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Research article

ANALYSIS OF THE ESSENTIAL OIL OF THE GERMANDER (*TEUCRIUM POLIUM* L.) AERIAL PARTS FROM THE NORTHERN REGION OF SAUDI ARABIA

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ABSTRACT: The chemical composition of the essential oil of the aerial parts of *Teucrium polium* L. collected during the flowering period from, northern region of Saudi Arabia, (Aljuf, kingdom of Saudi Arabia) has been studied by GC and GC/MS. 114 compounds were identified amounted to 88.51% of the total oils. The presence of sesquiterpenoid hydrocarbons (34.62%) characterizes the oil of Saudi Arabian germander. While, *T. polium* oil is also rich with hydrocarbons monoterpenes (26.08%), oxygenated monoterpenes (11.14%) and oxygenated sesquiterpenoids (11.79%). The dominant constituents (52.17%) wereγ-muurolene (8.72%), α-cadinol (5.93%), δ-cadinene (5.08%), β -pinene (4.58 %), β-gurjurene (4.43 %), α-limonene (4.29 %), α-Pinene (3.79 %),α-Thujene (3.69%), Spathulenol (3.42 %), p-cymene (2.95%) γ-cadinene (2.81%)and Sabinene (2.47 %).

Key words: *Teucrium polium* L. essential oil, Saudi Arabia, γ-muurolene, α-cadinol, δ-cadinene, β –pinene, β-gurjurene, α-limonene.

INTRODUCTION

Teucrium is a polymorphic and cosmopolitan genus of perennial plants, the genus is considered to be the largest of the Lamiaceae family in the Mediterranean area, with more than 300 species (Djabou et al., 2012). *Teucrium polium* L. is a perennial shrub, which is considered as an out-crossing species. The species can take 10-35 cm as a high; the crenate leaves are white, tomentose on both sides, with downwards rolled rounded-toothed margins and the stems are prostate (Lamnauer and Batanouny, 2005, Djabou et al., 2012). According to the same authors (Lamnauer and Batanouny, 2005), the flowers have a white or yellow corolla, in a globular inflorescence. The calyx is bell-shaped with 5 subequal flat, triangular or acuminate triangular teeth. The fruits are light-brown to dark brown nut lets with a latticed surface. The plant gives off a pleasant aromatic smell; flowering takes place from April until June. It shows a high variation both in morphological and ploidal levels (El Oualidi et al., 2002; Navarro and ElOualidi, 2000).
Teucrium ssp are most common in Mediterranean climates and Middle East (Moghtader, 2009). It usually develops in regions belonging to the semi-arid and arid bioclimates; it likes light and sun and well-drained soil. It grows widely on hillsides, sands and in arid places. (Lamnauer and Batanouny, 2005). The Saudi Arabia with its 2250 species richness comprises six species belonging to the genus (Collenette, 1999): *Teucrium hijazicum* Hedge & R.A. King, *Teucrium leucocladum* Boiss., *Teucrium oliverianum* Ging. exBenth, *Teucrium polium* L., *Teucrium popovii* R.A. King and *Teucrium yemense* Defl. According to Collenette (1999), 2 species belonging to the genus figure on the list of endangered species (*Teucrium popvii* R.A. King and *Teucrium hijazicum* Hedge & R.A. King).
It well known that *Teucrium* species are rich in essential oils (Cakir et al., 1998; Cozzani et al., 2005; Aburjai et al., 2006; Kabouche et al., 2007; Boulila et al., 2008; Moghtader, 2009; Menichini et al., 2009; De Martino et al., 2010). In Saudi Arabian folk medicine, germander (*T. polium*) is known under the vernacular name of “Jaada” or “Jaa'd.
Arial parts from the plant and infusion of tender are used by the local population of Bedouins for the treatment of the febrifuge, vermifuge, stomach and intestinal troubles. The plant is also used in a steam bath in colds and fevers. (Mossa et al., 2000; Rahman et al., 2004; Tariq et al., 1989). The essential oil of *T. polium* has been widely studied in several regions especially in Mediterranean regions (Menichini et al., 2009; Boulila et al., 2008; Belmekki et al., 2013; Djabou et al., 2012). In Middle East few reports focuses on the essential oil of the species (Moghtader, 2009; Aburjai et al., 2006; Cakir et al., 1998). In the present work we have studied the chemical composition of *T. polium* grown in wild habitat from the northern region of Saudi Arabia.

