J. Agric. Sci. Mansoura Univ., 30 (10): 6453 - 6465, 2005

BIOCHEMICAL AND BIOLOGICAL STUDIES ON CAT THYME (*Teucrium polium L.*)

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ABSTRACT

The present work assessed the potential of cat thyme (*Teucrium polium L.*) chloroform extract as a source of natural antimicrobial compounds. Lipid constituents of this extract were analyzed by gas-liquid chromatography (GLC). The fatty acid analysis indicated palmitic (20.35%), stearic (19.15%), pentadecanoic (15.16%) and oleic (7.78%), linoleic (9.55%), linolenic (3.61%) acids were the most prevalent saturated and unsaturated acids, respectively. The unsaponifiable matter consisted of C_{22} (21.27%) and C_{27} (17.25%) as major hydrocarbons and β-sitosterol (9.75%) as the major sterol. The phenolic compounds were analyzed by HPLC and the results showed that the predominant constituent was hydroquinone (26.47%) and the identified second main constituent was catechin (8.17%). Furthermore, apigenin was present as a trace constituent (0.92%).

Preliminary screening of the antimicrobial activity of cat thyme (*T. polium*) chloroform extract against some pathogenic and spoilage microorganisms representing some Gram-negative bacteria, Gram-positive bacteria, one yeast strain and eight fungal species were performed using disc-diffusion method. The extract was effective in inhibiting the growth of the organisms except for *Escherichia coli, Enterobacter aerogenase, Bacillus megaterium* and *Aspergillus niger*. Antimicrobial activity of the extract increased with increasing concentration. Additionally, antifungal activity of the extract was less potent than the antimicrobial activity.

Keywords: Cat thyme, *Teucrium polium L.*, Labiatae, phenolic constituents, lipid composition, antimicrobial activity.

INTRODUCTION

It was recognized long time ago that some plants have beneficial biological activity on humans. Most of the antimicrobial synthetic drugs have side effects and toxicity on the human body, in addition to the appearance of resistant strains of microorganisms (Shanson, 1999). This leads to return back to the folk medicines, which provide them a relatively rapid response against diseases, to avoid toxicity of the synthetic antimicrobial drugs and possibly reduce the costs of medicine.

Many plants grow widely in the Egypt which are used as folk medicine. Cat thyme (*Teucrium polium*) has been used as a traditional medicine in Saudi Arabia and Egypt for the treatment of different diseases such as antispasmodic, antirheumatic, carminative, diabetes mellitus, dyspepsia and flavoring agent (Al- Mougy *et al.*, 1992; Al- Sayed *et al.*, 1990 and Mohammad *et al.*, 1999). Antimicrobial activity of *Teucrium polium* chloroform extract was examined by Gulcin *et al.* (2003) using eleven microbial strains. The extract was effective in inhibiting the microbial growth at concentration range between 50-250 μg except for *Escherichia coli*. Antifungal activity of the extract was less potent than the antimicrobial activity. It has been reported that aqueous *T.polium* extract slightly inhibited the growth of yeast such as *saccharomyces cerevisiae* and *Yarrovia lipolytica* (Aggelis *et al.*, 1998). The antimicrobial effect of genus *Teucrium* might be due to the presence of diterpenoids (Carreiras *et al.*, 1989). In this respect, Verykokidou- Vitsaropoulou and Vajias (1986) isolated two methylated flavones; acacetin and salvigenin from a chloroform extract of leaves.

Eight known compounds (24α- ethylcholesta- 5, 25- dien - 3 β - ol; sitosterol, α amyrin; ursolic acid; apigenin; naringenin; pectolinarigenin and circilrol) and two new steroidal compounds (3 β - hydroxystigmast- 24, 25 dienal and 3 β - hydroxyl- 24α - ethylcholesta- 5, 25 dien 7- one) were isolated from the aerial parts of *Teucrium chamaedrys* subsp by Ulubelen *et al.* (1994). The aerial parts of *Teucrium montanum* contain clerostreol and clerosteryl acylglucosides as the major steroids (Kisiel *et al.*, 1995). In addition, *Teucrium* speices are characterized by DELTAS- fatty acids (Tsevegsuren *et al.*, 1997).

