Фітохімічні дослідження

ФАРМАЦЕВТИЧНИЙ ЧАСОПИС

http://ojs.tdmu.edu.ua/index.php/pharm-chas

UDC 615.014:582.929.4
DOI https://doi.org/10.11603/2312-0967.2022.1.13054

CHROMATOGRAPHIC PROFILES OF POLYPHENOLS AND TERPENOIDS IN THE HERBS OF SOME *THYMUS* L. REPRESENTATIVES

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INFORMATION

Надійшла до редакції / Received: 11.01.2022
Після доопрацювання / Revised: 17.01.2022
Прийнято до друку / Accepted: 18.01.2022

Key words:
Thymus pulegioides;
Thymus richardii;
Thymus vulgaris;
cultivar;
herb;
TLC;
GC/MS;
polyphenols;
terpenoids.

ABSTRACT

The aim of the work. To perform the identification of phenolic compounds and terpenoids in the aerial parts of *Thymus pulegioides* L. (cultivar '2/6-07'), *Thymus richardii* Pers. (cultivar 'Fantasy') and *Thymus vulgaris* L. (cultivar 'Jalos') using thin layer chromatography (TLC), as well as to conduct the gas-chromatographic analysis of *Thymus richardii* essential oil.
Materials and Methods. The TLC method was used in this study for the qualitative analyses of polyphenols and non-polar compounds in the aerial part of three Thyme (*Thymus* L.) species from the *Lamiaceae* Martinov family cultivated in Kherson region (Ukraine). The volatile compounds were determined in the essential oil of *Thymus richardii* by gas chromatography mass spectrometry (GC/MS) method.
Results and Discussion. Rosmarinic and caffeic acids were identified in the methanol extracts of all three *Thymus* representatives. Luteolin was revealed distinctly only in the *Thymus richardii* herb whilst the weak zone of apigenin was found only in *Thymus vulgaris*. The TLC-analysis of terpenoids showed the presence of the clear spot of thymol only in the methylene chloride extract of *Thymus vulgaris* aerial part. The weak zones of *β*-sitosterol were visible in the chromatograms of all three *Thymus* species. The major volatile constituent of the *Thymus richardii* essential oil was linalool (38.60 %), followed by linalyl acetate (18.26 %) and thymol (8.41 %). These predominant compounds possess significant therapeutic potential.
Conclusions. The used chromatographic techniques could be regarded as simple and reliable methods for the phytochemical authentication of the studied *Thymus* cultivars.

Introduction. The Thyme (*Thymus* L.) genus belongs to the *Mentheae* Dumort. tribe of *Nepetoideae* Burnett subfamily (*Lamiaceae* Martinov family). The *Thymus* genus counts more than 300 species of herbaceous plants and subshrubs [1]. They are characterized by the specific aromatic smell due to the accumulation of essential oil in the epidermal trichomes [2, 3]. Some species are known as medicinal plants widely used in

traditional medicine of many countries [4–6]. Thus, the raw materials of such species as *Thymus vulgaris* L. or *Thymus zygis* L. (*Thyme*) and *Thymus serpyllum* L. (Wild Thyme) are included in the European and Ukrainian Pharmacopoeias [7,8]. According to the European Pharmacopoeia [8], essential oil from the flowering herbs of *Thymus vulgaris* or *Thymus zygis* (or a mixture of both species) known as 'Thyme oil' should contain at least 36.0% of thymol.

Such species as *Thymus pulegioides* L. or *Thymus richardii* Pers. are unofficial ones and are only used in complementary medicine [9–12]. Due to natural processes of hybridization and diligent breeders' work, the new subspecies, cultivars and chemotypes of *Thymus* species gradually appear [13]. It encourages investigations of their chemical composition as well as biological properties [5, 6].

Phytochemical study of the main groups of biologically active substances in the raw materials of unofficial medicinal plants or cultivars opens up opportunities to determine their potential role in the development of new phytomedicines. Since plant raw materials are multicomponent mixtures of different compounds, their chromatographic analysis could be considered as the optimal method of the phytochemical evaluation [14, 15].