MATERIALS AND METHODS

Plant material

The starting material consists of flowering branches and fresh leaves collected from plants growing in Camel and Range Research Centre of Ministry of Agriculture, Al-Jouf (Kingdom of Saudi Arabia). The centre is located in the Northern region of Saudi Arabia (latitude 29°49′ 18.04 ′′ N, longitude 40°07′ 47. 52′′ E, located at 1035 km NW from Riayadh). The site belongs to the Mediterranean desertic –continental zone with a rainfall ranging between 50 and 150 mm/year and situated at an altitude of 350 m. (Figure 1).

Essential oils identification

The essential oils have been extracted from air-dried flowered stems (100 g) by hydrodistillation for 3 h, using a Clevenger-type apparatus. Oil yields were then estimated on the basis of the dry weight of plant material (v/w).
The essential oils were analyzed by Gas chromatography–mass spectrometry (GC–MS) using a HP 5975 C mass spectrometer (Agilent technologies) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m×250 µm coated with 5% phenylmethyl silicone, 95% dimethylpolysiloxane, 0.25 µm film thickness) was used. Oven temperature was programmed to rise from 60 to 220°C at a rate of 4°C/min; transfer line temperature was 23 0°C. The carrier gas was He with a flow rate of 0.8 ml/min and a split ratio of 50:1. Scan time and mass range were 1 s and 50–550 m/z, respectively. The identification of oil components was assigned by comparison of their retention indices (RI) relative to (C8–C22) n-alkanes with those of literature or with those of authentic compounds available in the authors' laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra (Adams, 2001). Determination of the percentage composition was based on peak area normalization without using correction factors. Analyses were performed in triplicate for the studied species.

RESULTS

The average percentage of the essential oil of the aerial parts (leaves and flowered stems) of Saudi Arabian *T. Polium* L. was light yellow with yields of 1.65% (v/w). Typical CG-MS chromatogram of the essential oil of Saudi Arabian germander is shown in Figure 2. The figure proves the high diversity of oil composition at individual level. The compounds from essential oils are grouped in Table 1 based on their chemical structures in which they are classified. The GC-MS analysis of the oils of *T. polium* resulted in 88.51% from 114 compounds. The highest percentage of compounds (Table 1) were hydrocarbon sesquiterpenes (34.62 %), followed by monoterpene hydrocarbons (22.39%), oxygenated sesquiterpenes (11.79%), oxygenated monoterpenes (11.14%), monoterpene ketones (4.61%), alcohols (1.48%), sesquiterpene alcohols (0.92%), Phenolics and other volatiles compounds (0.6%), and sesquiterpenes ketones (0.09%). In the hydrocarbon sesquiterpenes, the major compound of this population is γ-Muurolene with 8.72 % of the total oil.As shown by table 1, δ-cadinene and γ-cadinene are the two major components that followed γ-muurolene. These two hydrocarbon sesquiterpenes are represented respectively by 5.08% and 2.81 % of the total component of essential oil of Saudi Arabian germander.The amount presented here are higher than those described by Boulila et al (2008). Among the sesquiterpenes (47.44%), those oxygenated represent 11.79 % and are represented mainly by α-cadinol (5.93%), spathulenol (3.42%) and caryophyllene oxide (1.22%).
Monoterpenes represent 38.15% of the total identified compounds.The major predominated compounds of monoterpenehydrocarbons (22.39%) are β-pinene (4.58%), α- limonene (4.29%), α-pinene (3.7%), p- cymene (2.91%) and sabinene (2.74%). Within the oxygenated monoterpenes (11.14%), the major compounds of Saudi Arabian germander were 4-terpineol (3.72%), cis-Z-α-bisabolene (1.54%) and linalool (1.18%).In the case of monoterpene ketones (4.61%), the major compound was α—thujene (3.69%).The molecular structure of the major constituent (33 %) of essential oil *T. polium* L. is depicted in Figure 3.

