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ESSENTIAL OIL COMPOSITION OF TEUCRIUM L. (LAMIACEAE) COLLECTED FROM DIFFERENT LOCATIONS IN LIBYA.

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Abstract:-
A The essential oil was extracted from the dried shoots of plant materials, twelve compounds were identified by Gas chromatography–mass spectrometry (GC-MS) analyses revealed that essential oil contains mainly germacrene B, β-caryophyllene, Limonene, α-pinene, Germacrene D, β-Elemene, α-Copaene, α-Cadinol, Terpinen, Isoborneol, Camphene and Linalool. However, T. zanonii was specified by the presence of all assessed oils, while section Chamaedrys (T.barbeyanum) was characterized by the absence of α-pinene, α-cadinol and Isoborneol. Although, αpinene and α-cadinol were also absent in T.polium subsp. flavovirens, these were detected in the two other forms of T.polium. Contrary, Linalool and Terpinen were detected in the two forms of T.polium and not detect in subspecies. The Terpinen is also undetected in T.brevifolium, T.campanulatum and T.fruticans, which are belonged to section Teucrium.

Keywords:- *Teucrium, Lamiaceae Essential oil, GC/MS, Libya.*

INTRODUCTION

Lamiaceae is a cosmopolitan family with more than containing about 236 genera and has been stated to contain 6900–7200 species [1]. Fifty percent of the known species restricted to ten genera; *Clerodendrum, Hyptis, Nepeta, Plectranthus, Scutellaria*, *Salvia*, *Stachys*, *Thymus*, *Teucrium*, and *Vitex*. Lamiaceae is a cosmopolitan family and its aromatic species are economically important due to the essential oils they produce. *Teucrium* genus represent one of the most important genus in family Lamiaceae, which is also cosmopolitan and have about 300 species, distributed mainly in the Mediterranean Basin. Meanwhile, it is usually grow in poor soils with limited water resources and low nutritional requirements.

Consequently, *Teucrium* exhibits an importance from an economic point of view [2, 3, 4 and 5]. *Teucrium* species are often known for their medicinal utilization and exhibit interesting biological properties [6 and 7]. Lamiaceae are best known for the essential oils common to many members of the family. Evidence from archeological excavations showed that some species of this family, which are now known only as wild plants, had been cultivated at local scales in the past. Species of *Mentha*, *Thymus*, *Salvia*, *Origanum*, *Coleus* and *Ocimum* are used as food flavorings, vegetables and in industry [8].Lamiaceae family, are of interest to food manufacturers as consumers move towards functional foods with specific health effects. The anti-oxidative and antimicrobial effects are mainly due to phenolic components, such as Flavonoids, phenolic acids, and phenolic diterpenes [9]. A typical and most characteristic feature of most Nepetoideae is the production and accumulation of comparably large amounts of volatile monoterpenes, which are usually sequestered in specialized glands and trichomes. Few genera produce sesquiterpenes; furthermore, biologically active diterpenes have been found in some members of the Nepetoideae [10].

Teucrium belonged to tribe Teucrieae below the subfamily Ajugoideae [11]. It enclosed more than 300 species with a ubiquitous distribution around the world; Europe, North Africa, temperate regions of Asia and particularly in the Mediterranean basin. About 50 species were located in the later habitats [12, 13]. The genus has been widely studied during the last 30 years, both from the taxonomical and the phytochemical points of view, owing to their active secondary metabolites, mainly flavonoids [14] and neoclerodane diterpenoids [15]. Isolation and identification of some chemical constituents from *Teucrium apollinis* [16].

