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PHYTOCHEMISTRY AND BIOLOGICAL PROPERTIES INVESTIGATION OF *TEUCRIUM POLIUM* L.

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ABSTRACT

Aims: *To perform successive extraction of the dried plant of Teucrium polium L. belonging to Lamiaceae family, obtained from Saudi Arabia, using five extracting solvents to evaluate its antimicrobial activity comparatively. GLC analysis of the saponified and unsaponified parts of the ethereal fraction. The volatile content of Teucrium polium L were identified usingGC-MS of, and determine their structures. The chemical composition of the protein and ash contents of the plant were analizded. Phytochemical investigation of the successive five extracts.* ***Study Design:*** *Collection of Teucrium polium L from Saudi Arabia Mountains, successive solvent extraction, application of different research points, including antimicrobial activity, GLC analysis of the saponified and unsaponified ethereal parts, GC-MS analysis of the volatile content of Teucrium polium L, and determination of the protein and mineral contents of the plant material.* ***Methodology:*** *Teucrium polium L. was collected in spring 2014 from the mountain region of Alkurr Wadi, Tabuk area, Saudi Arabia. Teucrium polium L shade dried, and ground into powder. The plant was applied to successive extraction using petroleum ether, methylene chloride, ethyl acetate, methanol and water, respectively, at room temperature. The afforded extracts were tested against a set of pathogenic microorganisms for antimicrobial activity using the agar diffusion assay; the unsaponified and saponified fractions of petroleum ether extract were analyzed by GLC analysis, while the essential oil components of the plant have been determined by GC-MS analysis. The biochemical composition (protein and mineral contents) of the plant was determined as well. A phytochemical assay for the entire natural classes of the five extracts were determined as well.* ***Results:*** *Teucrium polium L. was collected from the mountain region of Alkurr Wadi, Tabuk area, Saudi Arabia. GLC analysis of the saponified and unsaponified parts of the ethereal fraction of T. polium L confirmed the existence of 20 fatty acid ester and 19 unsaponified hydrocarbons, among them 4 sterols: cholesterol, campesterol, stigmasterol, and β-sitosterol. the most abundant fatty acid was Linoleic acid (66.10 %) in the saponified fraction, while n-octadecane, (12.0%), n-heptacosane (11.73%), n-nonacosane (10.25%), n-pentacosane (8.87), n-tricosane (7.49), n-hexadecane (6.03%), and cholesterol (6.01%), represent the most abundant unsaponified components. The hydro-distillation wasused for volatile oil content of Teucrium polium L and determined by GC-MS analysis, indicating the existence of 29 diverse bioactive compounds, namely (-)-caryophyllene oxide (17.83%), farnesol (14.32%), spathulenol (11.88%), α-Curcumene (7.21%), caryophyllene (5.17%), 2,2,6- trimethyl-1- (2-methyl-cyclobut-2-enyl)- hepta-4,6-dien-3-one (3.15%) representing the most abundant constituents of the essential oil (EO). The chemical composition of the protein content and mineral of the ash content was studied. Finally, the phytochemical evaluation of the successive five extracts was emphasized as well. Based on the antimicrobial activity testing, the pet. ether fraction revealed the most potent activity against the diverse set of Gram positive (B. subtilis, S. aureus), Gram negative (p. auragenosa) bacteria, yeast (C. albicans) and fungi (S. servisia, A. niger).*

Conclusions: *T. polium L. is rich with several nutrient minerals and numerous diverse bioactive compounds, and hence it can be served as amale source of various medicinal compounds which can be exploited for their merchant production.*

KEY WORDS

Teucrium polium L; Phytochemistry; chemical composition; biological properties.