The first aim of this study was to perform the TLC-analysis of phenolic compounds and terpenoids in the aerial parts of three *Thymus* representatives cultivated in Ukraine: *Thymus pulegioides* (cultivar '2/6-07'), *Thymus richardii* (cultivar 'Fantasy') and *Thymus vulgaris* (cultivar 'Jalos'). The second aim was to conduct the GC/MS analysis of *Thymus richardii* essential oil.

Materials and Methods. The herbs of the studied species were harvested from the experimental plots in Kherson region (Ukraine) and shade dried for 6–7 days.

The raw materials for the TLC-analysis of polyphenols were macerated in methanol in a ratio of 1:10, using periodical shaking. The reference standards (rosmarinic acid, caffeic acid, apigenin and luteolin) were dissolved in methanol. The mobile phase was prepared using ethyl acetate, formic acid and water in a ratio of 15:1:1. TLC plates F_{254} (20x10 cm, with silica gel, Merck) were used in this study. The derivatization was conducted with 1% $AlCl_3$ solution. The detection of phenolic compounds was based on the natural fluorescence (UV-light at 366 nm).

The raw materials for the TLC-fingerprinting of terpenoids were macerated in toluene (ratio 1:10). Thymol and *β*-sitosterol were used as reference standards. The mobile phase consisted of methylene chloride and methanol (ratio 9:1). The detection of non-polar compounds was done using anisaldehyde solution. After the heating at 100-105 °C for 5 min, the spots were examined in daylight immediately.

Essential oil was separated from the *Thymus richardii* herb by hydrodistillation. Chromatograph GC/MS Agilent Technologies Hewlett Packard 6890N/5973 (USA) equipped with a ZB-5HT inferno column (30.0 m x 0.32 mm x 0.25 μm) was used for the component analysis of essential oil [6]. The flow rate of helium as a carrier gas was 2 mL/min. The temperature of the injector was 250 °C. The injected volume of the test solution was 1 μL; split-ratio 20:1. Compounds were identified by comparing their retention indexes and by interpretation of the mass spectra from a National Institute of Standards and Technology library.

Results and Discussion. The 'chromatographic fingerprints' of phenolic compounds in the investigated herbs are presented on Figure 1. Rosmarinic and caffeic acids were identified in the methanol extracts of all three species. The most intense light blue fluorescent zones at (R_f=0.72±0.02) corresponded to the reference standard of rosmarinic acid. The weaker light blue spots of caffeic acid were presented just above the rosmarinic acid one (R_f=0.80±0.02). Several weak additional zones in the different shades of a blue fluorescence were also presented along the chromatograms of the tested species. Flavonoid luteolin (R_f=0.83±0.02) was identified clearly only in the *Thymus richardii* (cultivar 'Fantasy') herb. The weak yellow zone of apigenin (R_f=0.86±0.02) was revealed only in the *Thymus vulgaris* (cultivar 'Jalos') aerial part. Several red spots of chlorophyll were observed just under the solvent front. Generally, rosmarinic acid with proven antioxidant, anti-inflammatory, antimicrobial, antiviral, hepatoprotective and other properties [16, 17] can be considered as the main biologically active polyphenol of all three studied species.

Rosmarinic acid was the predominant phenolic compound in the aerial parts of *Thymus serpyllum* L. and other *Thymus* species [4] as well as representatives of the *Melissa* and *Ocimum* genera (*Lamiaceae* Family) [14, 17]. Rosmarinic acid prevailed in the *Thymus pulegioides* aqueous and hydro-ethanolic extracts which possessed the antioxidant, anti-proliferative, neuroprotective, anti-aging and anti-diabetic activities [18].