The aim of the present work was to characterize the important chemical components of the *T. polium* chloroform extract and to illustrate its antimicrobial activity.

MATERIALS AND METHODS

Source of plant material

Teucrium polium L. belongs to labiatae family. It was collected at flowering stage from the region of Sinai, Egypt on April 2003. Dried aerial parts were ground and stored until analysis.

Chloroform extract of *T. polium*

Aerial parts of *T. polium* were extracted with chloroform- methanol mixture (2: 1, v/v) at room temperature by soaking for 48 h. The extract was partitioned with water in separating funnel, then the chloroform extract was removed by rotary evaporator at 40°C until dryness. The residual oil was weighed and kept at –20°C in a deep freezer for subsequent analysis.

Chemical composition of *T. polium* chloroform extract

Separation of fatty acids and unsaponifiables

Lipid materials were saponified with ethanolic KOH (20%, w/v) for 24 h at room temperature. The unsaponifiables were extracted three times with ether. The aqueous layer was acidified by HCL (6N) and the liberated fatty acids were extracted three times with ether. The combined extracts of each of unsaponifiables and fatty acids were washed several times with distilled water until the washings were neutral to methyl orange. Fatty acids were converted to methyl esters using ethereal solution of diazomethane (Vogel, 1975).

Fractionation and determination of fatty acid methyl esters and unsaponifiables

The methyl esters of the fatty acids, unsaponifiables and corresponding standard compounds were analyzed with a Pye Unicam PU 4550 capillary gas chromatograph equipped with dual flame ionization detectors. The separation conditions were exactly the same as reported by Farag *et al.* (1978).

Identification and determination of phenolic compounds in *T. pollum* chloroform extract by HPLC

Identification of individual phenolic compounds of the chloroform extract was performed on a Hewlett- Packard HPLC (Model 1100), using a hypersil C_{18} reversed- phase column (250 x 4.6 mm) with 5 µm particle size. The separation of phenolic compounds was conducted with constant flow rate of 1ml/ min with two mobile phases: (A) 0.5% acetic in distilled water at PH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with mobile phase (A) and ending with mobile phase (B) over 35 min, using an UV detector set at wavelength 254 nm. Phenolic compounds of the sample were identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to relative percentage.

Antibacterial activity

Microorganisms

Some pathogenic and food spoilage microorganisms representing Gram- negative bacteria (*Salmonella typhimurium*, *Shigella sp*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenase*), Gram- positive bacteria (*Listeria monocytogenes*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus sp* and *Sarcina sp*), acid fast bacteria (*Mycobacterium phlei*) and *Streptomyces sp* were useds in the present study. All strains were obtained from Microbiology Department, Faculty of Agriculture, Cairo University. These microorganisms were checked for purity and identity and always generated to obtain an active microorganism.

Media for bacterial growth

All bacteria strains were grown in a nutrient glucose agar medium (NGA) consisting of yeast extract (2.5g), tryptone (5g), agar (15g) and glucose (10g) per 1 liter tap water and adjusted to pH 7.0.

Disc diffusion method

Disc diffusion method was carried out to measure the antibacterial activity according to Sleigh and Timburg (1981). Base agar (melted agar) was overlaid with seed agar with inoculum of bacteria (1ml of 24 h old broth cultures) to yield a lown of growth. After solidification of seed agar, different concentrations (50, 250, 700 and 1000 µg/disc) of the chloroform extract of *T. polium L.* were individually added to Whatman No.1 filter paper discs (5mm diameter) in appropriate quantities in triplicates. Control plates were supplemented with filter paper discs after immersion in the solvent and dried for comparison. The contents were incubated at 37°C for 24 h and the inhibition zones of the microbial growth produced by different concentrations were measured.