INTRODUCTION

Teucrium (Lamiaceae) is a large and polymorphic genus with about 340 species widespread all around the world, which distributed mainly in Europe, North Africa and in the temperate parts of Asia, but mainly in the Mediterranean region [i-iv]. Traditionally, *T. polium* has been used for over 2000 age in traditional medical specialty due to its diuretic, diaphoretic, tonic, antipyretic, gastrointestinal disorders, inflammations, diabetes, rheumatism, cholagogic, hypoglycemic, insulinotropic, hypolipidemic, antinociceptive and antioxidant properties [i-ix]. Amid the most recent 40 years, diverse classes of mixes have been detached from different parts of T. polium of which the fundamental gatherings are terpenoid [x-xii] flavonoids [ii-iv]. It has been finding that these mixes have an expansive range of pharmacological activities including antioxidant [ii-v] anticancer [ii-v], antiinflammatory, hypoglycemic [13, ii-30], hepatoprotective, hypolipidemic [iii], antibacterial, antifungal and antiviral activities [ii-vii]. Saudi Arabia has an extraordinary hereditary decent variety as ecotypes of tree species. The western and southwestern areas of the nation are wealthy in local plant flora of developed harvests and therapeutic plants [ii]. Around 300 types of therapeutic plants are utilized in conventional medication in Saudi Arabia [xxxix]. The consequences of information examinations on the synthetic, pharmacological and toxicological qualities of T. polium bolster the view that this plant has helpful remedial properties. Be that as it may, additionally active components and further confirm their applicable pharmacological activities are justified.

In the present investigation, the phytochemistry and biological properties of *T. polium* L., belonging to Lamiaceae family, collected from Saudi Arabia was achieved. In accordance, a successive extraction of the dried plant using diverse solvents of subsequent polarities, namely, petroleum ether, methylene chloride, ethyl acetate, methanol and water, was equivalently carried out at room temperature. The resulted extracts were comparatively examined for antimicrobial activities. The phytochemical evaluation of the successive extracts was comparatively studied. The chemical composition of the protein content and minerals of the ash content was studied. Finally, the volatile oil content of *T. polium* L. was qualitatively and quantitatively estimated by GC-MS as well.

MATERIALS AND METHODS

Plant Material

The aerial parts of Teucrium polium L. (Lamiaceae) gathered from the mountain district of Alkurr Wadi, Tabuk zone, Saudi Arabia in spring 2014, and recognized by Dr Amal M. Fakhri Abdelsalam, Plant Culture, Biology Department, Tabuk University, Tabuk, Saudi Arabia. At that point the plant was shade dried and crushed into powder. The readied powder was kept in tight holders, shielded totally from light.

Proximate and Mineral Elements Composition:

Standard procedure as outlined by the Association of Official Analytical Chemists [40] used to find moisture, ash, crude protein, contents. The plant sample dried at 65ºC for 48 hrs, ground and wet digested using H_2SO_4: H_2O_2 method [41]. The overviews tests were then subjected to estimation of N (for protein) utilizing Micro-Kjeldahle strategy [42]; phosphorus measured utilizing the molybdenum blue technique and dictated by spectrophotometer [43]; potassium and magnesium controlled by Flame Photometer [42], whilea iron analized by atomic absorption photometer [44]

Phytochemical evaluation of *Teucrium polium* L.

The methanolic concentrate of the aeronautical parts of T. polium subjected to fundamental phytochemical tests to identify nearness of alkaloids, saponins, glycosides, tannins, triterpens, steroids, coumarins, flavonoids, sugars and proteins utilizing standard strategies. The methodology of screening in the present work benefits of our previous [45,46] as well as that of Wall et al [47] and Trease and Evans [48].

Analysis of saponified and unsaponified fractions in the unpolar fraction of *Teucrium polium* L.

The unpolar fraction (pet. ether fraction, 1.0 g) of *T. polium* L applied to saponification according to the method reported by Tsuda et al, 1960 [49] using 10 % methanolic potassium hydroxide for 3 hrs. The reaction mixture was then extracted with chloroform to separate the unsaponified fraction, and allowed to dry using sodium sulphate anhydrous, filtration and then concentrated *in vacuo* till dryness to afford the unsaponified fraction (0.471 g). Alternatively, the saponified free fatty acids present in the water phase methylated according to the method reported by Finar (Finar, 1967) [50], and then extracted with chloroform. The resulting chloroform exutract was then dried with sodium sulfate anhydrous, followed by filtration and concentration *in vacuo* to afford the corresponding fatty acid methyl esters (0.295 g). The constituents of both the saponifiable and unsaponifiable fractions identified by GLC spectrometry using Agilent Technologies 6890 Network GC system (USA) compared with the authentic samples.

