

PMR Determination of the Major Constituents of the Volatile Oil of *Thymus vulgaris* Growing in Saudi Arabia

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ABSTRACT. A proton NMR analytical procedure for the quantitative determination of the major constituents of the volatile oil of *Thymus vulgaris* L. (fam. Labiatae) growing in Saudi Arabia is developed. The constituents determined are phenols (thymol, carvacrol and others), p-cymene and γ -terpinene. The method is specific, accurate and rapid. It also can be applied for other thyme oils.

Thymus vulgaris is the source of the well known, volatile oil, oil of thyme. Thymol is the most valuable and main constituent of the oil. The thymol content of the oil is a measure of its quality and commercial value. Other main constituents of the oil are carvacrol, p-cymene and γ -terpinene. The oil has antiseptic, antispasmodic and carminative properties. Thymol is a powerful germicide and possesses antifungal and anthelmintic properties. The oil is assayed mainly for its total phenolic and thymol content. The main procedure used for such determination is that by the reaction of the oil with 5% potassium hydroxide in a cassia flask measuring the remaining non phenolic part of the oil (Guenther 1958). Other methods of analysis have also been used for the determination of thyme oil constituents such as TLC, GLC, GC/MS, combined TLC/GC, combined, GC/IR and combined GC/¹³C-NMR (Richard *et al.* 1975, Rovesti 1971, Formacek and Kabeczka 1982, Lawrence 1980, Montes Guyot *et al.* 1981).

The present work was undertaken with the aim of establishing the feasibility of using PMR spectroscopy for the quantitative analysis of the major constituents of the volatile oil of *Thymus vulgaris*.

Experimental

Materials

- Thymol (Riedel-De Haen, W. Germany).
- p-Cymene, Diethylmalonate (BDH Chemicals, Poole, England). Khellin (Kock-Light Laboratories Limited; Colnbrook Buck, England).
- Caffeine (Merck, West Germany).
- The volatile oil obtained by steam distillation of *Thymus vulgaris*.

Apparatus

All spectra were recorded at 37°C on a 60 MHz spectrometer (Varian T 60A, Varian AG, Palo Alto, California, U.S.A.) using deuterated chloroform as a solvent. Chemical shifts were measured relative to the internal reference tetramethylsilane at 0 ppm.

Method

An aliquot of the oil or the nonphenolic fraction (50-400 mg) was accurately weighed in a glass stoppered test tube. The accurately weighed amount of the internal standard (25-150 mg) was then added. The mixture was then dissolved in one ml of deuterated chloroform, 0.5 ml of the solution was transferred into an NMR tube. The spectrum was obtained adjusting the spin-rate to eliminate the spinning side bands as much as possible. The peaks of interest in each case were integrated at least three times and the average integrals were taken for calculations. The percentage of the desired compounds were then determined using the following general formula:

$$\% \text{ w/w of the desired compound} = \frac{\text{AI}}{\text{AII}} \times \frac{\text{E. WI}}{\text{E. WII}} \times \frac{\text{mg II}}{\text{mg Oil}} \times 100$$

AI = integral value of the signal representing the desired compound.

AII = integral value of the signal representing the internal standard used.

E. WI = formula weight of the desired compound (thymol = 150.21/6 = 25.035, p-cymene = 134.21/4 = 33.55).

E. WII = formula weight of the internal standard used (Khellin = 260.24/6 = 34.37; Caffeine = 194.19/3 = 64.73; Diethylmalonate = 160/4 = 40).

mg II = weight in mg of internal standard used.