In Libya, Lamiaceae was represented by 22 genera and 65 species [17]. *Teucrium* represented in the Flora of Libya by 13 species; *T.apollinis, T.barbeyanum, T.brevifolium,*
T.campanulatum, T.compactum, T.davaeanum, T.divaricatum, T.flavum, T.fruticans, T.linivaccarii, T.pilosum, T.polium and T.zanoni [17]. *Alternatively,* [18] pointed out 15 taxa; *T.alpesre subsp.alpesre, T.alpesre subsp.gracile, T.apollinis, T.brevifolium, T.compactum, T.davaeanum, T.divaricatum, T.fruticans, T.libyaca, T.lini-vaccarii subsp.linivaccarii, T.lini-vaccarii subsp.racemosa, T.microphyllinus, T.pilosum, T.polium and T.zanonii*.[19] reveal that Teucrium in Libya is represented by 11 specics , *T. apollinis, T. barbeynum, T. brevifolium, T. campanulatum, T. capitatum, T. davaeanum, T. fruticans, T. linivaccarii, T. polium form 1, T. polium form 2, T. polium subsp. flavovirens, and T. zanonii.* The aim of this study was comparative the chemical composition of the essential oils of eleven taxa belongs to twelve species of Teucrium collected from Libya including five endemic species.

Materials and Methods

The plant specimens were gathered in order to cover most habitats within the range of distribution of the genus in Libya from 12 different locations; Shahhat Susah, Lathroun RasElhellal, Wadi-Errieg, Awllad Hezzam village (Garian), Wadi El-Quttarh, El-Rabtta ElAssbeh, Quasser El-Khyaar (Wadi Jelabow), Sirut, Al-Hemida Escarpment, Tarhonah and Dryannah. The essential oil was extracted from the dried shoots of samples of the target species (50 g) by hydro-distillation method for three hours using Clevenger-type apparatus. The extracted oils were dehydrated over anhydrous sodium sulphate (Josiah *et al*., 2005). The essential oils were analyzed with Gas chromatography-mass spectrometry (GC-MS) (Thermo Electron Corporation) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32 mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260 °C, the oven temperature was programmed to increase from 130 to 280 °C at a rate of 4 °C/min. All the data of the present study were subjected, where appropriate , to standard one-way analysis of variance (ANOVA) and student's t-test (t-value < 0.05 was considered as significant) using the COSTAT 2.00 statistical analysis software of CoHort Software Company [20]. Where a significant difference was detected by ANOVA test, pairwise comparisons of means were performed using Least Significant Differences (LSD) at 0.05 probability level.

Results and discussion

Teucrium (Ajugoideae, Teucrieae) is a cosmopolitan and polymorphic genus with more than 300 species in Europe, North Africa, temperate regions of Asia and particularly in the Mediterranean basin [7 and 12]. A comparative study on the chemical composition of the essential oils of *Teucrium* was carried out and presented in Table 1. 12 compounds were identified and the major ones were germacrene B with a percentage of about 17.98% in *Teucrium fruticans*, and 27.01% in *T. capitatum*. β-caryophyllene attained a percentage of about 22.17% in *T. apollinis* and 25.27% in *T. campanulatum*. Limonene was one of the most important compounds which attained a percentage of about 7.03% in *T. lini-vaccarii* and 8.35% in *T. polium*. α-pinene attained a high value of about 2.5% *T. campanulatum* and not detected in *T. barbeyanum, T. brevifolium*, *T. lini-vaccarii* and *T. polium* subsp *flavoveriens*. Germacrene D slightly differed among the studied species, where the minimum was about 2.99% in *T. captatum* and the maximum was 4.81% in *T. barbeyanum*. Similarly, β-Elemene attained a minimum value of about 2.01% in *T. barbeyanum* and the maximum (4.74%) was in *T. apollinis*. A percentages of about 3.05% and 4.84% of α-Copaene were achieved in *T. zanoni* and *T. fruticans* respectively. α-Cadinol was not detected in *T. barbeyanum*, *T. davaeanu*m and *T. capitatum* and *T. polium* subsp.*flavoveriens* and attained a percentage of about 2.72% in *T. brevifolium*. Linalool was not detected in *T. brevifolium*, *T. campanulatum* and *T. polium* form1, but achieved a value of about 0.96% in *T. capitatum*. Terpinen was not detected in *T. brevifolium*, *T. campanulatum*, *T. polium* form1 and *T. fruticans* but achieved a value of about 0.29% in *T. zanonii*. Isoborneol was not detected in *T. apollinis*, *T. barbeyanum*, *T. polium* form 2, *T. fruticans* and *T. polium subsp.flavovirens* but achieved a value of about 0.12% in *T. polium* subsp.*flavoveeriens*. Camphene attained the minimum value (0.09%) in *T. apollinis* and the maximum one (0.59%) in *T. fruticans* (Figure 1 and 2).