For analysis the unsaponified fraction, Agilent Technologies Network GC system (USA) served having the following characteristic and conditions: **Oven**: Initial temperature 80ºC, initial time 1.00 min, Rate 8.00ºC/min, Final temperature: 300^{0}C, final time 15 min. **Front Inlet**, mode: Splitless, initial temperature 250ºC. **Column**: HP-5% phenyl methyl siloxane, maximum temperature 325ºC, Nominal length 30.0 m, nominal diameter 320.0 mm, nominal film thickness 0.25 mm, initial flow 20 mL/min. Back detector (FID) temperature: 300ºC. Used gases: Carrier gas Nitrogen (44.5 mL/min), flame gases: Hydrogen (25.0 mL/min) and Air (250 mL/min).

The same GC equipment (Agilent Technologies 6890 Network GC system (USA)) has served for the analysis of the saponified fraction using the following conditions: **Oven**: Initial temperature 50ºC, initial time 2.00 min, rate 10, 8, 5, 6ºC /min, final temperature: 70, 170, 200, 240ºC, final time 2.0, 9.0, 5.0, and 15 min. **Front Inlet**, mode: Splitless, initial temperature 250ºC. **Column**: capillary column, Agilent 19091J-413, HP-5% phenyl methyl siloxane, maximum temperature 325ºC, Nominal length 30.0 m, nominal diameter 320.0 mm, nominal film thickness 0.25 mm, initial flow 1.5 mL/min. **Back detector** (FID) temperature: 280ºC. Used gases: Carrier gas Nitrogen (44.5 mL/min), flame gases: Hydrogen (25.0 mL/min) and Air (250 mL/min).

Essential oil (EO) Preparation

Dry aerial parts (100 g) of T. polium L. were subjected to the hydrodistillation of 4 h, utilizing a cleavenger-type contraption, as per the technique prescribed by the European Pharmacopeia to deliver oils [51]. The acquired broke up basic oil in hexane was concentrated, and the managed EO was dried over anhydrous sodium sulfate and put away at 4oC until tried and investigated

Gas chromatography–mass spectrometry (GC/MS) analysis

The essential oil (EO) detected by gas chromatography. The chromatograph (Agilent 6890 UK) outfitted with a HP-5MS hairlike segment (30 × 0.25 mm ID × 0.25 mm film thickness) and the information taken under the accompanying conditions: beginning temperature 50°C, temperature slope 5°C/min, 240°C/min to 300°C (holding for 3 min), and injector temperature at 290°C. The carrier gas was helium and the split proportion was 0.8 mL-1/min. For affirmation of examination results, basic oil was additionally broke down by GC/MS (Agilent 6890 gas chromatograph furnished with an Agilent 5973 mass-specific locator; Agilent UK) and indistinguishable fine segment and investigative conditions from above. The MS was kept running in electron ionization mode with ionization energy of 70 eV [52].

Biological Activity

Antimicrobial Activity

Antimicrobial assays conducted utilizing the disc-agar method [53, 54] against diverse sets of microorganisms. Extracts (pet. ether, methylene chloride, ethyl acetate, methanol and water) of *T. polium* L dissolved in their corresponding solvents. Aliquots of 10 μL for each extract, saturated on filter paper (5 mm, Wattman no. 1, Schleicher and Schüll, Germany) discs and dried for 1 h min at room temperature under disinfected conditions. The discs put on inoculated plates agar and incubated for 24 h at 35°C for bacterial, yeast and fungi. The two yeasts and bacteria were developed on supplement agar medium (g/L): Beef separate, 3; peptone, 10; and agar, 20 at pH 7.2. Contagious strains were developed on potato dextrose agar medium at pH 6. The width of hindrance zone estimated in mm

RESULTS AND DISCUSSION

Physicochemical evaluation of *Teucrium polium* L.

A successive extraction of dried *T. polium* L. is using five solvents namely, petroleum ether, methylene chloride, ethyl acetate, methanol and water, respectively, was carried out. In accordance, diverse extracting ratios based on the nature and the existence constituents were displayed (Table 1).

