

CONSIDERATIONS REGARDING THE TAXONOMY OF THE GENUS *THYMUS* IN ROMANIA

Rodica BEICU, Alina NEACȘU, Ilinca-Merima IMBREA
Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of România”,
Aradului St. 119, Timișoara 300645, România
rodicabontescu@yahoo.com

Abstract: The species of *Thymus* is taxonomically classified in the Lamiaceae (Labiatae) family, a large family recognized for the large number of medicinal and aromatic species found here. The species of the Lamiaceae family are intensively studied in the chemical-pharmaco-dynamic aspect and widely used for therapeutic purposes in medicine and traditional medicine. An important aspect, when we study a species under different aspects (botanical, biochemical, genetic), is the correct taxonomic determination. This can be difficult especially if the studies target species are from spontaneous flora. A misidentification of the genus or species leads to an incorrect reporting of the scientific results of the research, with repercussions on the entire academic community, when comparative results for the same species obtained from different areas will be reported. In the last period of time, the genetic and biochemical researches are constituted in additional and complex taxonomic instruments, which come to complement and clarify the classical taxonomy. The paper presents in comparison the difficulties of determining the *Thymus* genus in the flora of Romania, comparing with the species of the genus recognized at European level. On the one hand, it is noted that Euro+Med Plantbase does not recognize the presence on the territory of our country of some species, reported in its own determinants as being present in us. On the other hand, certain species mentioned in the indigenous plant identification books, are not recognized as being present in the flora of Romania by European recognized database (*T. roegneri* not mentioned in our country for distribution). Also, some species are variously synonymized or considered subspecies as is the case of *T. pannonicus* treated in European flora as a subspecies for *T. pulegioides*. Taxonomic interpretation of the species belonging to the genus *Thymus* is also difficult, due to the high variability of populations with very different morphological characteristics, but also because of hybridization.

Key words: *Thymus*, Lamiaceae Family, medicinal plant, Romanian flora

INTRODUCTION

The family Lamiaceae (Labiatae) includes over 200 genera, including the genus *Thymus*, considered one of the important genera along with *Salvia*, *Stachys*, *Teucrium*, *Nepeta*, etc. (MORALES, 1997) The literature data indicate a number of 215 species of the genus *Thymus*. (MORALES, in STAHL-BISKUP & SAEZ, 2002) The medicinal importance and the use of this plant as a culinary herb have brought it to the attention of botanists since ancient times. As a crop, the species *Thymus vulgaris* L. is commonly known as the garden thyme or lemon thyme. The species has been cultivated since ancient times, being recognized as an important medicinal and seasoning plant. This species spontaneously grows in Spain, Greece, Italy, southern France. (MUNTEAN *et al.*, 2007)

From a phytotherapeutic point of view, the above-ground flowering part of the wild thyme it used - *Serpylli herba*, which contains a mixture of all the spontaneous species growing in our country (*T. pannonicus*, *T. glabrescens*, *T. pulegioides*, *T. austriacus*, etc.). (CIULEI *et al.*, 1993)

The identification of spontaneous species with significant phytomedicinal properties can open new paths for capitalization, either by cultivating them or by applying techniques of over-seeding and direct harvesting from the area.

Wild thyme is recommended in respiratory disorders (cough), gastrointestinal disorders, influenza, having stomachic, antiseptic, choleric effects. Antimicrobial effect of

spontaneous thyme species compounds has been demonstrated for Gram-positive and Gram-negative bacteria (ROTA *et al.*, 2008), but their antifungal effect has also been demonstrated (MAKSIMOVIĆ *et al.*, 2008; PAVEL *et al.*, 2010). In addition to the flavoring properties determined by the active constituent ingredients (PETROVIC *et al.*, 2017), the essential oils also have strong antioxidant properties. (GRIGORE *et al.*, 2010)

MATERIAL AND METHODS

The dichotomous keys of plant identification books are based on the morphological characteristics of the species, and in many cases, the identification of species and especially of subspecies, is extremely difficult, due to the similarity of these characteristics. In addition, the taxa in the case of the wild thyme are differently synonymized according to the work that stood at the basis of the taxonomic identification. In this paper, the taxa of the genus *Thymus* from the most used local plant identification books (*Flora of Romania vol. VIII*, 1961; *Illustrated Flora of Romania*, CIOCARLAN, 2009; *Vascular plants from Romania*, SÂRBU *et al.*, 2013), and from the most used and recognized online database for European flora, *Euro+Med Plantbase* (<http://ww2.bgbm.org/EuroPlusMed/query.asp>).