Figure 1.Map of Saudi Arabia, the collection site in Camel and Range Research Center of Ministry of Agriculture, Al-Jouf (CRRC)

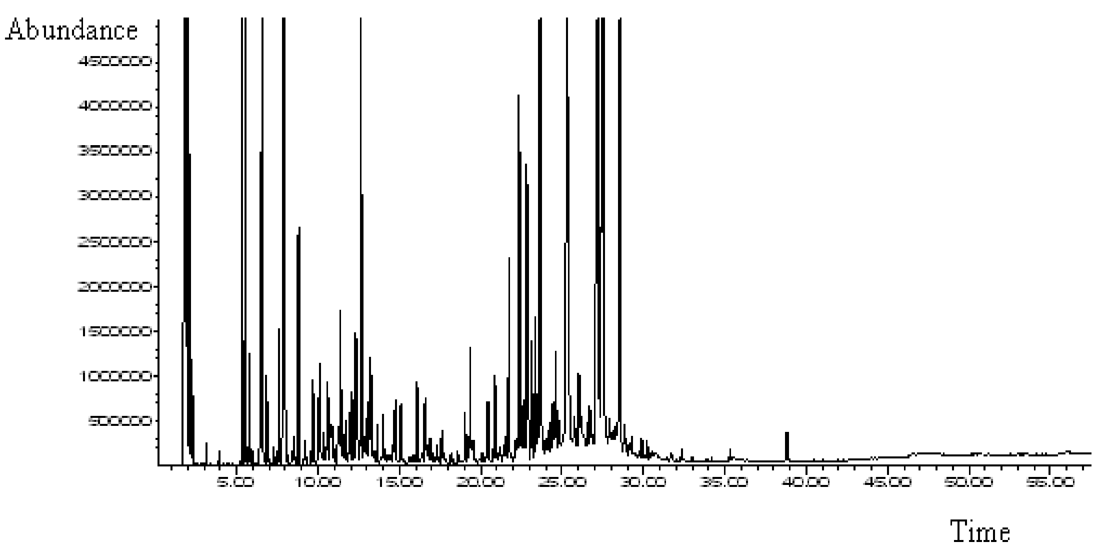
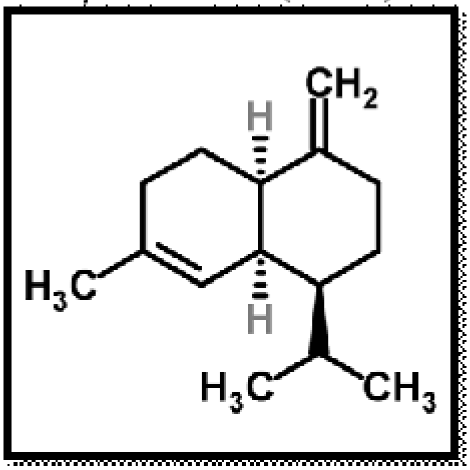
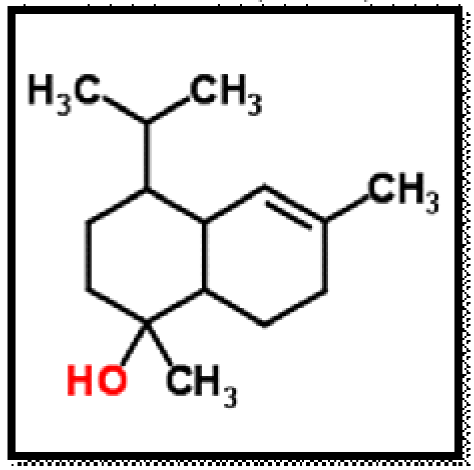


Figure 2.Typical CG-MS chromatogram of the essential oil of Saudi Arabian germander (*T. polium* L.)