Table 1: Physicochemical evaluation of *Teucrium polium* L. extracts (100 g dry weight)

Extracting solvent	Extractive value %
Petroleum ether	1.90
Methylene chloride	2.20
Ethyl acetate	1.15
Methanol	4.15
Water	10.12

Proximate and mineral element composition of *Teucrium polium* L.

Generally, the nutritional properties of *T. polium* L. is usually determined by their biochemical composition such as protein and mineral contents. Proximate composition of *T. polium* L. is shown in Table 2. Ash content was significantly high (13.25%). The high percentage of water- soluble residue (8.50%) in the ash of *T. polium* L was evaluated. Regarding the soluble protein content, a remarkably high ratio detected in *T. polium* L. (2.345%).

It is interesting to note that the phosphorus (1.498 %) and iron (2.015 %) contents were remarkably high in *T. polium* L, while moderate contents of potassium (0.575%) and magnesium (0.344%) are observed in Table 3. Table 3 showed the presence of the inorganic magnesium, potassium, iron and phosphorus in *T. polium* L.

In another study, the potassium (17.10%) and magnesium (5.20%) contents of *T. polium* leaves from Oman, were higher as compared to the present study [55]. On the other hand, iron (0.84%) was significantly lower than the reported data.

Table 2. Proximate composition of *Teucrium polium* L.

Composition	% of dry weight
Ash	13.25
Moisture	8.50
Total protein	2.345

Table 3. Mineral element composition of *Teucrium polium* L.

Composition	% of dry weight
P	1.498
K	0.575
Mg	0.344
Fe	2.015

Phytochemical evaluation of *Teucrium polium* L

The previous phytochemical screening displays the presence the phyto-compound such as steroids, alkaloids, tannins, glycosides, flavanoids, saponins, triterpenes, fixed oils, carbohydrates, proteins, and coumarins in the different extracts of the aerial part of *T. polium* L. (Table 4). The carbohydrates and saponins found only in the water extract, while flavanoids , proteins, tannins and glycosides were detected in the methanol and water extracts. Alkaloids were found in all samples except petroleum ether extracts. Triterpenes and coumarins were present in methylene chloride and ethyl acetate extracts, while steroids and fixed oils were found in petroleum ether, methylene chloride and ethyl acetate extracts. In agreeing to our results, Al-kufaishi and Al-Mashhedy [56] reported the phytochemical screening of hot, ethanolic and cold extracts of *T. polium*

to find the presence of flavonoids, tannins, triterpenes, glycosides, saponins. Furthermore, alkaloids, saponins and tannins were identified in the plant collected from Iran [57].

Table 4: Phytochemical evaluation of *Teucrium polium* L.

	Plant constituent	Pet. ether	Methylene chloride	Ethyl acetate	Methanol	Water
1	Steroids	+	+	+	-	-
2	Flavonoids	-	-	-	+	+
3	Alkaloids	-	+	+	+	+
4	Carbohydrates	-	-	-	-	+
5	Proteins	-	-	-	+	+
6	Tannins	-	-	-	+	+
7	Glycosides	-	-	-	+	+
8	Saponins	-	-	-	-	+
9	Triterpenes	-	+	+	-	-
10	Fixed oils	+	+	+	-	-
11	Coumarins	-	+	+	-	-

Evaluation of fatty acids and sterols in *Teucrium polium* L.

Lipids form a large group of natural compounds, which are mostly glyceride esters of long chain carboxylic fatty acids. The most important members of this class are widely distributed throughout the animal and vegetable kingdom. An application of the ethereal extracts of dried *T. polium* L. to saponification using 10 % methanolic potassium hydroxide followed by estimation on the bases of GLC analyses revealed the existence of twenty fatty acid esters (Table 5). Linoleic acid represents the most abundant fatty acid (66.10 %), followed by arachidic acid (6.94 %), oleic acid (6.78 %), linolenic acid (3.88 %), palmitoleic acid (3.75 %), and tricosanoic acid (2.78 %). On the other hand, nineteen unsaponified compounds were determined starting with n-hexadecane (C_{16}) and ended by n-triacontane (C_{30}), along with four steroidal compounds, namely, cholesterol, campesterol, stigmasterol, and *β*-sitosterol (Table 6). In accordance, n-octadecane, (12.00%), n-heptacosane (11.73%), n-nonacosane (10.25%), n-pentacosane (8.87), n-tricosane (7.49), n-hexadecane (6.03%), and cholesterol (6.01%), represent the most abundant unsaponified components in the unpolar fraction of *T. polium* L. (Table 6). In the other study, Capasso [58] identified the aerial parts of *T. polium* by gas chromatographic and mentioned presence stigmasterol, betasitosterol, brassicasterol, cholesterol and campesterol from the aerial parts of *T. polium*.