Results and Discussion

The PMR spectrum of the isolated thyme oil shows among other peaks a high field doublet centered at 1.20 ppm represents the methyl functions of the isopropyl group ($-\text{CHMe}_2$). This group is a common function for the major constituents of the oil namely: thymol (I), carvacrol (II), p-cymene (III) and γ -terpinene (IV) and have very close molecular weights. Also the spectrum shows two aromatic protons multiplet at 6.70-6.30 ppm for thymol and carvacrol and their isomers. Therefore, the determination of the total components having an isopropyl function was based on the integration of the six proton methyl doublet centered at 1.20 ppm. Khellin (V) was utilized as an internal standard since it provides resonance signals (six proton methyl singlet at 4.03 and 4.16 ppm) in a sufficiently downfield position from the signals chosen for the oil and pure thymol to allow for interference-free determination. Since all the compounds involved in the assay are freely soluble in deuterated chloroform, it is the solvent of choice. A series of standard mixtures containing pure thymol and khellin in deuterated chloroform were prepared and assayed (Table 1). The results show that the method is accurate and precise with a

Table 1. PMR assay of thymol in standard mixtures

Sample No.	Internal standard "Khellin" ^a (mg)	Thymol ^b		
		Added (mg)	Found (mg)	% Recovery w/w
1	50.00	50	49.75	99.5
2	50.00	75	78.18	104.24
3	50.00	100	101.80	101.80
4	50.00	125	128.11	102.488
5	50.00	150	157.26	104.84
6	50.00	200	202.05	101.025
7	50.00	250	249.60	99.84
8	50.00	250	265.17	106.068
9	50.00	300	307.23	102.41
10	50.00	350	348.25	99.50
Average recovery = 102.17%				
S.D. = ± 2.30				

a for "Khellin", the integrated six proton two methyl singlet are at 4.03 ppm and 4.16 ppm.

b for thymol, the integrated six proton methyl doublet are at 1.20 ppm.

standard deviation of ± 2.30 . The oil was assayed by the above procedure which proved to be reproducible (Table 2) with an average recovery of 94.47% of total

Table 2. PMR assay of total isopropyl derivatives in oil

Sample No.	Internal standard "Khellin" ^a (mg)	Thyme oil ^b		
		Added (mg)	Found (mg)	% Recovery w/w
1	50.38	50.35	49.70	93.16
2	50.42	77.18	72.50	93.99
3	50.53	100.74	94.80	94.13
4	50.62	209.03	199.60	95.50
5	49.99	251.05	240.80	95.83
6	50.19	302.22	284.80	94.23
Average recovery =				94.47%
S.D. =				±1.003

a for "Khellin", the integrated six proton two methyl singlet are at 4.03 ppm and 4.16 ppm.

b for thymol, in thyme oil, the integrated six proton methyl doublet are at 1.20 ppm.

isopropyl components calculated as thymol with a standard deviation ± 1.003 . The determination of thymol in synthetic mixtures and in the oil was based on the integration of the two proton aromatic multiplet at 6.70 - 6.30 ppm. Caffeine (VI) was used as an internal standard since it provides a three proton methyl singlet at 3.9 ppm. The results show that the method is accurate and precise with an average recovery of 101.42 ± 1.48 for the synthetic mixtures (Table 3). The total phenols in

Table 3. PMR assay of thymol in standard mixtures

Sample No.	Internal standard Caffeine ^c (mg)	Thymol ^b		
		Added (mg)	Found (mg)	% Recovery w/w
1	50.24	51.17	52.17	102.60
2	50.22	100.78	103.80	103.00
3	50.10	155.81	159.49	102.37
4	50.04	249.12	204.25	100.56
5	50.29	303.25	305.60	100.78
6	50.05	351.33	348.34	99.15
Average recovery =				101.42%
S.D. =				±1.4894

b for thymol, in thyme oil, the integrated six proton methyl doublet are at 1.20 ppm.

c for caffeine, the integrated three proton methyl singlet are at 3.90 ppm.

the oil were determined by the above procedure (Table 4), with an average recovery of $73.98\% \pm 3.49$. This value (73.98%) is an estimate of the thymol, carvacrol and other related phenols. The determination of p-cymene was based on the integration of its four proton aromatic singlet resonating at 7.20 ppm. Diethylmalonate (VII) was used as an internal standard since it provides a four

Table 4. PMR assay of thymol in oil

Sample No.	Internal standard Caffeine ^c (mg)	Thymol oil ^b		
		Added (mg)	Found (mg)	% Recovery w/w
1	49.91	55.94	39.85	71.24
2	49.97	111.51	82.44	73.93
3	50.44	211.37	163.96	77.56
4	49.98	299.85	231.87	77.33
5	50.36	402.19	380.81	69.82
Average recovery =				73.98%
S.D. =				± 3.4948

^b for thymol, in thyme oil, the integrated six proton methyl doublet are at 1.20 ppm.