Table 1. Variation in the percentages of some major compounds of the essential oils extracted from 12 taxa of Teucrium L. distributed along the different locations in the study area in Libya.

Species	α-pinene	Limonene	β-Caryophyllene	Germacerene- B	Germacerene-D	Camphene	Linalool	Isoborneol	α-Copaene	β-Elemene	Terpinen	α-Cadinol
T.apollinis Maire et Weiller	0.53^{b}	8.14^{c}	22.17^{ab}	26.31^{d}	4.11^{l}	0.09^{ij}	0.96^{g}	0.00	4.03^{e}	4.74^{k}	0.29^{h}	2.01
T.barbeyanum Aschers	0.00	8.33^{d}	24.54^{h}	23.12i	4.81ab	0.34^{k}	0.88^{c}	0.00	4.56^{e}	2.01^{cb}	0.09^{b}	0.00
T.brevifolium Schreber	0.00	8.04^{h}	24.83^{ij}	19.93l	3.85f	0.36^{b}	0.00	0.32^{c}	3.97^{b}	4.23^{k}	0.00	2.72^{j}
T. campanulatum L.	2.50^{e}	7.09^{k}	25.27^{i}	21.33^{h}	3.37^{b}	0.45^{cb}	0.78^{d}	0.00	4.07^{j}	4.28^{g}	0.00	2.55^{h}
T. capitatum L.	1.08^{c}	8.19^{a}	23.44^{b}	27.01^{h}	2.99^{c}	0.45^{hi}	0.96^{e}	0.00	4.06^{j}	3.97^{L}	0.16^{k}	2.01e
T.davaeanum Coss.	2.11^{b}	8.32^{i}	23.17^{d}	25.17^{h}	4.11^{k}	0.08^{a}	0.00	0.25^{f}	4.42^{cb}	3.29^{j}	0.18^{g}	0.00
T. fruticans L.	2.01^{h}	8.09^{c}	22.94^{a}	17.98^{b}	3.09^{k}	0.59^{k}	0.91^{j}	0.00	4.84^{e}	4.06^{dh}	0.00	1.29^{a}
T.lini-vaccarii Pamp.	0.00	7.03^{a}	23.38^{g}	21.11^{e}	3.26^{k}	0.57^{L}	0.92^{f}	0.19^{cb}	3.65^{h}	2.94^{ij}	0.25^{ab}	2.11^{b}
T.polium L. form1	1.07^{a}	7.93^{c}	24.94^{j}	20.22^{h}	4.27^{e}	0.49^{b}	0.00	0.28^{g}	4.23^{e}	3.93^{f}	0.00	2.05^{d}
T.polium L. form2	0.19^{i}	8.35^{f}	24.47^{e}	20.43^{b}	4.38^{cb}	0.34^{a}	0.84^{k}	0.00	3.89^{j}	3.03^{g}	0.11^{c}	0.00
T. polium subsp.*flavovirens* (Batt.) S. Pucch	0.00	9.01^{e}	24.18^{h}	22.48^{a}	4.47^{j}	0.36^{a}	0.85^{h}	0.41^{e}	4.38^{k}	4.06^{b}	0.00	0.00
T. zanonii Pamp.	2.33^{d}	7.26^{c}	24.28^{g}	21.92^{i}	4.06^{cd}	0.53^{k}	0.00	0.00	3.05^{e}	4.21^{a}	0.12^{g}	1.73^{h}
Mean	**1.47**	**7.98**	**23.96**	**22.25**	**3.89**	**0.83**	**0.88**	**0.236**	**3.79**	**3.72**	**0.17**	**2.05**