The TLC-analysis of non-polar compounds showed the presence of the clear spot of aromatic alcohol thymol (R_f=0.84±2) in the methylene chloride extract of *Thymus vulgaris* herb only (Fig. 2). This finding corresponds to the literature date about the dominant components of the essential oil of this species [6]. The weak violet zones of *β*-sitosterol (R_f=0.75±2) were visible in the chromatograms of all three species. The presence of the faint spots of *β*-sitosterol was also found in the raw materials of other species from the genera *Satureja, Lophanthus* and *Dracocephalum* of *Lamiaceae* family [19]. Furthermore, the different additional violet, blue and green zones of fluorescence were presented in the chromatograms of the tested samples which reflected the specific 'chromatographic fingerprint' of terpenoids.

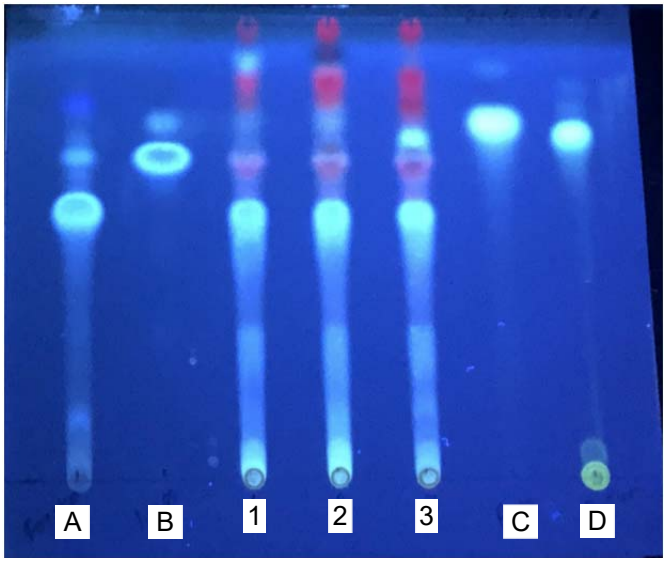

Figure 1. The image of typical TLC-chromatograms of polyphenols in the herbs of three *Thymus* representatives in UV-light at 366 nm: *1* – *Thymus pulegioides* (cultivar '2/6-07'); 2 – *Thymus vulgaris* (cultivar 'Jalos'); 3 – *Thymus richardii* (cultivar 'Fantasy'); A – standard of rosmarinic acid; B – standard of caffeic acid; C – standard of apigenin; D – standard of luteolin. Mobile phase: ethyl acetate, formic acid and water (15:1:1); derivatization with 1% $AlCl_3$ solution.

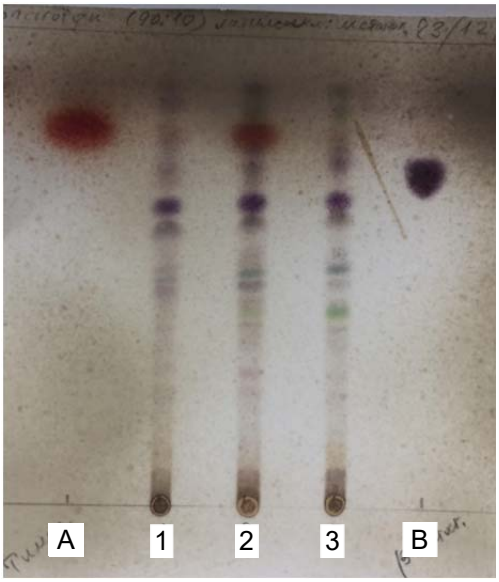

Figure 2. The image of typical TLC-chromatograms of terpenoids in the herbs of three *Thymus* representatives after derivatization with anise aldehyde solution in daylight: *1* – *Thymus pulegioides* (cultivar '2/6-07'); 2 – *Thymus vulgaris* (cultivar 'Jalos'); 3 – *Thymus richardii* (cultivar 'Fantasy'); A – standard of thymol; B – standard of *β*-sitosterol. Mobile phase: methylene chloride and methanol (9:1).