Antifungal activity

Fungi

Trichoderma viride (EMCC 107) and *Aspergillus niger*, *Pythium ultinum*, *Fusarium oxysporium*, *Rhizoctonia solani* and *Alternaria solani* were obtained from MIRCEN, Ain Shams University and Department of Plant Pathology, Institute of Plant Pathology ARC, respectively. *Trichoderma reesei* (NRRL 11236) and *phanerochaete chrysosporium* (NRRL 6361) were obtained from Microbiology Department, Faculty of Agriculture, Cairo University. *Saccharomyces cerevisiae* (0-14) was obtained from Institute of Bakery, Moscow. All strains were checked for purity and identity according to Raper and Fennel (1965).

Spore suspension

Test organisms were grown on a potato- dextrose- agar (Difco) slants for 10 days at 28°C. Spores were harvested by adding sterilized water. The suspension was adjusted spectrophotometrically to an absorbance of 0.6 at 450 nm (Irobi and Daramola, 1993).

Disc assay

Petri plates (9 cm diameter) containing 12 ml of pentose- dextrose- agar medium were seeded with 1.0 ml of 24 h old broth cultures of the inocula of the examined fungi. After solidification, plates were provided with filter paper discs (5 mm diameter) previously autoclaved, impregnated with different concentrations i.e. 50, 250, 700 and 1000 µg/disc of the plant extract, dried and immersed in the fungal spore suspension of the test organism. Control plates were supplemented with filter paper discs after immersion in the solvent and dried for comparison. Plates were incubated at 30°C ± 1°C for 3 days and the diameters of inhibition zones were measured. Results were recorded as mean values of three replicates representing the diameter of radial growth in mm.

Minimum inhibitory concentration (MIC) method

The minimum inhibitory concentration of the *T. polium* chloroform extract was carried out on extract- non sensitive pathogenic fungi (*Aspergillus niger*, *Pythium ultinum*, *Fusarium oxysporium* and *Rhizoctonia solani*) and extract- sensitive pathogenic fungi (*Alternaria solani*) for comparison as reported by Irobi and Daramola (1993) and Natarajan *et al.* (2003). Different concentrations of the extract (50, 250, 500 and 1000 µg) were thoroughly mixed with sterilized potato dextrose broth (PDB;100 ml) in Erlenmeyer flasks (500 ml). The flasks were inoculated with 1 ml inocula of the examined fungal strains and incubated at 30°C for 14 days. Suitable controls were also included, PDB with 1.0 ml inoculum served as positive control and PDB without inocula served as negative control. The inhibitory effect was expressed by the relative values to the growth observed in control without plant extract and was designated as the mycelia dry weight (g/100 ml).

Determination of mycelia dry weight

The mycelia were harvested by filtration through pre- weighed Whatman No.1 filter paper. The filter paper containing the mycelium was washed several times with distilled water, dried at 100°C for 24 h and weighed, then the percentage of inhibition was calculated.

Statistical analysis

The data obtained from the present work were statistically analyzed according to the procedure outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical analysis of *Teucrium polium L.* chloroform extract indicates the presence of 17.48% as crude lipids.

Fatty acids and unsaponifiable matter of aerial parts of *T. polium*.

Table (1) shows the fatty acid composition of aerial parts of *T. polium* under study. The obtained results showed that *T. polium* chloroform extract contained high amounts of saturated acids. Moreover, the presence of palmitic, stearic, pentadecanoic acids (20.35%, 19 25% and 15.16%, respectively). Furthermore, it containes oleic, linoleic and linolenic acids (7.78%, 9.55% and 3.61%, respectively) as unsaturated acids make this study particularly interesting, because these acids have an important role in membrane lipid formation. Linoleic acid is accepted as vitamin F and is the precursor of arachidonic acid which has an important role in the synthesis of prostaglandins having various biological activities and existing nearly in all of the organs in the organisms (Sener *et al.*, 1985). Lenolnic acid has strong antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus* and that linolenic acid combined with .linolenic acid alone (Lee *et al*, 2002). The amount of the saturated fatty acids was about 3.3 times as great as of unasaturated ones. Furthermore, the increament saturated to unsaturated ratio lipids led to negative effect on the microbial activity (Henri et al., 2003).