Table 5: **GLC analysis results of saponified fraction present in the unpolar extract of *Teucrium polium* L.**

No.	Name of compound	RT	%
1	Henedecanoic acid (11:0)	16.91	0.47
2	Dodecanoic acid (12:0)	18.20	0.33
3	Tridecanoic acid (13:0)	19.80	0.15
4	Myristic acid (14:0)	21.90	1.92
5	Pentadecanoic acid (15:0)	24.89	0.22
6	Palmitic acid (16:0)	28.11	0.69
7	Palmitoleic acid (16:1)	30.98	3.75
8	Heptadecanoic acid (17:0)	31.54	0.44
9	Stearic acid (18:0)	32.38	0.80
10	Oleic acid (18:1)	33.87	6.78
11	Linoleic acid (18:2)	34.95	66.10
12	Linolenic acid (18:3)	35.46	3.88
13	Arachidic acid (20:0)	36.05	6.94
14	Homo-g-linolenic acid (20:3)	40.80	0.79
15	Arachidonic acid (20:4)	41.26	1.47

No.	Name of compound	RT	%
16	Behenic acid (22:0)	44.63	0.41
17	Brassidic acid (22:1)	46.28	0.97
18	Tricosanoic acid (23:0)	48.56	2.78
19	Tetradecosanoic acid (24:0)	50.41	0.64
20	Nervonic acid (24:1)	51.41	0.47

RT; retention time

Table 6: **GLC analysis results of the unsaponified fraction present in the unpolar extract of *T. polium* L.**

#	Name of compound	RT	%
1	n-Hexadecane (C_{16})	12.15	6.03
2	n-Heptadecane (C_{17})	13.68	1.72
3	n-Octadecane (C_{18})	15.54	12.00
4	n-Nonadecane (C_{19})	16.34	4.71
5	n-Eicosane (C_{20})	17.30	3.98
6	n-Heneicosane (C_{21})	19.06	4.97
7	n-Docosane (C_{22})	20.68	3.10
8	n-Tricosane (C_{23})	21.64	7.49
9	n-Tetracosane (C_{24})	22.69	3.57
10	n-Pentacosane (C_{25})	23.57	8.87
11	n- Hexacosane (C_{26})	24.35	2.85
12	n-Heptacosane (C_{27})	25.35	11.73
13	n- Octacosane (C_{28})	26.25	7.60
14	n- Nonacosane (C_{29})	27.88	10.25
15	n-Triacontane (C_{30})	28.60	2.68
16	Cholesterol (C_{27})	29.46	6.01
17	Campesterol (C_{28})	30.35	0.90
18	Stigmasterol (C_{29})	30.74	0.74
19	*β*-Sitosterol (C_{29})	31.44	0.80

GC-MS analysis of the essential oil of *Teucrium polium* L.

An extraction of the volatile oil of *T. polium* L. (100 g air dried plant) was carried out using volatile oil-water distilling system, and the afforded oil was extracted with n-hexane, and the latter was concentrated at room temperature affording colourless oil. GC-MS analysis of the obtained volatile oil established the existence of thirty volatile compounds having diverse chemical structures as shown in Table 7. The essential oil (EO) was extracted by the hydrodistillation of dried parts of *T. polium* and were analyzed by GC–MS. GC–MS analysis enabled the identification of a total of 29 constituents, representing 100% of the oil. The relative concentrations of the identified volatile components (%), retention time, molecular weight and molecular formula are presented in Table 6. The results of GC/MS analysis of *T. polium* EO in Table 6, revealed the presence of sesquiterpenens are the major components with percent 83.19 %. The main constituents of the sesquiterpenens were found as caryophyllene oxide (17.83%), farnesol (14.32%), spathulenol (11.88%), α--curcumene (7.21%), ledene oxide (7.07 %) and caryophyllene (5.17%). The percentage of compounds were oxygenated sesquiterpenes (60.71%), followed by hydrocarbon sesquiterpenes (22.48 %), oxygenated monoterpenes (4.67%), and other volatile compounds (12.14%). A study on oil obtained from *Teucrium polium* grown in Iran revealed the presence of sesquiterpenes, as major components [59]. Germacrene D (13.2%), β-caryophyllene (18%), spathulenol (10.4%) and bicyclogermacrene (9.0%) were the major identified components. Conversely, the *T. polium* essential oil composition grown in Algeria [60] was dominated by hydrocarbon compounds which contained a high percentage of hydrocarbon sesquiterpenes (46.81%). Similar results of higher amounts hydrocarbon