The analysis of available scientific sources indicates very limited data about phytochemical peculiarities of *Thymus richardii*. For instance, only one article was found in the authoritative international database PubMed on February 1, 2022 after searching words "*Thymus richardii*" [10]. We suppose that essential oil of *Thymus richardii* deserves significant attention from the point of view of the determination of its component composition and chemotype features.

The GC/MS analysis of the *Thymus richardii* (cultivar 'Fantasy') essential oil is presented in Table 1 and in Figure 3. It was revealed the dominating of the acyclic monoterpenoids such as linalool (38.60 %) and linalyl acetate (18.26 %) followed by aromatic alcohol thymol (8.41 %). Moreover, the significant

Table 1
Chemical composition of the *Thymus richardii* (cultivar 'Fantasy') essential oil revealed by GC/MS

Component	Retention index	Content [%] ± SD %
1-octen-3-ol	982	0.28±0.03
3-octanone	988	0.04±0.00
β-myrcene	992	0.04±0.01
3-octanol	995	0.05±0.00
terpinolene	1017	0.07±0.00
p-cymene	1026	0.93±0.02
eucalyptol (1,8-Cineole)	1032	0.93±0.03
y-terpinene	1061	0.44±0.01
cis-*β*-terpineol	1070	0.26±0.01
cis-linalool oxide	1074	1.19±0.03
trans-linalool oxide	1089	1.08±0.01
linalool	1100	38.60±0.37
thujone	1114	0.30±0.03
camphor	1148	5.94±0.03
borneol	1169	6.32±0.08
lavanduol	1178	0.54±0.01
terpinen-4-ol	1180	5.33±0.05
α-terpineol	1193	3.41±0.08
borneol acetate	1225	0.16±0.01
nerol (cis-geraniol)	1233	0.25±0.08
thymol methyl ether	1246	0.46±0.08
linalyl acetate	1264	18.26±0.09
bornyl acetate	1287	0.19±0.01
lavanduol acetate	1291	1.34±0.02
thymol	1299	8.41±0.10
carvacrol	1305	1.30±0.32
nerol acetate	1366	0.58±0.05
geranyl acetate	1385	1.02±0.07
caryophyllene	1423	0.52±0.01
β-farnesene	1436	0.33±0.01
β-bisabolene	1511	0.07±0.02
neryl propionate	1547	0.14±0.01
caryophyllene oxide	1589	0.55±0.02

contents of borneol (6.32 %), camphor (5.94 %) and terpinen-4-ol (5.33 %) were also detected in the studied essential oil.

The predominant components of *Thymus richardii* (cultivar 'Fantasy') essential oil are significantly different from those found in the essential oils of its other populaо tions [20, 21]. Thus, the EsO of plants harvested in 6 different *Thymus richardii* localities from Spain (Majorca, Valencia, Ibiza), Bosnia-Herzegovina (Konjic, Borci) and Italy (Sicily) were investigated by Llorens et al. [20]. They found that the main compounds in the most samples were monoterpenoids *p*-cymene and *y*-terpinene. The principal constituent in the samples from Majorca was sesquiterpene *β*-bisabolene (>40%). Furthermore, Italian scientists Bader et al. [21] detected 36 compounds in the essential oil of *Thymus richardii* subsp. *nitidus* (Guss.) Jalas, and sesquiterpene *β*-bisabolene (32.3 %), as well as aromatic monoterpenoids carvacrol