Table (1): Fatty acid composition (%)of aerial parts of *Teucrium polium L.*

Fatty acid	Concentration (%)
Lauric acid (12:0)	6.42
Tridecanoic acid (13:0)	0.97
Myristic acid (14:0)	2.75
Pentadecanoic acid (15:0)	15.16
Palmitic acid (16:0)	20.35
Palmitoleic acid (16:1)	2.08
Heptadecanoic acid (17:0)	0.85
Stearic acid (18:0)	19.25
Oleic acid (18:1)	7.78
Linoleic acid (18:2)	9.55
Linolenic acid (18:3)	3.61
Arachidic acid (20:0)	8.24
Heneicosanoic acid (21:0)	0.68
Doeicosanoic acid (22:0)	2.31

Few studies were conducted to elucidate the composition of unsaponifiable matter of aerial parts of *T. polium* (Ulubelen *et al.*, 1994 and Gasper *et al.*, 1996). Table (2) shows the unsaponifiables of aerial parts of *Teucrium polium*. The present data indicated that the unsaponifiable matter was fractionated by GLC into 25 different compounds of which 17 hydrocarbons and two sterols were identified. Aerial parts of this plant contained $C_{22}$and C_{27} as the major hydrocarbons (21.27 % and 17.25%), while C_{17}, C_{18}, C_{20}, C_{21}, C_{24}, C_{25}, C_{26}, C_{28}, and C_{29} beside six unknown hydrocarbons were present as minor compounds. Two sterols were separated and identified, i.e, campestrol (1.01%) and ß-sitosterol (9.75 %) in aerial parts of *T. polium* oil.

Lichtfouse (1999) found that the straight chain hydrocarbons (C_{27} – C_{33}) and plant sterols could be used for selective preservative of microbial strains.

Table (2): Unsaponifiable matter composition (%) of *Teucrium polium L.*

Component	Concentration (%)
C_{15} (n-pentadecane)	0.11
C_{16} (n- hexadecane)	0.44
C_{17} (n- heptadecane)	1.44
Unknown	0.21
C_{18} (n-octadecane)	2.99
Unknown	4.84
Unknown	0.47
C_{19} (n- nonadecane)	0.91
Unknown	1.13
C_{20} (n- eicosane)	1.24
C_{21} (n- hencosane)	2.24
C_{22} (n- docosane)	21.27
C_{24} (n- tetracosane)	1.41
Unknown	5.10
C_{25} (n- pentacosane)	5.22
C_{26} (n- hexacosane)	9.17
C_{27} (n-heptacosane)	17.25
C_{28} (n- octacosane)	6.55
C_{29} (n- nonacosane)	2.73
C_{30} (n-triacontane)	0.65
C_{31} (n- hentriacontane)	0.78
Unknown	2.57
C_{32} (n-dotriacontane)	0.52
Campesterol	1.01
B- Sitosterol	9.75

Phenolic compounds in the aerial parts of *T. polium* chloroform extract

Table (3) shows the phenolic compounds composition of *T. polium* chloroform extract. The lack of certain standard compounds did not allow for the complete identification of these compounds in the extract. The results showed that the extract contained 21 phenolic compounds and 11 of them were identified. For simplicity, the concentration of the identified phenolic compounds can be classified into three categories, i.e. trace (< 1%), minor (< 10%) and major (> 10%) components. The extract of aerial parts of *Teucrium polium* contained hydroquinone (26.47%) as the major compound, while pyrogallic acid, gallic acid, protocatechoic acid, p- hydroxybenzoic acid, chlorogenic acid, catechin, phenol and vanillin occurred as minor compound. In addition, ferculic acid and apigenin occurred as trace compounds (0.67 and 0.92%, respectively). These results are nearly agreed with the data of Kawashty *et al.* (1999) and Safaei and Haghi (2004).