Means followed by the same letter(s) in the same column are not significant varied according to $LSD_{0.05}$ value

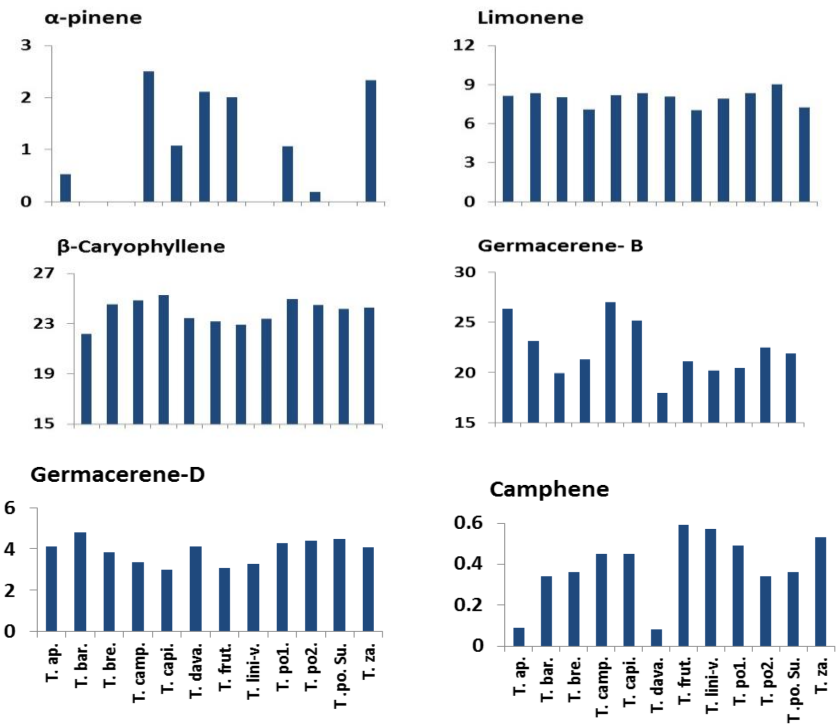

Figure 1.Variation in the percentages of six essential oil components (α-Pinene, Limonene, βCaryophyllene, Germacerene- B, Germacerene-D and Camphene) in 12 taxa of *Teucrium* L. (T.ap. = *T.apollinis*, T.bar. = *T.barbeyanum*, T.brev. = *T.brevifolium*, T.camp. = *T.campanulatum*, T.capi. = *T.capitatum*, T.dava. = *T.davaeanum*, T.fr. = *T.fruticans*, T.lini. = *T.lini-vaccarii*, T.po1. = *T.polium* form1, T.po2. = *T.polium* form2, T.po.sub. = *T.polium* subsp.*flavovirens*, T.za. = *T.zanonii*) collected from the different locations in the study area in Libya.

