EVALUATION OF THE ANTIBACTERIAL ACTIVITIES OF *FERULA HERMONIS* (BOISS.)

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ABSTRACT

Ferula hermonis of the genus Ferula belongs to the family Umbelliferae. The genus Ferula has been extensively studied, due to the high occurrence of active ingredients in it, and which made many species of it be used in traditional medicine. Ferula hermonis Boiss. was not among the investigated species, even though it has a strong reputation as an aphrodisiac and as a general stimulant. This study focuses, first on the composition of the volatile oil and its active elements through the analysis by gas chromatography: the volatile oil of flowering tops, roots and seed was found to be very rich, but with a predominance of α-pinene, 38%, 31% and 51.3% respectively. The effectiveness of the Ferula hermonis extracts against some pathogenic germs was also evaluated, and then compared to that of usual antibiotic discs. Results showed resin extract of Ferula hermonis to be highly effective against gram-negative bacteria while root's oil extract to be highly effective against gram-positive bacteria. 50µl/ml of roots oil extract would inhibit within 10 minutes 6000 germs of Staphylococcus aureus; and 10µl/ml would inhibit within 1 hour 16000 germs of Streptococcus fecalis. 10µl/ml of Resin extract would inhibit 2400 germs of Salmonella typhi within 1 hour, and within 24 hours 50µl/ml and 10µl/ml of resin extract would inhibit respectively 15300 germs of Escherichia coli and 3500 germs of Pseudomonas aeruginosa. In general Ferula hermonis was found to be strongly bactericidal and its activity strongly exceeded that of usual antibiotic discs.

Keywords: *Ferula hermonis,* α-pinene, antibacterial effect

INTRODUCTION

In a time where most chemical additives showed dangerous side effects, there is a general tendency towards going back to nature, especially food and nutrition wise. Hence, the need to study wild plants became a must, in order to try to clear up the mystery that surrounds their beneficial effects; the same effects that made them long ago being used as major ingredient in our grandmothers healing recipes.

In this scope, the root of Shirish El Zallouh, Latin name *Ferula hermonis,* has a very strong reputation as a general stimulant, nervous activator against neurasthenia, general weakness, strain, fatigue and for retardation of the early appearance of symptoms of old age. It is also suspected to have antibacterial and antioxidant activities, which could make it to be valorized as a natural food additive in replacement to chemical ones.

Ferula hermonis Boiss. is a glabrous plant ramified more or less verticilate in the top, branches of the inflorescence are nude at the base. Inferior leaves have oval edges, are ample, glaucous and very glabrous. Primary divisions have thick petioles, the others are sub-sessile having very small lobes, very multiple, linear forked at the summit. Superior leaves are gradually reduced at their sheath, this latter being very full, glabrous, shiny cym-formed to pointed. Styles are deflected, longer than the depressed stylopod. The fruit is ovoid 10 mm long, 5mm width, and brown purplish. Dorsal coasts are equidistant filamentous thread-like and projecting, the lateral ones near the narrow margin. It is a plant with strong smell, exuding sometimes small quantities of orangish resin (Mouterde, 1970).

The *Ferula hermonis* Boiss. (*Fh*) is an endemic plant that can be found in a very restricted eco-geographic area: Mount Hermon in Lebanon, at 2000 m of altitude, (70Km from Beirut) (Tohmé & Tohmé, 2002) or mount Blodan in Syria, these two places constitute its unique and exclusive natural habitat. Soil analysis of samples taken at the bottom of the plant in the previously mentioned location showed a non-calcareous soil (CO_3Ca, 0.6%) having very low salinity, a loamy clayey texture with a pH7 neutral; this soil is mostly characterized by it's extremely high content in potassium 1420 ppm, whereas the normal range is 100-400ppm (soil analysis at LARI-Fanar, 2001).

The root of *Ferula hermonis* Boiss., commonly known in Lebanon and Syria as "Shirsh -el-Zallouh", has been used in folk medicine as an aphrodisiac (Abourashed, 2000). A recent study conducted on the oil extracted from the seeds of *Ferula hermonis* tested its efficacy in enhancing erectile function and toxicity in the male rats: the sexual activities assessed by penile erection index were proven to be dose dependent ; acute and sub-acute toxicity were observed at 880 times the LD50 (12 mg/kg). It is reputed to enhance male sexual behavior; however there is no scientific verification. There was a decrease in total body weight; in addition there was a significant decrease in cholesterol level (El-Taher, 2001).