RESULTS AND DISCUSSIONS

Sixty six species divided into two subgenera, *Coridothymus* with one species *T. capitatus* and *Thymus* with 8 sections, are mentioned in *Flora Europaea*, JALAS (1972). The species found in Romania are included in two sections of the subgenus *Thymus*: section *Hyphodromi* with *T. zygoides* and section *Serpyllum* with *T. sibthorpii*, *T. pannonicus*, *T. glabrescens*, *T. longicaulis*, *T. paecox*, *T. pulcherrimus*, *T. comosus*, *T. bihoriensis*, *T. pulegioides*, *T. alpestris*, *T. serpyllum*. In the current electronic database *Euro+Med Plantbase*, which also includes the species of *Flora Europaea*, the two species mentioned as possible, are found to be present in Romania (*T. sibthorpii*, *T. serpyllum*).

In the spontaneous flora of Romania the number of species reported to be present, varies according to the bibliographic sources studied. The main data from the Romanian plant identification books of reference is analyzed below.

Thus, in the *Flora of Romania vol. VIII* (1961), the material presented for the genus *Thymus* was edited by GUȘULEAC. He points out 19 species of the genus, of which one species is cultivated (*T. vulgaris*) and 18 are spontaneous. Thus, spontaneous species are: *T. callieri*; *T. zygoides*; *T. marschallianus* with two subspecies *marschallianus* and *toševii*; *T. pannonicus*; *T. serpyllum*; *T. glabrescens*; *T. austriacus*; *T. pulegioides* with three subspecies: *montanus*, *subcitratus* and *chamaedrys*; *T. alpestris*; *T. dacicus*; *T. longicaulis*; *T. illyricus*; *T. jankae*; *T. balcanus*; *T. marginatus*; *T. pulcherrimus*; *T. comosus*. Three hybrid species are mentioned separately: *T. xporcii* Borb. *marschallianus* x *pulegioides* (f. *porcii*, f. *juranyianus*, f. *pillisiensis*, f. *cibinensis*); *T. xschurii* Gușul *marschallianus* x *comosus* (f. *claudiopolitanus*); *T. xbaumgarteni* Gușul *pulegioides* x *comosus* (f. *schistosus*).

According to CIOCARLAN (2009), the genus groups together 17 species (16 spontaneous): *T. vulgaris*; *T. comosus*; *T. bihoriensis*; *T. pulcherrimus*; *T. alpestris*; *T. pulegioides* with subspecies *montanus*; subspecies *pulegioides* (*T. p.* subspecies *chamaedrys*); *T. zygoides*; *T. jankae*; *T. balcanus*; *T. longicaulis*; *T. alternans*; *T. comptus*; *T. callieri*; *T. serpyllum*; *T. glabrescens* with subspecies *glabrescens* and subspecies *pilosus*, *T. sibthorpii*, subspecies *toševii*; *T. pannonicus* (*T. marschallianus*): subspecies *pannicus* and subspecies *auctus*. (CIOCARLAN, 2009)

According to SÂRBU *et al.*, 2013, the genus comprises 18 species of which one cultivated species and one species whose presence is considered doubtful in the spontaneous flora (*T. serpyllum*). The mentioned species are similar to those appearing in CIOCÂRLAN (2009), with the mention that some species are differently synonymized. Two species appearing in the Flora of Romania are not mentioned in Ciocârlan's work (*T. austriacus* and *T. dacicus*). (SÂRBU *et al.*, 2013)

In the online database for European flora, *Euro+Med Plantbase* (<http://ww2.bgbm.org/EuroPlusMed/query.asp>), some species are not mentioned in distribution as being present in Romania (*T. roegneri*, *T. praecox* subspecies *jankae*, *T. praecox* subspecies *polytrichus*, *T. comptus*). For *T. pulegioides* are mentioned two subspecies: *pilisensis* (mentioned only in Romania and Ukraine) and *pannonicus*, which appear as species in the local plant identification books.

All of these inconsistencies pose great taxonomic problems. It is known that, from a medicinal point of view, all species of the genus are used under the generic name of *T. serpyllum*. However, it is necessary to accurately determine the species, especially in scientific research, in order to compare data with similar ones in the literature. Reporting the chemical composition, the genetic imprint, as well as the flora and vegetation studies requires precise identification of the species in the field. The accuracy of the results and the accuracy of the interpretation are closely related to the taxonomic interpretation.