^c for caffeine, the integrated three proton methyl singlet are at 3.90 ppm.

proton methylene quartet centered at 4.40 ppm. The results indicate that the method is accurate and precise with an average recovery of $99.68\% \pm 3.11$ in the synthetic mixtures (Table 5). The determination of p-cymene in the oil is

Table 5. PMR assay of p-cymene in standard mixtures

Sample No.	Internal standard diethyl ^d malonate (mg)	p-Cymene ^e		
		Added (mg)	Found (mg)	% Recovery w/w
1	152.65	55.58	53.05	95.40
2	149.66	75.73	75.03	99.10
3	152.85	103.82	100.40	96.70
4	152.60	122.35	125.02	102.20
5	149.23	150.54	154.60	102.70
6	152.83	204.13	208.30	102.00
Average recovery =				99.68%
S.D. =				± 3.111

^d for diethylmalonate, the integrated four proton methylene quartet are at 4.40 ppm.

^e for p-cymene, the integrated four proton aromatic singlet are at 7.20 ppm.

impracticable because of the interference of the aromatic proton signals from other oil constituents particularly thymol, carvacrol and other related phenols. However, this difficulty was overcome by the determination of p-cymene in the nonphenolic fraction of the oil where the four proton aromatic singlet shows up at 7.20 without any interference. The average recovery is $23.85\% \pm 1.23$ (Table 6), *i.e.* the amount of p-cymene in the oil is 4.88% ($23.85 \times 20.49/100$). γ -Terpinene content of oil was obtained by difference ($20.49 - 4.88 = 15.61$). The amounts of major constituents of the oil as determined by PMR spectroscopy are shown in Table 7.

Table 6. PMR assay of p-cymene in non-phenolic fraction of the oil

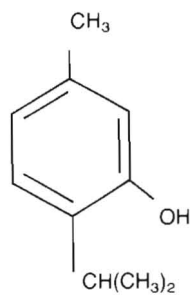
Sample No.	Internal standard diethyl ^d malonate (mg)	Non-phenolic portion ^e		
		Added (mg)	Found (mg)	% Recovery w/w
1	152.47	22.59	5.77	25.60
2	153.54	58.00	12.92	22.30
3	149.98	89.48	22.19	24.80
4	149.64	147.38	34.13	23.20
5	150.05	121.72	30.19	24.80
6	151.10	158.10	35.97	22.70
7	150.23	181.16	42.83	23.60
Average recovery =				23.85%
S.D. =				± 1.23

^d for diethylmalonate, the integrated four proton methylene quartet are at 4.40 ppm.

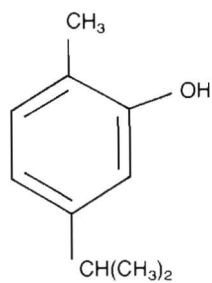
^e for p-cymene, in the non-phenolic proton, the integrated four proton aromatic singlet are at 7.20 ppm.

Table 7. Percentages of major constituents of thyme oil

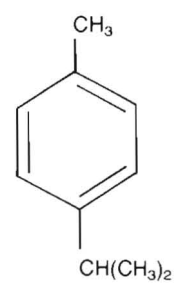
Components	% w/w
Isopropyl derivatives	94.47
Other derivatives	5.53
Thymol, carvacrol and related phenols	73.98
p-Cymene	4.88
γ -Terpinene	15.61