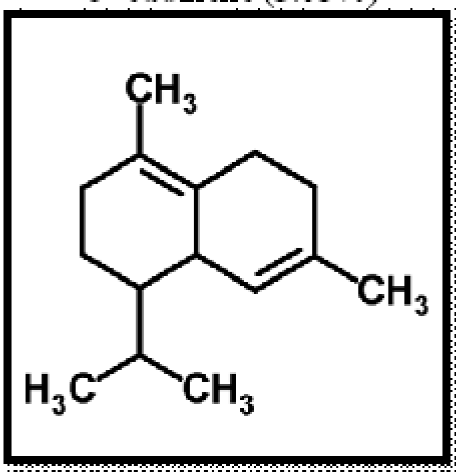
γ -muurolene (8.72%)



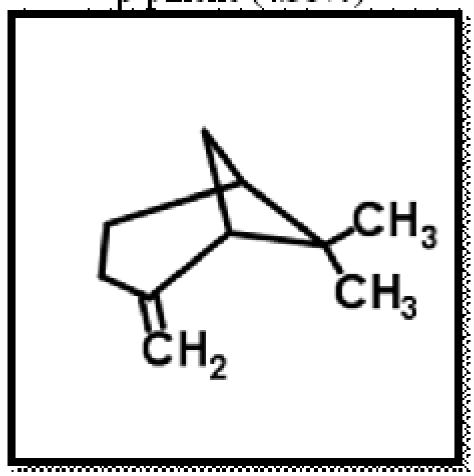
α-cadinol (5.93 %)



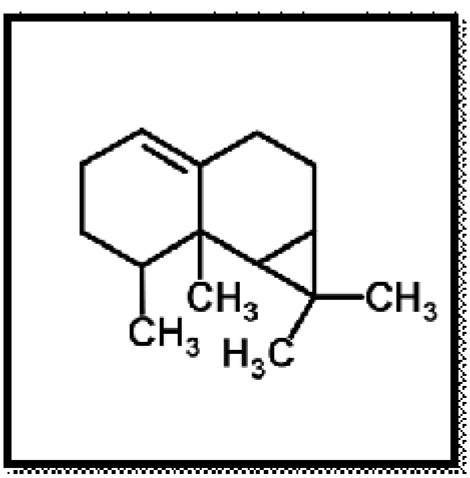
δ--cadinene (5.08%)



β-pinene (4.58%)



β-gurjurene (4.43%)



α-limonene (4.29%)

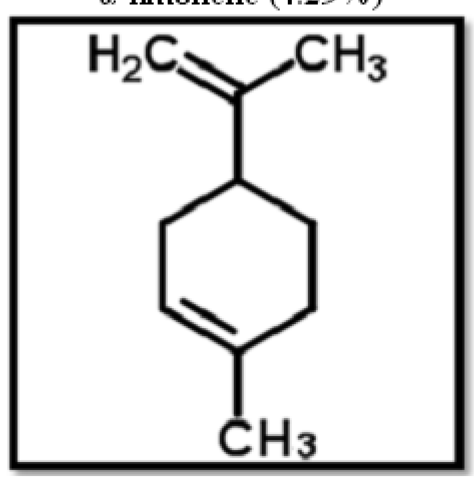


Figure 3.Major components (>33 %) of *Teucrium polium* L. in Saudi Arabia

Table-1. Percentage composition of essential oil of Saudi Arabian germander (*T. polium* L.) and their retention indices.