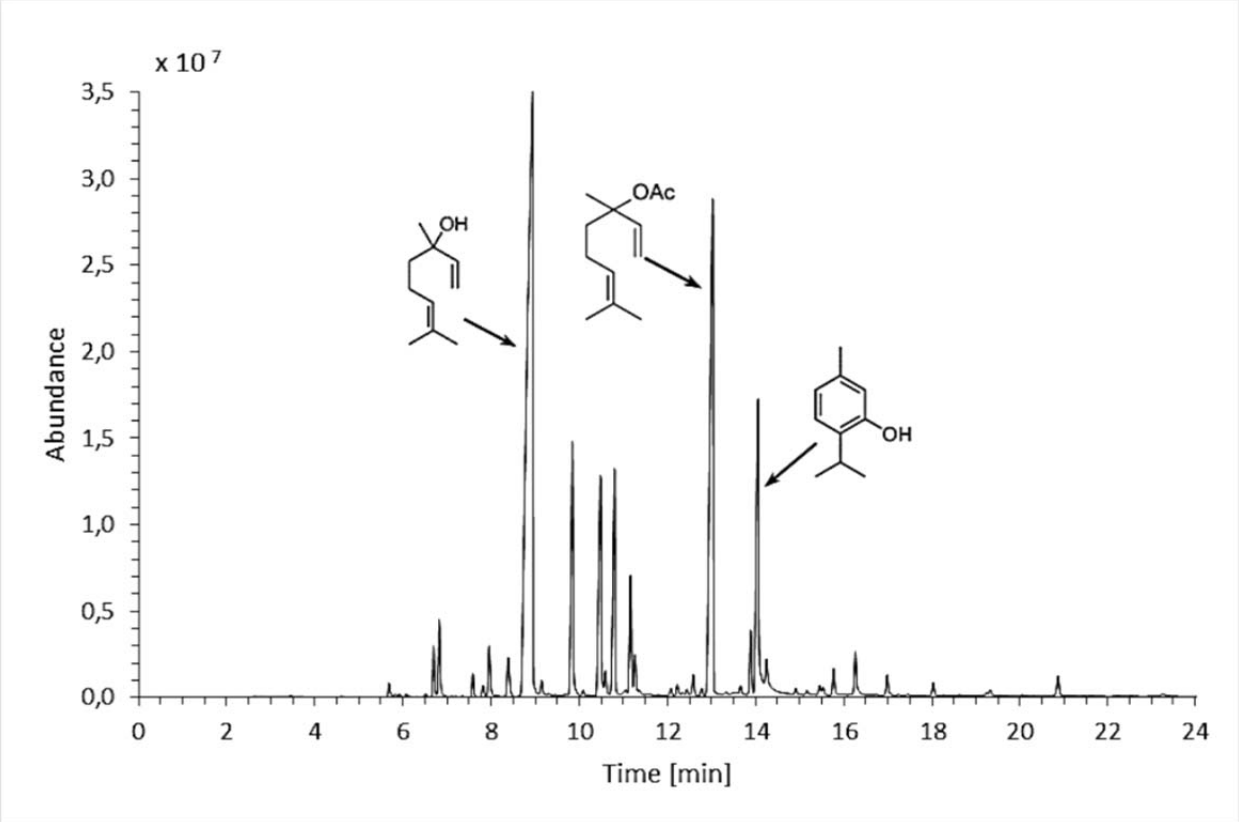

Figure 3. The typical GC/MS chromatogram of the *Thymus richardii* (cultivar 'Fantasy') essential oil.

(13.1 %) and methyl-thymol (12.4 %) predominated among them.

It should be mentioned, that the main volatile compounds of Pharmacopoeial Thymes [7, 8] are thymol and carvacrol. On the other hand, the predominant compounds of Ukrainian *Thymus pulegioides* essential oil were α-citral (27.10 %) and *β*-citral (17.11 %) [6].

Such minor components of the *Thymus richardii* (cultivar 'Fantasy') essential oil as lavanduol acetate (1.34 %), lavanduol (0.54 %) and borneol acetate (0.16 %) could be considered as its specific chemotaxonomic markers.