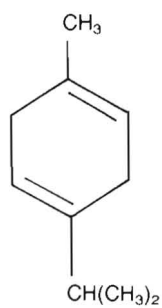
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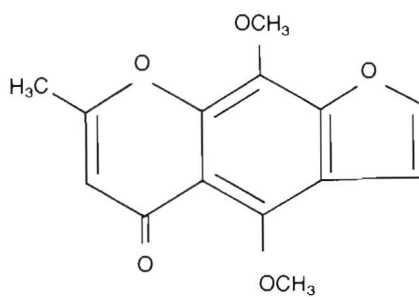
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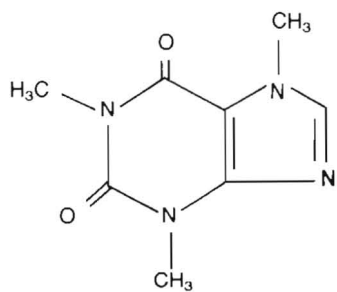
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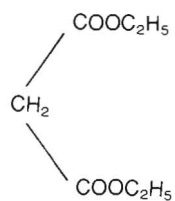
IV



V



VI



VII

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تقدير المكونات الرئيسية للزيت الطيار - لنبات الزعتر - الذي ينمو في المملكة العربية السعودية بواسطة الرنين المغناطيسي النووي

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تم استخدام الرنين المغناطيسي النووي لاستنباط طريقة للتقدير الكمي للمكونات الرئيسية للزيت الطيار في نبات الزعتر الذي ينمو بالمملكة العربية السعودية. والمكونات التي قدرت هي الفينولات (الثيمول - الكارفاكرول وأخرى) والباراسيمين والجاماتربينين. وتحتوي جميع هذه المركبات، على مجموعة ايزوبريل ولها وزن جزيئي متقارب. وتقدير هذه المواد يعتمد كلياً على قياس البروتونات الستة في مجموعة الايزوبريل. وهي تقع عند ٢, ١ جزء في المليون في طيف الرنين المغناطيسي النووي لها. وقد استخدم مركباً الخليلين والكافيين كمادة قياسية داخلية. وكانت النتائج دقيقة ونوعية، ومتوسطها ١٧, ١٠٢ ± ٢, ٣ و ٤٢, ١٠١ ± ١, ٤٩ للمخاليط القياسية و ٤٧, ٩٤ ± ١, ٠٣ في عينة الزيت. كما اعتمد تقدير الفينولات (الثيمول والكارفاكرول وأخرى) على قياس اثنين من بروتونات الحلقة العطرية التي تقع عند ٦, ٣ - ٦, ٧ جزء في المليون في طيف الرنين المغناطيسي النووي وأستخدم الكافيين مادة قياسية داخلية، وكانت النتائج دقيقة بمتوسط ٤٢, ١٠١ ± ١, ٤٩ و ٩٨, ٧٣ ± ٣, ٤٩ للمخاليط القياسية وعينة الزيت على التوالي. أما ما يحتويه الزيت من الباراسيمين والجاماتربينين فقد تم تعيينه بالفرق وكان ٤٩, ٢٠٪. وتم تقدير الباراسيمين في الزيت بعد استخلاص الفينولات على أساس قياس البروتونات الأربعة لحلقة العطرية والتي تقع عند ٢, ٧ جزء في المليون في طيف الرنين المغناطيسي النووي. واستخدم مركب ثنائي ايثيل مالونات مادة قياسية داخلية، وكانت النتائج دقيقة بمتوسط ٦٨, ٩٩ ± ٣, ١١ و ٨٥, ٢٣ ± ١, ٢٣ في المخاليط القياسية والزيت

على التوالي. وبذلك تكون كمية الباراسيمين في الزيت ككل ٤,٨٨٪
(٢٣,٨٥ \times $\frac{٢٠,٤٩}{١٠٠}$) وتم حساب كمية الجاماتربينين بالفرق حيث بلغت ١٥,٦١
(٤٩,٨٨ - ٢٠,٤٩). وبذلك تم التوصل بهذه الطريقة إلى تقدير النسب المئوية
للمكونات الرئيسة لزيت الزعتر. علاوة على ذلك فإن طيف الرنين المغناطيسي
النووي للزيت يقدم وسيلة للتعرف بدقة على مكوناته واكتشاف الشوائب فيه إن
وجدت.