sesquiterpenes were obtained by Bezić et al. 2011 [61] and Djabou et al. 2012 [62].

Table 7: **GC-MS analysis of the essential oil (EO) of *T. polium* L.**

No.	Compound Name	Rt	M.Wt.	M. F.	(%)
1	Myrtenol	20.83	152	$C_{10}H_{16}O$	0.83
2	2-Pinen-4-one	21.24	150	$C_{10}H_{14}O$	1.01
3	Thymol	24.21	150	$C_{10}H_{14}O$	0.78
4	α-Terpinenyl acetate	25.99	196	$C_{12}H_{20}O_2$	0.77
5	Eugenol	26.30	164	$C_{12}H_{12}O_2$	1.28
6	Daydream dendrene	27.41	202	$C_{15}H_{22}$	1.09
7	Caryophyllene	28.38	204	$C_{15}H_{24}$	5.17
8	Isocaryophyllene	28.80	204	$C_{15}H_{24}$	1.58
9	Trans-a'-farnesene	29.42	204	$C_{15}H_{24}$	1.68
10	e-Elemene	30.73	204	$C_{15}H_{24}$	0.86
11	9,12-Octadecadiynoic acid methyl ester	30.97	290	$C_{19}H_{30}O_2$	1.18
12	α-Curcumene	31.19	204	$C_{15}H_{24}$	7.21
13	1,3,5,7- Tetramethyl-adamantene	31.42	192	$C_{14}H_{24}$	0.78
14	Cadinene; Cadina-3,9-diene	31.53	204	$C_{15}H_{24}$	1.82
15	α-Calacorene	32.13	200	$C_{15}H_{20}$	1.49
16	2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)- hepta-4,6-dien-3-one	32.64	218	$C_{15}H_{22}O$	3.15
17	Caryophyllene oxide	32.83	220	$C_{15}H_{24}O$	17.83
18	Farnesol	32.95	222	$C_{15}H_{26}O$	14.32
19	Spathulenol	33.45	220	$C_{15}H_{24}O$	11.88
20	12-Heptadecyn-1-ol	33.69	252	$C_{17}H_{32}O$	2.24
21	Leden oxide	34.18	220	$C_{15}H_{24}O$	7.07
22	Cedr-8-ene	34.81	204	$C_{15}H_{24}$	1.58
23	Himachalol	35.09	222	$C_{15}H_{26}O$	1.71
24	Aromadendrene-oxide	35.48	220	$C_{15}H_{24}O$	1.73
25	α-Bisabolol	35.87	222	$C_{15}H_{26}O$	2.46
26	Cadalin	36.00	198	$C_{15}H_{18}$	2.02
27	1-(3,3,6a-Trimethyl-1a,2,3,5,6a,6b-Hexahydro-1H-6-oxa-cyclopropa [e]inden-5-yl) -ethanone	36.20	220	$C_{14}H_{20}O_2$	1.00
28	1-Isopropyl-4,8-dimethylspiro [4.5]dec-8-en-7-one	37.37	220	$C_{15}H_{24}O$	3.71
29	6,10,14-Trimethyl-2-pentadecanone	40.34	268	$C_{18}H_{36}O$	1.77

Chemical composition of the EO of the aerial parts of *T. polium* L. grow in Jordan was determined by GC/MS. The major components determined were 8-cedren-13-ol (24.8%), beta-caryophyllene (8.7%), germacrene D (6.8%) and subinene (5.2%) [63].