Hence, the crude oil from the plant *Ferula hermonis* was proven to enhance erectile function in rats; however it becomes toxic if used for a long period of time (El-Taher, 2001). This aphrodisiac property is a common one for most of the species of the genus Ferula (*Umbelliferae*) which are actually also reputed to exhibit antimicrobial activity against gram-positive bacteria (Abourashed, 2000). In fact a recent literature survey revealed the anti-microbial activity of the essential oil of *Ferula narthex* and of the gum resin of *Ferula gumosa*. Also, in 1998, M. Al-Yahya isolated three anti-bacterial sesquiterpenes from *Ferula communis*, which exhibited significant activity against gram-positive bacteria as well as against Mycobacterium organisms (Al-Yahya, 1998).

The final outcome of this study is an ecological one: to preserve bio-diversity, as nowadays with the late fame of this root, a 100% natural alternative and rival to "Viagra", it is being collected at an alarming rate after having endured long ages of pasturing; so it is very strongly endangered species and whose habitat is being threatened by destruction.

By giving *Ferula hermonis* a food and pharmaceutical industrial importance, ways to protect it and preserve it will be pointing out. In fact it is the scope of this study to: try to elucidate the chemical composition of this root, grains and its resin then, to evaluate antibacterial activity trying to find fields of applications for them. The potential biological activities of *Ferula hermonis,* were conducted at LARI Fanar laboratories (Medicinal plants laboratory).

MATERIALS & METHODS

Samples collection of *Ferula hermonis*

Fresh *Ferula hermonis* roots were gathered from EL Cheikh Mountain, Mount Hermon, at 1900- 2000m of altitude (Lebanese part) and from Blodan (the other versant of the mountain) in Syria. In autumn season only root parts were available. Flowering tops and seeds were collected in April-May and October respectively.

Steam distillation

Steam distillation is a mean of separating and purifying organic compounds (Furniss, 1991). Fresh roots and seeds were washed, grinned, macerated in distilled water and then subjected to steam distillation by Clevenger apparatus (Pharmacopée Européenne, 1989) in order to extract the essential oil. The duration of each extraction was around six hours. The oil was stored in the fridge at 4° for 24 hours, in dark bottle in order to protect it from light exposure.

Resin extract of *Ferula hermonis*

The resin was collected from the rhizomes of the plants. It has the same organoleptic characteristics (odor, taste and smell) as the roots. 1gr of (*Fh*) resin was dissolved in 1ml of absolute ethanol. The ethanol alone had to be considered; so 2 dilutions of bacteria ($10^{-5}$and 10^{-6}) were selected to be tested with the highest amount of ethanol (500µl). This test was conducted in 2 replications each time. In this case the dilutions adopted were: 5µl/ml, 10µl/ml and 50µl/ml of pure resin.

The identification of the volatile oil fraction using the Gas Chromatograph

Gas Chromatography is a method that consists of separating the various essential oils' constituents (Pradeau, 1992). This method allows a qualitative as well as quantitative evaluation of the essential oils found in the volatile fraction. The apparatus used is Shimadzu 17A, Automatic flow controller, Auto- injector AOC 20. Whilst for the condition, that was applied to it, it was according to Hilan *et al*. (1998). The Standard essential oils were purchased from Sigma.

The bacterial identification and count and the Inhibition test

The reference pathogenic organisms that were tested with the various extracts of *Ferula hermonis* were: *Staphylococcus aureus, Streptococcus fecalis, Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa* (Faculty of Public Health of the Lebanese University). They were isolated from patients hospitalized after having been subjected to foodborne toxi-infections. Each germ was given a laboratory reference number. These strains were first pre-cultured on specific culture media: Nutrient broth, Mannitol agar, SS agar, Mac-Conkey agar, Nutrient agar, Bile-Esculine agar, Kligler Iron agar, and Mûller Hinton agar (Biolife s.r.l-V. Le Monza, 272, 20128 Milano- Italy).The method applied was according to Hilan *et al.* (1997). The experiments were conducted 3 times for each bacterium with (*Fh*) roots and seeds essential oil, and with (*Fh*) resin extract. All the manipulations were conducted

aseptically to avoid any foreign contamination, which would result in inaccurate results (Hilan & Sfeir, 2001).

The antibiogram

This test is conducted in the scope of evaluating the antibiotic resistance of the germs that are used in this study, then to compare it with the effect of (*Fh*) oil. The antibiotic discs used for comparison with antimicrobial effect of *Ferula hermonis* oil were: Gentamycin 10 (GN), Kanamycin 30µg (K), Chloramphenicol 30µg (C), Ampicillin 10µg (Amp), Ciprofloxacin 5µg (Cip), Streptomycin 10µg (S) and Nalidixic acid 30µg (NA). The media used was the Müller Hinton Agar (MHA):

After 18 hours of incubation at 37ºC, the number of total germs in the three count dishes was determined, and then the diameter of the inhibition area of the strain tested with antibiotics was measured using a ruler.