Table (3): Phenolic compound composition (%) of chloroform extract of *Teucrium Polium L.*

Component	RT*	Concentration (%)
Unknown	2.279	6.10
Unknown	2.456	3.31
Unknown	2.820	1.69
Unknown	3.032	9.80
Unknown	3.964	4.28
Unknown	4.696	11.63
Pyrogallic acid	5.476	2.31
Hydroquinone	6.076	26.47
Gallic acid	7.443	3.61
Unknown	8.483	5.07
Protocatechoic acid	10.846	3.79
Unknown	12.694	1.24
P- Hydroxy benzoic acid	14.709	1.30
Chorogenic acid	15.696	1.08
Catechin	17.517	8.17
Phenol	18.479	1.33
Vanillin	19.354	3.36
Ferulic acid	22.803	0.67
Apigenin	27.358	0.92
Unknown	43.867	1.96
Unknown	45.154	1.91

*** RT: refers to retention time (min.)**

Antibacterial activity

Discs containing the chloroform extract of *Teucrium polium* at different concentrations (50-1000 µg/disc) caused inhibition zones in plates inoculated with most of bacterial strains under investigation. *Esherichia coli*, *Enterobacter aerogenase* (Gram – ve) and *Bacillus megaterium* (Gram + ve) tolerated the influence of compounds in the extract. These results are illustrated in Table (4). A similar observation with *Escherichia coli* was recorded with *Pseudomonas aerugenosa* grown at 50, 250, 700 µg/disc concentrations of *T. polium* extract which possessed weak inhibitory effect (2 mm diameter) at 1000 µg/disc. In contrast, the chloroform extract of *T. polium* exhibited the highest inhibitory effect (1-8 mm) against *Salmonella typhimurium*, *listeria monocytogenes* and *Micrococcus sp* at all concentrations. The inhibitory effect of chloroform extract against the growth of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina sp* (Gram + ve), *Mycobacterium phlci* (acid fast bacteria) was more pronounced at high concentration (700-1000 µg/disc) with large inhibition zones ranged between 1- 6 mm. On the other hand, the growth inhibition zones were increased at 700 and 1000 µg / disc with *Micrococcus sp* (1 - 4 mm).In addition, *Streptomyces sp* was more sensitive than *Mycobacterium* phlei in this regard. These results are in line with those of results of Gulcin *et al.* (2003).

Table (4): Antibacterial activity of chloroform extract of *Teucrium polium L.*

Bacterial Strain	Concentration (µg/disc)					LSD at P= 0.01
	Control	50	250	700	1000	
			Gram – negative bacteria			
Salmonella typhimurium	0^a	$1.00^b \pm 0.00$	$3.00^c \pm 0.58$	$3.00^c \pm 0.00$	$8.00^d \pm 1.00$	1.58
Shigella sp	0^a	ND	$1.00^b \pm 0.58$	$3.00^c \pm 0.00$	$5.00^d \pm 0.58$	1.12
Pseudomonas aeruginosa	0	ND	ND	ND	$2.00^b \pm 0.00$	-
Escherichia coli	0	ND	ND	ND	ND	-
Enterobacter aerogenase	0	ND	ND	ND	ND	-
			Gram – Positive bacteria			
Listeria monocytogenes	0^a	$1.00^b \pm 0.00$	$1.33^b \pm 0.57$	$3.33^c \pm 0.58$	$7.33^d \pm 0.58$	1.37
Bacillus cereus	0^a	ND	$1.00^b \pm 0.00$	$3.00^c \pm 0.58$	$4.23^d \pm 0.58$	1.12
Bacillus megaterium	0	ND	ND	ND	ND	-
Bacillus subtilis	0^a	ND	ND	$3.00^b \pm 0.58$	$3.00^b \pm 1.62$	2.35
Staphylococcus aureus	0^a	ND	ND	$3.00^b \pm 0.58$	$5.00^c \pm 0.58$	1.12
Micrococcus sp	0^a	$1.00^b \pm 0.00$	$1.33^b \pm 0.58$	$4.62^c \pm 0.58$	$4.00^c \pm 1.00$	1.77
Sarcina sp	0^a	ND	ND	$3.00^b \pm 0.00$	$6.00^c \pm 1.00$	1.37
Streptomyces sp	0^a	ND	$1.00^b \pm 0.00$	$3.00^c \pm 0.58$	$5.00^d \pm 0.58$	1.12
			Acid Fast bacteria			
Mycobacterium phlei	0^a	ND	ND	$1.33^b \pm 0.58$	$3.33^c \pm 0.58$	1.12

ND: No detected activity at this concentration.
The numbers in each row followed by the same letter is not significantly different at P = 0.01.
Each value represents the mean of 3 replicates (Mean ± SE).