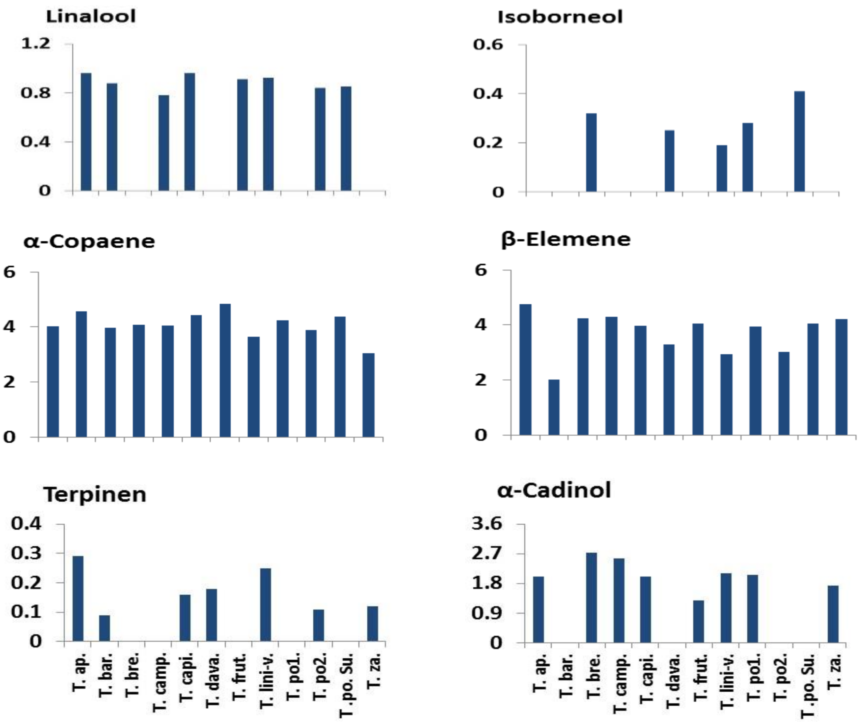

Figure 2. Variation in the percentages of six essential oil components (Linalool, Isoborneo,α-Copaene, β-Elemene,Terpinen and α-Cadinol) in 12 taxa of *Teucrium* L. (T.ap. = *T.apollinis*, T.bar. = *T.barbeyanum*, T.brev. = *T.brevifolium*, T.camp. = *T.campanulatum*, T.capi. = *T.capitatum*, T.dava. = *T.davaeanum*, T.fr. = *T.fruticans*, T.lini. = *T.lini-vaccarii*, T.po1. = *T.polium* form1, T.po2. = *T.polium* form2, T.po.sub. = *T.polium* subsp.*flavovirens*, T.za. = *T.zanonii*) collected the different locations in the study area in Libya.

The results explained that ecological factors play an important and unique role in qualitative and quantitative of the all studied characters, this is because the 12 different locations reflect specific regime for each location independently, these congruent with various literature showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions [21, 22, 23]. [24,25] reported variations in the yield and chemical profile of essential oils from *Mentha longifolia* and *Tagetes minuta* populations, collected from different geographical locations. Such differences could be associated to the varied soil textures and possible adoption response of different populations, resulting in different chemical products being formed, without morphological differences being observed in the plants [26].