The biological activities of volatile compounds found in the *Thymus richardii* (cultivar 'Fantasy') essential oil are very substantial. For instance, linalool as a monoterpene alcohol presented in many *Lamiaceae* species causes the antimicrobial efficacy of their essential oils [6, 22]. Linalool also possesses anxiolytic, antidepressant, anticancer, hepatoprotective, renal protective, and lung protective activities [23]. Rai et al. [24] revealed the antipsoriatic effect of Lavander essential oil and its predominant components linalool and linalyl acetate. Moreover, the essential oil of *Salvia sclarea* which contained 38.67 % of linalyl acetate and 20.42% of linalool was effective against *Staphylococcus epidermidis* [25]. The noticeable antiseptic properties are inherent in thymol [5], which is the third predominant component of the *Thymus richardii* (cultivar 'Fantasy') essential oil.

Conclusions. 1. The TLC analysis of polyphenols in the herbs of all the studied *Thymus* species demonstrates the presence of distinct zones of rosmarinic acid which has significant therapeutic properties. The TLC-fingerprints of terpenoids showed the presence of clear spot of aromatic alcohol thymol in the *Thymus vulgaris* (cultivar 'Jalos') herb. The weak zones of *β*-sitosterol were visible in the chromatograms of all three species. The used TLC-techniques could be regarded as simple and reliable methods for phytochemical authentication of three *Thymus* representatives.

2. Linalool followed by linalyl acetate and thymol could be considered as the main biologically active markers of the *Thymus richardii* (cultivar 'Fantasy') essential oil investigated by GC/MS. The obtained data of GC/MS analysis are important for planning further pharmacological studies of *Thymus richardii* (cultivar 'Fantasy') essential oil.

Конфлікт інтересів: відсутній.

Conflicts of interests: authors have no conflict of interest to declare.

ХРОМАТОГРАФІЧНІ ПРОФІЛІ ПОЛІФЕНОЛІВ І ТЕРПЕНОЇДІВ У ТРАВІ ДЕЯКИХ ПРЕДСТАВНИКІВ РОДУ ЧЕБРЕЦЬ (*THYMUS* L.)

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Мета роботи. Провести ідентифікацію поліфенолів та терпеноїдів у траві *Thymus pulegioides* L. (сорт «2/6-07»), *Thymus richardii* Pers. (сорт «Fantasy») і *Thymus vulgaris* L. (сорт «Jalos») з використанням методу тонкошарової хроматографії (ТШХ), а також здійснити газохроматографічний аналіз ефірної олії *Thymus richardii*.
Матеріали і методи. Метод ТШХ було використано для якісного аналізу поліфенолів і сполук неполярної природи в надземній частині трьох представників роду Чебрець (*Thymus* L., родина *Lamiaceae* Martinov), які заготовляли в умовах культури на території Херсонської області. В ефірній олії *Thymus richardii* методом газової хроматографії з мас-спектрометрією визначено склад і вміст летких сполук.
Результати й обговорення. У метанольних витягах трави усіх трьох досліджуваних видів роду *Thymus* ідентифіковано розмаринову та кофейну кислоти. Лютеолін було чітко помітно тільки на хроматограмі *Thymus richardii*, тоді як слабку зону апігеніну виявлено лише в *Thymus vulgaris*. ТШХ-аналіз терпеноїдів показав наявність тимолу лише в метиленхлоридному екстракті надземної частини *Thymus vulgaris*. Слабкі зони *β*-ситостеролу були помітні на хроматограмах усіх трьох видів роду *Thymus*. Методом газової хроматографії з мас-спектрометрією встановлено, що основними компонентами ефірної олії *Thymus richardii* були ліналоол (38,60 %), ліналілацетат (18,26 %) і тимол (8,41 %), які мають значний терапевтичний потенціал.
Висновки. Використані методи хроматографічного аналізу можна вважати простими й надійними для застосування у фітохімічній ідентифікації сировини досліджуваних представників роду *Thymus.*

Ключові слова: *Thymus pulegioides; Thymus richardii; Thymus vulgaris*; сорт; трава; тонкошарова хроматографія; газова хроматографія з мас-спектрометрією; поліфеноли; терпеноїди.

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