Antifungal activity

Table (5) elucidates the antifungal activity of the chloroform extract of *T.polium* at various concentrations (50-1000 µg/disc) against some pathogenic fungi and one yeast strain using the filter paper disc agar diffusion technique. The data for the inhibition zones (mm) of various microorganisms under study indicated that the extract at 700 and 1000 µg/disc had highly effect against *Trichoderma viride* and *Saccharomyces cerevisiae*. The extract at 1000 µg/disc concentration showed moderate effect against *Rhizoctonia solani, Fusarium oxysporium, Pythium ultinum* and *phanerochaete chrysosporium* (2-3 mm). On the other hand, the increase in the concentration of extract led to an increase in the inhibitory action towards the growth of *Trichoderma reesei* and *Alternaria solani* organisms. In contrast, *Aspergillus niger* was non- sensitive to the extract at concentration range of 50-1000µg/disc.

Table (5): Antifungal activity of chloroform extract of *Teucrium polium L.*

Fungal Strain	Concentration (µg/disc)					
	Control	50	250	700	1000	LSD at P= 0.01
Trichoderma viride	0^a	ND	ND	$6.00^b \pm 0.58$	$8.00^c \pm 0.58$	1.12
Trichoderma reesei	0^a	ND	2.00 ± 0.00	$2.00^b \pm 0.58$	$3.00^b \pm 0.00$	0.79
Phenerochaete chrysosporium	0^a	ND	ND	ND	$2.33^b \pm 0.58$	0.79
Aspergillus nigir	0^a	ND	ND	ND	ND	-
Pythium ultimum	0^a	ND	ND	ND	$2.33^b \pm 0.58$	0.71
Alternaria Solani	0^a	$1.00^b \pm 0.00$	$1.33^b \pm 0.58$	$1.33^b \pm 0.58$	$4.00^c \pm 1.00$	1.77
Fusarium oxysporium	0^a	ND	ND	ND	$3.33^b \pm 0.58$	0.79
Rhizoctonia solani	0^a	ND	ND	ND	$3.00^b \pm 0.58$	0.79
Saccharomyces Cereuisiae	0^a	ND	ND	$6.00^b \pm 0.58$	$8.00^c \pm 1.00$	1.58

ND: refers to detected activity at this concentration.
The numbers in each row followed by the same letter are not significantly different at P = 0.01.
Each value represents the mean of 3 replicates (Mean ± SE).

Table (6) shows the effects of different concentrations (50,250,500 and1000 µg/100ml) of chloroform extract of *T. polium* on the growth of some pathogenic fungi represented as mycelial dry weight (g/100 ml medium) and the minimum inhibitory concentration (MIC) was determined. The results of the present work indicated that the extract at all concentrations (50-1000 µg/100ml) caused significant gradual decrease in the mycelia dry weight for all tested fungi except *Fusariun oxysporium* fungi. It is worth mentioning that the extract higher levels from 250 to 1000 µg/100ml completely prevented the

Alternaria solani from growth. For *Rhizoctonia solani,* only 1000µg/100ml prevents growth. The MIC of both fungi was 50 µg/100ml. On the other hand, this extract had no potent effect on the growth of *Pythium ultimum* and *Aspergillus niger* at low concentrations of 50- 250 µg/100ml, while the highest levels (500-1000 µg/100ml) significantly decreased the mycelia growth compared with the control experiment and MIC for these strains were 500 µg/100ml.