Currently, plant identification books are based on the morphological characteristics of the species, and in many cases, the identification of species and subspecies is extremely difficult due to the similarity of these characteristics. The local plant identification books differentiate the species of the genus based on the morphology of the twigs (cylindrical or square-edged); presence/absence and distribution of the bristles (*Holotriches*, *Goniotriches*, *Allelotriches*); pilosity and leaf venation (camptodromous, pseudo-edged, edged); the presence or absence of sterile shoots. In Flora Europaea (1972) Jalas also considers characteristics regarding the morphology of the calyx (length, pilosity) and the inflorescence bracts. Subspecies, varieties and forms have very close characteristics, sometimes interpretable, contributing to confusion between species, and wrong identifications. This fact also emerges from the comparative table (Table 1) in which we can easily observe uncertain species, synonymized as other species, or species not listed in the Flora of Europe's electronic database as present in Romania, but described in our plant identification books.

In some cases, new taxa can be reported for Romania, but due to the similarities between them, they can be easily wrongly identified, and the results reported incorrectly.

Tabel 1

Comparative data on the taxonomy of the species of the genus *Thymus* in Romania and in the online database *Euro+Med Plantbase*

<i>Flora of Romania</i> vol. VIII, GUȘULEAC, 1961	<i>Illustrated Flora of</i> <i>Romania,</i> CIOCÂRLAN, 2010	<i>Vascular plants from</i> <i>Romania,</i> SÂRBU <i>et al.</i> , 2013	<i>Euro+Med Plantbase</i> (http://ww2.bgbm.org/EuroPlusMed/query.asp)
<i>T. glaucus</i>	<i>T. comptus</i> (<i>T. glaucus</i>)	<i>T. comptus</i> (<i>T. glaucus</i>)	<i>T. comptus</i> (<i>T. glaucus</i>) not mentioned in Ro
<i>T. callieri</i>	<i>T. callieri</i> (<i>T. glabrescens</i> ssp. <i>urumovii</i>)	<i>T. roegneri</i> (<i>T. callieri</i> , <i>T. glabrescens</i> ssp. <i>urumovii</i>)	<i>T. roegneri</i> (<i>T. alternans</i> , <i>T. callieri</i>) not mentioned in Ro
<i>T. zygioides</i>	<i>T. zygioides</i>	<i>T. zygioides</i>	<i>T. zygioides</i>
<i>T. marschallianus</i> ssp.	<i>T. pannonicus</i> (<i>T. marschallianus</i>) ssp.	<i>T. pannonicus</i> (<i>T. marschallianus</i>) ssp.	<i>T. pulegioides</i> ssp. <i>pannonicus</i> (<i>T. pannonicus</i> , <i>T.</i>