S.No	Oxygenated monoterpenes: Compounds	RI	Percentage of the Compounds (%)	S.No	Monoterpene ketones: Compounds	RI	Percentage of the Compounds (%)
1	**linalool**	1098	**1.186** (±0.781)	17	trans pinocamphone	1141	tr
2	α -thujone	1105	0.546 (±0.167)	18	eucarvone	1146	0.126 (±0.05)
3	α-campholenal	1122	0.335 (±0.155)	19	p-menth-8-en-3-ol	1149	tr
4	β- fenchol	1123	0.31 (±0.034)	20	β-terpineole	1159	tr
5	*trans*-pinocarveol	1137	0.933 (±0.247)	21	pinocarvone	1160	0.363 (±0.095)
6	**4-terpineol**	1159	**3.723** (±0.361)	22	carvomenthenediol	1176	0.08 (±0.092)
7	α-terpineol	1189	0.106 (±0.123)	23	verbenone	1204	0.14 (±0.161)
8	Myrtenal	1193	0.873 (±0.17)	24	carvone	1239	0.236 (±0.13)
9	Myrtenol	1196	0.066 (±0.076)	25	perillaldehyde	1245	0.055 (±0.017)
10	cis-carveol	1217	0.145 (±0.132)	26	phytol	2111	tr
11	Geraniol	1235	0.113 (±0.025)	27	trans-phytol	1988	0.066 (±0.067)
12	bornyl acetate	1284	0.41 (±0.129)				
13	carvacrol	1314	tr				
14	β-bisabolene	1513	0.77 (±1.022)				
15	α-bisabolene	1536	0.09 (±0.011)				
16	**cis-Z- α-bisabolene epoxide**	1814	**1.54** (±0.727)				
	TotalOxygenated monoterpenes: 11.14%				**Total Monoterpene ketones: 0.92 %**		

RI: Retention index on HP-INNOWAX capillary column.tr: Traces (compounds %<0.05%).

(Continued Table 1)

	Monoterpenehydrocarbons (26.09 %): Compounds	RI	Percentage of the Compounds (%)		Phenolic compounds and other volatiles compounds (0.60%): Compounds	RI	Percentage of the Compounds (%)
28	santolinatriene	908	0.063 (±0.005)	54	thymol	1293	0.196 (±0.227)
29	α-pinene	938	3.7 (±2.64)	55	dibutyl ester	1300	0.053 (±0.061)
30	Camphene	953	0.233 (±0.198)	56	p-thymol	1308	0.195 (±0.005)
31	β-thujene	971	0.116 (±0.12)	57	hexahydrofarnesyl acetone	1835	0.075 (±0.017)
32	Sabinene	973	2.473 (±1.15)	58	palmitic acid	1957	0.086 (±0.1)
33	β-pinene	980	4.583 (±1.586)		SesquiterpenoidAlcohols (0.923 %)		
34	β-myrcene	993	0.79 (±0.392)	59	cuparene	1504	0.49 (±0.565)
35	β-cymene	1011	0.15 (±0.193)	60	elemol	1548	0.213 (±0.246)
36	α- terpinene	1014	0.783 (±0.269)	61	D- viridiflorol	1612	0.12 (±0.034)
37	p- cymene	1025	2.913 (±0.757)	62	α –bisabolol	1685	0.1 (±0.115)
38	α- limonene	1030	4.29 (±2.587)	63	farnesol	1713	tr
39	β-ocimene	1032	0.176 (±0.052)	64	2,4-decadienal	1999	tr
40	γ-terpinene	1054	1.28 (±0.392)		Sesquiterpenoidsketones(0.1 %)		
41	α- terpinolene	1089	0.713 (±0.211)	65	β –ionone	1428	0.1(±0.111)
42	p-cymenene	1090	Tr		Oxygenated sesquiterpenoids(11.80 %)		
43	trans-α-ocimene	1097	0.053 (±0.061)	66	trans-β-caryophyllene	1435	0.743 (±0.277)
44	trans sabinene hydrate	1098	0.08 (±0.092)	67	caryophyllene oxide	1582	1.22 (±0.207)
45	α—thujene	1021	3.69 (±1.742)	68	spathulenol	1578	3.423 (±1.418)
	Alcohols (1.42 %)			69	ledol	1602	0.48 (±0.15)
46	verbenol	1137	0.43 (±0.33)	70	α-cadinol	1649	5.93 (±0.161)
47	menth-3-en-8-ol	1145	0.103 (±0. 119)				
48	p-cymen-8-ol	1179	0.316 (±0.042)				
49	p-mentha-2,8-dien-1-ol	1187	0.116 (±0.12)				
50	cis-piperitol	1203	0.08 (±0. 092)				
51	p-cymen-7-ol	1289	0.106 (±0.013)				
52	p-mentha-1,4-dien-7-ol	1325	0.27 (±0.011)				
53	cedr-8-en-13-ol	1668	tr				