Thirty-seven components were detected in the EO obtained from the aerial part of Turkish *T. polium* [64]. The major components identified were beta-pinene (18%), beta-caryophyllene (17.8%), alpha-pinene (12%), caryophyllene oxide (10%), myrcene (6.8%), germacrene D (5.3%), limonene (3.5%) and spathulenol (3.3%). In a recent study in Iran [1] has been found the oil to contain spathulenol (14.65%) at such high percentage, spathulenol was reported as an important constituent of *T. polium* (10.4%) [59] and in the EO obtained from the aerial parts of Turkish *T. polium* (3.3%) [64]. Furthermore, spathulenol the major component from the essential oils of *T. polium* collected during flowering stage from West of Iran was 15.06 %.

Whereas, in the present study the EO has been found to contain spathulenol with percentage 11.88%.

Biological Activities

Diverse antimicrobial activity testing of the successive five extracts of *T. polium* L. was carried out comparatively (10 µL/disc) against eleven microbial tests on the bases of agar diffusion method (Table 8). In accordance, pet. ether extract showed the most potent activity against all test organisms (except the Gram negative bacterium *E. coli*), representing Gram positive bacteria (*S. aureus* [14 mm], *B. subtilis* [14 mm]), Gram negative bacteria (*P. aeruginosa* [14 mm]), the yeast *C. albicans* [15 mm], and the fungi *S. cerevisiae* [12 mm] and *A. niger* [8 mm].

Table 8: **Antimicrobial (40 µL/disc (∅ 5 mm; [mm]) activity testing for the successive extracts of *Teucrium polium* L.**

***T. polium* extract**	**Diameter of inhibition (mm)**						
	BS[a]	**SA[b]**	**PA[c]**	**EC[d]**	**CA[e]**	**SC[f]**	**AN[g]**
Pet. Ether	14	14	14	-	15	12	8
Methylene Chloride	-	8	8	-	-	-	9
Ethyl acetate	8	9	8	-	-	-	-
Methanol	9	9	10	-	-	10	14
Water	8	9	9	-	-	18	-

[a]*Bacillus subtilis*, [b]*Staphylococcus aureus*, [c]*Pseudomonas auragenosa*, [d]*Escherichia coli,* [e]*Candida albicans,* [f]*Saccaromyces cerevisiae,* [g]*Aspergillus niger*

CONCLUSIONS

The phytochemistry and biological activity investigation of *T. polium* L. belonging to Lamiaceae family, obtained from Saudi Arabia has been carried out. A successive extraction of the dried plant using petroleum ether, methylene chloride, ethyl acetate, methanol and water was equivalently performed, and the afforded extracts were comparatively studied against a set of diverse pathogenic microorganisms for antimicrobial activity. *T. polium* has high percent ash and protein content. Additionally, *T. polium* contained either high or moderate amount of P, Fe, K and Mg. GLC analysis of the saponified and unsaponified parts of the ethereal fraction of *T. polium* L. confirmed the existence of 20 fatty acid ester and 19 unsaponified hydrocarbons, among them 4 sterols: cholesterol, campesterol, stigmasterol, and *β*-sitosterol. Linoleic acid represents the most abundant fatty acid (66.10 %) in the saponified fraction, while n-octadecane, (12.00%), n-heptacosane (11.73%), n-nonacosane (10.25%), n-pentacosane (8.87), n-tricosane (7.49), n-hexadecane (6.03%), and cholesterol (6.01%), represent the most abundant unsaponified components. The volatile oil content of *T. polium* L. indicated the existence of 29 diverse bioactive compounds: among them caryophyllene oxide (17.83%), farnesol (14.32%), spathulenol (11.88%), α-Curcumene (7.21%), caryophyllene (5.17%), 2,2,6-trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one (3.15%), which represent the most abundant constituents of the essential oil. Finally, the phytochemical evaluation of the successive five extracts was emphasized as well. Extract of the pet. ether fraction revealed the most potent activity against a diverse set of Gram positive (*B. subtilis, S. aureus*), Gram negative bacteria (*P. auragenosa*), yeast (*C. albicans*) and fungi (*S. cerevisiae*, *A. niger*).

Conflicts of Interest

The authors declare no conflict of interest.

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