<i>marschallianus</i>	<i>pannonicus</i>	<i>pannonicus</i>	<i>marschallianus</i>)
<i>toševii</i>	<i>auctus</i>	<i>auctus</i>	
<i>T. pannonicus</i>	<i>T. pannonicus</i> ssp. <i>pannonicus</i>	<i>T. pannonicus</i> ssp. <i>pannonicus</i>	
<i>T. serpyllum</i>	<i>T. serpyllum</i>	? <i>T. serpyllum</i>	<i>T. serpyllum</i>
<i>T. glabrescens</i>	<i>T. glabrescens</i> ssp.	<i>T. glabrescens</i>	<i>T. odoratissimum</i> (<i>T. glabrescens</i> , <i>T. pilosus</i> , <i>T. austriacus</i> , <i>T. comptus</i>)
	<i>glabrescens pilosus</i> (<i>T. austriacus</i>)		
<i>T. austriacus</i>	included as ssp. of <i>T. glabrescens</i>	<i>T. odoratissimum</i> (<i>T. pilosus</i> , <i>T. austriacus</i>)	
<i>T. pulegioides</i> ssp.	<i>T. pulegioides</i> ssp.	<i>T. pulegioides</i> ssp.	<i>T. pulegioides</i> ssp.
<i>montanus</i>	<i>montanus</i>	<i>montanus</i>	<i>montanus</i> – not mentioned in Ro. <i>pulegioides</i> (<i>T. porcii</i>) <i>chamaedrys panonicus pilisiensis</i> (<i>T. xporcii</i> f. <i>pillisiensis</i>) mentioned only in Ro. și Uk.
<i>subcitratus chamaedrys</i>	<i>pulegioides</i> (<i>T. p.</i> ssp. <i>chamaedrys</i>)	<i>pulegioides chamaedrys</i>	
<i>T. alpestris</i>	<i>T. alpestris</i>	<i>T. alpestris</i> (<i>T. serpyllum</i> ssp. <i>alpestris</i>)	<i>T. alpestris</i>
<i>T. dacicus</i>	-	<i>T. dacicus</i> (<i>T. porcii</i>)	<i>T. dacicus</i> (<i>T. serpyllum</i> ssp. <i>dacicus</i>) mentioned in Ro., not synonymized with <i>T. pocii</i>
<i>T. longicaulis</i>	<i>T. longicaulis</i> (<i>T. serpyllum</i> L. var. <i>pinifolius</i> Heuffel)	<i>T. longicaulis</i> (<i>T. pinifolius</i>) ssp.	<i>T. longicaulis</i> (not synonymized with <i>T. pinifolius</i>) ssp.
		<i>longicaulis</i> <i>ilyricus</i> (<i>T. ilyricus</i>)	<i>longicaulis</i> (<i>T. ilyricus</i>) <i>chaubardii</i> not mentioned in Ro.
<i>T. ilyricus</i>	-	<i>T. longicaulis</i> ssp. <i>ilyricus</i> (<i>T. ilyricus</i>)	<i>T. longicaulis</i> ssp. <i>longicaulis</i>
<i>T. jankae</i>	<i>T. jankae</i>	<i>T. praecox</i> ssp. <i>jankae</i> (<i>T. jankae</i> , <i>T. skorpilii</i>)	<i>T. praecox</i> ssp. <i>jankae</i> (<i>T. jankae</i>) not mentioned in Ro.
<i>T. balcanus</i>	<i>T. balcanus</i>	<i>T. praecox</i> ssp. <i>polytrichus</i> (<i>T. balcanus</i> , <i>T. alpigenus</i>)	<i>T. praecox</i> ssp. <i>polytrichus</i> (<i>T. balcanus</i> , <i>T. alpigenus</i>) not mentioned in Ro.
<i>T. marginatus</i>	<i>T. bihoriensis</i> (<i>T. marginatus</i>) end. Carp.	<i>T. bihoriensis</i> (<i>T. marginatus</i>)	<i>T. bihoriensis</i> (<i>T. marginatus</i>) mentioned only in Ro., endemic
<i>T. pulcherrimus</i>	<i>T. pulcherrimus</i> end. Carp.	<i>T. pulcherrimus</i>	<i>T. pulcherrimus</i>
<i>T. comosus</i>	<i>T. comosus</i> end. Carp.	<i>T. comosus</i>	<i>T. comosus</i> mentioned only in Ro.; end. Carp.
-	<i>T. alternans</i>	<i>T. alternans</i>	<i>T. roegneri</i> (<i>T. alternans</i> , <i>T. callieri</i>) not mentioned in Ro.

-	<i>T. sibthorpii</i> ssp. <i>tosevii</i>	<i>T. sibthorpii</i> ssp. <i>tosevii</i> (<i>T. serpyllum</i> ssp. <i>serpyllum</i> var. <i>marschallianus</i>)	<i>T. sibthorpii</i> (<i>T. tosevii</i>) mentioned in Ro., but not with ssp.;
---	---	--	--

Abbreviations: end. Carp. – Carpathian endemism; Ro-România; UK-Ukraine

The taxonomic interpretation of species belonging to the genus *Thymus* is very difficult, especially due to the increased variability of the populations, with extremely varied morphological characteristics, but also due to species hybridization.

Genetic and biochemical studies are sometimes necessary, in order to confirm the taxonomy of species and subspecies, the results obtained confirming classification or suggesting modifications. For example, the biochemical studies performed by ADZET *et al.* (1981) confirm remarkable phytochemical differences between *T. capitatus* and other species of the genus, but also similarities (the presence of certain characteristic flavones), supporting the division proposed by JALAS (1972) on the two subgenera *Coridothymus* and *Thymus*. In this case, however, it is a differentiation at species level, which is easier to prove genetically and biochemically. Even some molecular studies may prove inconclusive when trying to differentiate at subspecies level (FEDERICI *et al.*, 2013).

CONCLUSIONS

The spontaneous species of the genus *Thymus* reveal a great taxonomic diversity, indicating a natural evolution that has allowed the adaptation to different habitats and has created the premises of a divergent evolution based on a great genetic variability. This species biodiversity can contribute to the understanding of fundamental problems related to their origin, evolution and phylogenetic relationships.