	Sesquiterpenoid hydrocarbons			Sesquiterpenoid hydrocarbons			
	Compounds	RI	Percentage of the Compounds (%)		Compounds	RI	Percentage of the Compounds (%)
71	α –phellandrene	1002	1.85 (±2.762)	99	γ-muurolene	1478	8.72 (±9.595)
72	1,3,8-paramenthatriene	1108	0.19 (±0.173)	100	α curcumene	1483	tr
73	α-campholene aldehyde	1112	tr	101	β -himachalene	1489	tr
74	β-pinone	1135	0.236 (±0.068)	102	β-guaiene	1492	0.185 (±0.017)
75	α-longipinene	1347	0.063 (±0.073)	103	zingiberene	1493	0.36 (±0.415)
76	α-Cubebene	1352	0.34 (±0.323)	104	α –muurolene	1499	1.85 (±0.771)
77	α-Copaene	1377	0.356 (±0.148)	105	bicyclogermacrene	1507	1.213 (±0.301)
78	β-bourbonene	1385	0.393 (±0.174)	106	α –farnesene	1512	0.225 (±0.167)
79	β- cubebene	1387	1.64 (±0.36)	107	γ-cadinene	1515	2.81(±3.277)
80	β elemene	1393	0.096 (±0.01)	108	β- cadinene	1518	0.105 (±0.028)
81	α gurjunene	1407	0.49 (±0.23)	109	β - Sesquiphellandrene	1521	0.43 (±0.057)
82	β- caryophyllene	1408	0.27 (±0.161)	110	δ-cadinene	1526	5.083 (±4.262)
83	α cedrene	1409	0.15 (±0.01)	111	α-calacorene	1541	0.086 (±0.1)
84	bergamotene	1411	0.79 (±0.369)	112	germacrene B	1559	0.56(±0.17)
85	longofolene	1416	0.79 (±0.369)	113	β-gurjunene	1596	tr
86	thujopsene	1426	0.073 (±0.084)	114	germacrone	1693	tr
87	trans- α- bergamotene	1432	0.36 (±0.415)				
88	α –guaiene	1437	0.165 (±0.144)				
89	aromadendrene	1437	0.716 (±0.484)				
90	β humulene	1440	0.115 (±0.04)				
91	α-humulene	1452	0.366 (±0.264)				
92	(*E*)-β-Farnesene	1456	tr				
93	α Caryophyllene	1462	0.395 (±0.305)				
94	alloaromadendrene	1463	0.455 (±0.075)				
95	alloaromadendrene oxide	1465	0.32 (±0.323)				
96	γ –gurjunene	1472	0.39 (±0.10)				
97	α amorphene	1473	0.353 (±0.407)				
98	germacrene D	1477	1.64 (±0.361)				
	Total Sesquiterpenoid hydrocarbons: 34.62 %						