With respect and comparsion between several studies, regarding with composition of the essential oils of different speciecs of *Tecrium* showed that; the major compounds of *T.*
yemense were α -cadinene (34.9%), β -caryophyllene (22.7%), α -humulene (6.1%), α selinene (5.4%) and two unidentified esquiterpenes (16.5%). The oil was characterized by a high content of sesquiterpene (90.4%), while monoterpenes accounted for 3.8% of the identified compounds. α -Cadinene, β- caryophyllene, and or α -humulene were found as main components in the oils of *T. montanum* from Serbia [27]. *T. alopecurus* from Tunisia [28] and *T. polium* from Jordan [29]. Besides, α -cadinene was reported to be one of the main constituents in the oils of *T. ramosissimum* (20.0%) from Tunisia [28], *T. stocksianum* (12.413.9%) from United Arab Emirate (UAE) [29] *T. libanitis* (5.3-9.7%) from Spain [30], *T. capitatum* (3-9.8%) from Portugal [31]and *T. montanum* (6.3%) from Turkey [33], but the amount of α -cadinene in the above- mentioned oils was lower than its quantity (34.9%) in our sample. β-Caryophyllene as the second major oil component was found in oils of other *Teucrium* species such as *T. turredanum* (16-33%) from Spain [31], *T. orientale L.* var. *orientale*, (7.2-28.8%), *T. pestalozzae* (27.6%), *T.antitauricum* (27.6%), *T. orientale* var. *puberulens* (21.7%) *T. chamaedrys* subsp. *lydium* (19.7%) from Turkey [33, 34, 35]. *T. scordium* (22.8%), *T. orientale* L. var. *orientale*, (9.3%) from Iran [36,37], *T. salviastrum* (19.1-24.1%) from Portugal [38], *T. fruticans* (12-22%) and *T. scorodonia* L. subsp. *Scorodonia* (25.2%) from Italy [38,39].,*T. turredanum* (4.7-10.1%) and *T. polium* (4.3%) [29], *T. ramosissimum*. α -Cadinene (19.97%), α -cadinol (9.93%) and germacradien-4-α -1-ol (8.68%) were the major compounds [42].
Altitude seems to be one more important environmental factor influencing the content and chemical composition of an essential oil.[43] .Climatic factors such as heat and drought were also related to the essential oil profiles obtained [21, 44].
Qualitative and quantitative differences were reported in essential oils of all the studied species and these may be due to the genetic, differing chemotypes, drying conditions and geographic or climatic factors. [44], explained these differences to the distinct habitat in which the plant has been collected and it seems that the geographical origin of studied species greatly influences the oil quality [40]. [46], reported the variation in the quality and quantity of the essential oil obtained from *Artemisia annua* at different developmental growth stages including preflowering, flowering and post-flowering.

[47, 48], investigated the chemical intraspecific variability among plants of *Thymus hyemalis* L. and Spanish *T. vulgaris* subsp. *vulgaris* shrubs.

The higher essential oil yield from *Thymus vulgaris* was reported in spring and over the nine month harvesting period, thymol was found to vary from 31.5-52.4% [49]. With respect to the harvesting time, the thyme essential oils were richer in oxygenated compounds in the spring, followed by summer, autumn and winter [50].

Another important factors may be added which affect the yield and composition of essential oil include part of plant used [24 and 51]; length of exposure to sunlight[52 and 53], availability of water, height above sea level [54], plant density [53, 55], time of sowing [54] and the presence of fungal diseases and insects [55 and 56]. All evidences which used in this study prove without a doubt aggressive effective by ecological factors.

In general, the major components of the essential oils showed significant parentages among all the studied species. Monoterpens was represented by α-pinene, Limonene, Camphene, Linalool and Terpinen while Sesquiterpens include β-Caryophyllene, Germacerene- B, Germacerene, Isoborneol, α-Copaene, β-Elemene and α-Cadinol. It is worth mentioning that Limonene was detected in all the studied species but varies in the parcentage from high to low as the *following* order: *T. polium* subsp.flavovirens> *T. polium*> *T. barbyeanum*> *T. davaeanum*> *T. capitatum*> *T. apollinis* > *T. fruticans* > *T. brevifolium*> *T. polium*> *T. zanonii*> *T. campanulatum* > *T. lini-vaccarii*.

Receptiveness should be taken into consideration the time, season, organ used, prevailing environmental factors, soil moisture and further other factors affecting on the chemical composition of the extracted oils. In addition, the different methods of extraction, accuracy and methods of evaluation should be considered.

However, *T. zanonii* is specified by the presence of all assessed oils, while section Chamaedrys (*T.barbeyanum*) is characterized by the absence of α-pinene, α-cadinol and Isoborneol. Although, α-pinene and α-cadinol are also absent in *T.polium* subsp. *flavovirens*, these are detected in the two other forms of *T.polium*. Contrary, Linalool and Terpinen are detected in the two forms of *T.polium* and not in its subspecies. Terpinen is also undetectable in *T.brevifolium*, *T.campanulatum* and *T.fruticans*, which are belonged to section Teucrium.

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