Field reconfirmations are needed for certain species in our country, as well as chemotaxonomic and molecular studies, especially for those whose presence in Romania is uncertain. Molecular and biochemical analyses, although costly and complex, provide valid answers in taxonomy, contributing to the clarification of some problems related to the correct taxonomic classification.

BIBLIOGRAPHY

- ADZET T., MARTINEZ F., 1981 – Flavonoids in the Leaves of *Thymus*: a Chemotaxonomic Survey. *Biochemical Systematics and Ecology*, 9(4): 293-295.
- CIOCĂRLAN V., 2009 – Flora ilustrată a României. Ed. Ceres, București, pp: 662-667 .
- CIULEI I., GRIGORESCU E., STĂNESCU U., 1993 – Plante medicinale. *Fitochimie si Fitoterapie* (Vol. 2). Editura Medicală, București, pp: 165-169.
- FEDERICI S., GALIMBERTI A., BARTOLUCCI F., BRUNI I., DE MATTIA F., CORTIS P., LABRA M., 2013 – DNA barcoding to analyse taxonomically complex groups in plants: the case of *Thymus* (Lamiaceae). *Botanical Journal of the Linnean Society*, 171(4): 687–699.
- GRIGORE A., PARASCHIV I., MIHUL A., CORINA B., DRAGHICI E., ICHIM M., 2010 – Chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods. *Romanian Biotechnological Letters*, 15(4): 5436-5443.
- GUȘULEAC M., 1961 – *Thymus* L., In SĂVULESCU TR. (Redactore Principali), Redactore Tomi NYÁRÁDY E.I., Collarobatores Tomi VIII: BELDIE AL., BUIA AL., GRINȚESCU GH., GRINȚESCU I., GUȘULEAC M., MORARIU I., NYÁRÁDY A., NYÁRÁDY E.I., PAUCĂ A., PRODAN I., RĂVĂRUȚ M., ȚOPA EM., Flora României, vol. VIII, Ed. Acad. Române, București, pp: 301-334.

- JALAS J., *Thymus* L., In: TUTIN T.G., HEYWOOD V.H., BURGESS N.A., MOORE D.M., VALENTINE D.H., WALTERS S.M., 1972 – Flora Europaea, Vol. 3, Cambridge University Press, Cambridge, pp: 172-182.
- MAKSIMOVIĆ Z., MILENKOVIĆ M., VUČIĆEVIĆ D., RISTIĆ M., 2008 – Chemical composition and antimicrobial activity of *Thymus pannonicus* All. (Lamiaceae) essential oil. Cent. Eur. J. Biol., (2): 149–154.
- MORALES R., 2002 – The history, botany and taxonomy of the genus *Thymus*, in STAHL-BISKUP E., SAEZ F. Thyme - The genus Thyme. London Taylor and Francis, 1-44.
- MUNTEAN L. S., TĂMAȘ M., MUNTEAN S., MUNTEAN L., DUDA M., VÂRBAN D., FLORIAN S., 2007 – Tratat de plante medicinale cultivate și spontane. Ed. Risoprint, Cluj-Napoca, pp: 438-444.
- PAVEL M., RISTIĆ M., SVETIĆ T., 2010 – Essential oils of *Thymus pulegioides* and *Thymus glabrescens* from Romania: chemical composition and antimicrobial activity, J.Serb.Chem. Soc., 75 (1): 27-34.
- PETROVIC S., UŠJAK L., MILENKOVIC M., ARSENIJEVIC J., DROBACA M., DRNDAREVIC A., NIKETIC M., 2017 – *Thymus dacicus* as a new source of antioxidant and antimicrobial metabolites, Journal of Functional Foods, 28(2017): 114-121.
- ROTA M. C., HERRERA A., MARTÍNEZ R. M., SOTOMAYOR J. A., JORDÁN M. J., 2008 – Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*, 19(7): 681-687.
- SĂRBU I., ȘTEFAN N., OPREA A., 2013 – Plante vasculare din România. Determinator ilustrat de teren. Ed. Victor B. Victor, pp. 666-674.
- STAHL-BISKUP E., 1991 – The Chemical Composition of *Thymus* Oils: A Review of the Literature 1960 – 1989, Journal of Essential Oil Research, 3(2): 61-82.
- *** <https://ww2.bgbm.org/EuroPlusMed/query.asp>