broad IR peak around 3426 cm^{-1} (figure 5.a/b/c) represents a hydroxy group which indicates that the solvent ethanol is still present. This assumption is confirmed by the ^{1}H-NMR spectrum (figure 5.d) where a quartet is clearly visible at 3,7 ppm.

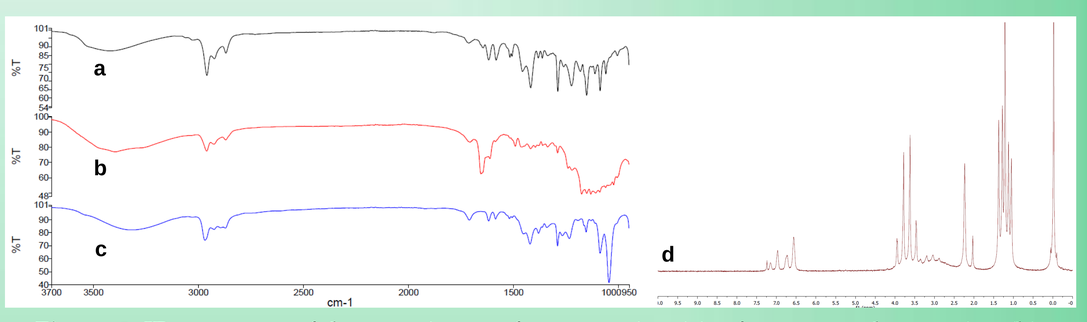

Figure 5: IR-spectrum of thymoquinone after extraction (a), after a second extraction (b), after a subsequent column chromatography (c) and ^{1}H-NMR-spectrum of thymoquinone after column chromatography (d)

Conclusion

Thymol has a lot of applications and can be isolated, purified through many ways from thyme. However, the most efficient way in our study is by hydrodistillation followed by column chromatography with grinded thyme. Various ways of synthesising the anti-inflammatory agent thymoquinone were performed, but the oxidation by $KMnO_4$ yielded the best results with a conversion rate of 17,9%. This low yield can be explained by the reaction having by-products. The synthesis of the color indicator thymolphtaleine was also successful.

References

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2. H;Roex. Organische chemie: LAB. Editie 2018-2019. Leuven: ACCO; 2018. 146 p.
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