Table (6): Influence of chloroform extract of *Teucrium polium L.* on the growth of some pathogenic fungi.

Fungal strain	Concentration (µg/100ml)					
	Control	50	250	500	1000	LSD at P= 0.01
ythium ultimum	0.39[a] ± 0.03	0.36[a] ± 0.02	0.36[a] ± 0.04	0.26[b] ± 0.01	0.18[c] ± 0.03	0.07
Alternaria Solani	0.40[a] ± 0.02	0.05[c] ± 0.00	0.00[d]	0.00[d]	0.00[d]	0.02
Aspergillus nigir	0.63[a] ± 0.04	0.60[a] ± 0.02	0.60[a] ± 0.05	0.54[b] ± 0.03	0.44[c] ± 0.01	0.09
Fusarium Oxysporium	0.57[a] ± 0.02	0.55[a] ± 0.01	0.54[a] ± 0.04	0.54[a] ± 0.03	0.52[a] ± 0.02	0.07
Rhizoctonia solani	0.20[a] ± 0.01	0.11[b] ± 0.02	0.04[c] ± 0.00	0.02[c] ± 0.01	0.00[d]	0.026

The numbers in each row followed by the same letter are not significantly different at P = 0.01.
Each value represents the mean of 3 replicates (Mean ± SE).

Figure (1) illustrates the influence of chloroform extract on the growth inhibition (%) of some non sensitive pathogenic fungi at different concentrations (50-1000 µg/100ml). The extract at the range of 250-1000 µg/100ml possessed strong inhibitory action on Alternaria *solani* (100%) and the same effect was noticed on *Rhizoctoni solani* (100%) at 1000 µg/100ml concentration. In addition, this extract possessed very weak (30.2%) and moderate (53.8%) inhibitory action on *Aspergillus niger* and *pythium ultimum* growth, respectively at high level (1000 µg/100ml).

In general, the extract exhibited strong antimicrobial and antifungal activities toward microorganisms under study. Further studies are needed for testing the application such compounds in controlling microorganisms. These effects may be due to presence of the phenolic compounds, linolenic acid or the presence of hydrocarbons (C_{22}-C_{27}) and sterols compounds in *T. polium L.* extract.