DISCUSSION

The main goal of this study was to evaluate the chemical composition of Saudi Arabian germander. The yield of oil was one of the most highest values (1.65). However, Aburjai et al. (2006), Kabouche et al. (2007) and Belmekk et al (2013) obtained, respectively, a yield of 0.8 % (w/w), 1.7 % (w/w) and 0.21 % (w/w). γ-Muurolene, the main constituent of the essential oil in this study, is an important constituent identified in most *Hypericum* species (Guedes et al., 2005).This is the second report describing the presence of γ-Muurolene as a major component of *T. polium* L. and its subspecies. Moreover, Ashnagar et al., (2007) reported that γ-Muurolene is the major predominated compounds of Iranian germander oil. In the same way Vokou and Bessiere (1985), Pérez-Alonsoet al., (1993), Cakir et al., (1998) and Djabou et al., (2012) mentioned the presence of this hydrocarbon sesquiterpenes (γ-Muurolene) with the percentages varying from 0.1%, to 1.9%. However previous study on same species never detected γ-Muurolene as a component of essential oil of germander (Aburjai et al., 2006; Kabouche et al., 2007; Boulila et al., 2008). Nevertheless, Kabouche et al (2007) mentioned 3β-a hydroxy-α-muurolene as one of major component of *T. polium* L. in Algeria with 22.5 % . In addition, essential oil from leaves of a medicinalplant from Brazilian flora (*Xylopialaevigata*) dominated by γ-Muurolene as a major component displayed cytotoxicity to all tumor cell lines tested (Quintans et al., 2013). By the end of the present investigation, the quality and quantity of the material of essential oil of Saudi Arabian germander had some differences and similarities with the cases reported in other regions. In the literature 25 studies, established the chemical composition of *T. polium*oils (Aburjai et al., 2006; Al-Qudah et al., 2011; Antunes et al., 2004; Ashnagar et al., 2007; Bezic et al., 2011; Cakir et al., 1998; Cozzani et al., 2005; De Martino et al., 2010; Eikani et al., 1999; Hammoudi and HadjMahammed, 2010; Hassan et al., 1979; Kabouche et al., 2007; Kamel and Sandra, 1994; Kovacevic et al., 2001; Menichini et al., 2009; Moghtader, 2009; Pérez-Alonso et al., 1993; Sarer and Konuklugil, 1987; Stanciu et al., 2006; Tomi and Casanova, 2006; Vahdani et al., 2011; Vokou and Bessiere, 1985; Bahramikia and Yazdanparast, 2012; Djabou et al., 2012; Belmekki et al., 2013).

According to Djabou et al., (2012) and whatever to the origin of the plant, the main components were hydrocarbon monoterpenes such as limonene, α- and β-pinene; hydrocarbon sesquiterpenes such as β-caryophyllene, γ-muurolene, γ-cadinene and germacrene D; and oxygenated sesquiterpenes such as α- and τ-cadinol, patchouli alcohol, caryophyllene oxide and 8-cedren-13-ol. To the best of our knowledge, essential oil from *T. polium* L. in Saudi Arabia was never reported in literature. The dominant constituents (Figure 3) were:γ-muurolene (8.72 %), α-cadinol (5.93 %), δ-cadinene (5.08 %), β -pinene (4.58 %), β-gurjurene (4.43 %), α-limonene (4.29 %), α-Pinene (3.79 %), α-Thujene (3.69%), Spathulenol (3.42 %), p-cymene (2.95%) γ-cadinene (2.81%) and Sabinene (2.47 %). Interestingly, it has been found that several compounds isolated from different parts of *T. polium* possess a broad spectrum of pharmacological effects including antioxidant, anticancer, antiinflammatory, hypoglycemic, hepatoprotective, hypolipidemic, antibacterial and antifungal (Bahramikia and Yazdanparast., 2012) .The present investigation show a high percentage of those compounds such aγ-muurolene which showed cytotoxicity to all tumor cell lines (Quintans et al., 2013). Nevertheless, α-cadinol, δ-cadinene, β-pinene were dominant compounds of Saudi Arabian *T. polium* which showed important antiproliferative effects (Lampronti et al., 2006). Germacrone is another compound, despite it law percentage in the oil of Saudi Arabian germander, until it was detected as a trace compound (less than 0.05%), this sesquiterpenoid hydrocarbons was reputed for its high anti-tumor effect (Liu et al., 2013). The observed differences and variability in the essential oil of Saudi Arabian *T. polium* may be probably due to different environmental and genetic factors. In Saudi Arabia, populations of germander are widely distributed in southern area such as Asir Mountains in its related chain of escarpments and in degraded areas in northern regions. Moreover, the genetic structure study will be with a great importance for understanding the genetic fitness and for developing conservation programs for this genotype in Saudi Arabia.

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