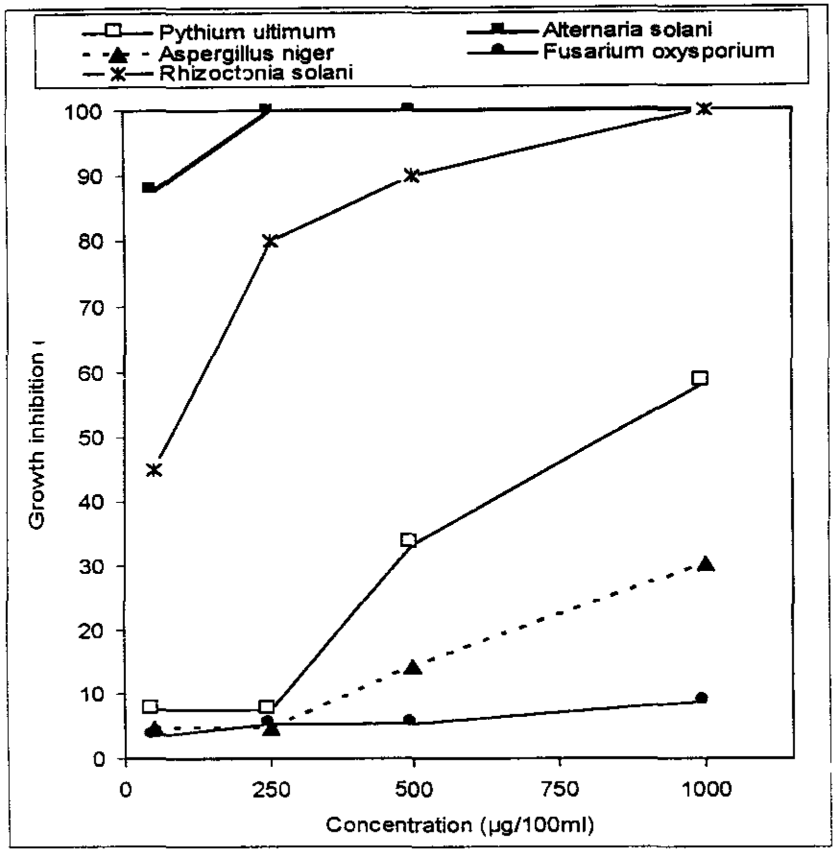

Figure (1): Influence of chloroform extract of *T. polium* on growth inhibition (%) of some pathogenic fungi.

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دراسات بيوكيميائية وبيولوجية على نبات الجعدة

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قد يتعرض الانسان لعدوى ميكروبية أو تسمم غذائي ، ويعالج بعقسا قير قد يكون لهـا آثار جانبية سامة ، ولهذا بذ لـــت العديد من المحاولات لإيجاد بديل علاجـي مـن المصـادر الطبيعية مثل النباتات الطبية . ولذلك تهدف هذه الدراسة إلى تقييم كيماوي وميكروبي لنبات الجعدة ، حيث تم استخدام المستخلص الزيتي للأجزاء الهوائية للنبـات ودرس تـأثيره المضـاد لنمـو الميكروبات الممرضة باستخدام سلالات بكتيرية سالبة وأخرى موجبة لجرام ، وسلالات فطريـة ونوع واحد من الخمائر ، وذلك بتركيزات تتراوح ما بين ٥٠- ١٠٠٠ جزء من المليون بطريقـة التشرب القرصي .

وقد أظهرت النتائج أن لهذا المستخلص الزيتي لنبات الجعدة تأثير مثبط قـوي لنمـو البكتيريا عدا بكتيريا الإشريشيا كولاي والانتيرو بكتر أيروجينيز والباسيلس ميجاتيريم ، كذلك كان له تأثير مضاد لنمو الفطريات و الخميرة تحت الدراسة عدا فطر الاسبراجلس نيجر ، ولوحظ أن هذا التأثير يزداد زيادة معنوية بزيادة التركيز حتى ١٠٠٠جزء من المليون .

وعند إجراء تحليل كيماوي للمستخلص بجهاز التحليل الكروماتوجرفي الغازي للمكونات الليبيدية ، وجد انه يحتوى على نسب مرتفعة من حمض البالمتيك (٢٢,٣٥%) وحمض الاستيارك (١٩,٢٥%) وحمض البنتاديكانويك (١٥,١٦%) كأحماض دهنية مشبعة باللاضـافة إلـى نسـبة متوسطة من حمض الأولييك (٧,٧٨%) و حمـض اللينوليـك (٩,٥٥%) و حمـض اللينولينـك (٣,٦١%). وقد ثبت من البحوث السابقة ان هذه الأحماض لها تأثير مثبط للنشاط البكتيري .كذلك لوحظ احتوائها على هيدروكربونات طويلة السلسلة بنسبة عالية وهـي $C_{٢٢}$ (٢١,٢٧%) و $C_{٢٧}$ (١٧,٢٥%) و بيتاسيتوستيرول بنسبة مرتفعة (٩,٢٥%) ، وقد ثبت علميا ان لهذه المركبات تاثير مضاد للنشاط الميكروبي .

أيضا تم تحليل المركبات الفينولية في هذا المستخلص بواسطة جهاز HPLC ، ووجد أنه يحتوي على مركبات فينولية عديدة ، أهمها مركـب الهيـدروكينون (٢٦,٤٦%) والكاتتشـين (٨,١٧%) بالاضافة إلى الأبيجينين والفريوليك ، وربما يرجع التأثير المضاد للميكروبـات إلـى احتواء المستخلص على المركبات الفينولية. وتوصي هذه الدراسة باستخدام المستخلص في مقاومة العديد من الميكروبات الممرضة في الأوساط المختلفة وبالتالي علاج العديد من الأمراض المتسببة عن نمو هذه الأنواع من الميكروبات .