Knowing the total area of the Petri dish (πR^2), the number of total germs and the area inhibition (πr^2); the count of inhibited germs was determined, this value was then compared to the one obtained with the various extracts of *Ferula hermonis* (Hilan et *al,* 1998). The number of germs (n) inhibited by each type of antibiotic is calculated according to the formula: n= (a*N)/A, and the results are expressed in germs/ml.

- A: is the area of the Petri dish (πR^2) in cm^2.
- a: is the area of the inhibition disc (πr^2) in cm^2.
- N: is the number of germs per ml.
- n: is the number of inhibited germs per ml.

The statistical analysis for the evaluation of the antibacterial activities

The multivariate analysis of variance for the influence of the 3 sources (roots, seeds and resin *Fh* extracts) on the inhibited number of the 2 gram positive and 3 gram negative bacteria were evaluated.

A statistical validation based on "the Z- test" for the comparison between the lowest number of germs inhibited by *Fh* essential oils extracts from (root, seeds and resin) and the highest number of germs inhibited by the most efficient antibiotic at a P-value of 5%, was also evaluated.

RESULTS & DISCUSSION

Steam distillation

Essential Oil yield from leaves, stems and flowers was very low; only the steam distillation of roots and seeds of (*Fh*) gave a significant yield: 0.5% of essential oil.

Results of the Gas Chromatography GC

The computing integrator gave the retention times, the peaks area and the percentage for every component identified by the GC (Table 1).

It is very obvious that flowering top and roots are very rich, where their essential oil comprises about 78 and 71 components respectively, only 32 were identified. A predominance of α-*pinene* was noticed in the case of flowering top and roots were this component showed the highest area percentage amongst all the others: 38% and 31% respectively. Other 3 major components were identified: *carvacrol* (5.50%), *linalyl acetate* (16.0%) and 1,8- *cineole* (6.0%). The presence of sesquiterpen hydrocarbon was also detected (Table 1). This was in fact previously proven to exist in the *Ferula hermonis* crude extracts (Galal, 2000) (Galal *et al*., 2000). On the other hand, in the case of seeds' essential oil, only 3 components were shown, but high in quantity: the α-*pinene* (51.3%), *β-pinene* (42.5%) and *camphene* (6.2%) (Table 1).

TABLE 1

Chromatography Profile of *Ferula hermonis* Boiss.
(Comparison between E.O. of Fresh Flowering Tops, Roots & Seeds)

	* E.O. Standards	E.O. Flowering Top	E.O.Roots	E.O. Seeds
1	***α- Pinene***	38.0	31.0	51.3
2	***(-)- Camphene***	0.50	0.20	6.2
3	***B- Pinene***	0.60	1.25	42.5
4	***Sabinene***	2.00	1.60	-
5	***β- Myrcene***	1.10	0.70	-
6	***α- Terpinene***	-	1.20	-
7	***(+)Limonene***	2.50	1.50	-
8	***1,8- Cineole***	1.50	6.00	-
9	***Verbenol***	1.00	0.30	-
10	***γ- Terpinene***	0.50	2.50	-
11	***P- Cymene***	0.40	0.70	-
12	***(+)- Fenchone***	0.05	0.65	-
13	***(-)-α- Thujone***	0.10	-	-
14	***(+)- Menthone***	-	0.06	-
15	***Camphor***	-	0.50	-
16	***(-)- Linalool***	0.50	0.20	-
17	***Linalyl acetate***	1.00	16.0	-
18	***α- Cariophyllene***	0.20	0.20	-
19	***(-)- Myrtenal***	-	0.06	-
20	***(+)- Menthol***	0.10	0.15	-
21	***(±)- Lavandulol***	2.50	4.50	-
22	***α- Terpineol***	0.15	0.20	-
23	***Borneol***	1.50	2.50	-
24	***Geranyl acetate***	2.20	0.05	-
25	***Citronellol***	0.06	3.20	-
26	***Nerol***	-	0.10	-
27	***Anethole***	1.20	0.15	-
28	***Geraniol***	0.07	-	-
29	***Thymol***	0.08	0.05	-
30	***Carvacrol***	5.50	3.15	-
31	***α- Bisabolol***	0.75	0.04	-
32	***Trans- Farnesol***	0.85	0.06	-
Totals Components		**78**	**71**	**3**

***** E.O. (Essential Oil)

Results of Inhibition tests of gram positive bacteria

TABLE 2

Number of Gram Positive Bacteria Remaining after Inhibition Test

Time	**0'**	**10'**	**1h**	**24h**	**10'**	**1h**	**24h**	**10'**	**1h**	**24h**
Concentration of E.O.		**5 µl/ml**			**10 µl/ml**			**50 µl/ml**		
***Fh* roots extract**										
***Staphylo. aureus*: 5.5x10^6 /ml**	5500	1200	420	30	130	20	0	0	0	0
	550	35	3	0	0	0	0	0	0	0
	55	3	0	0	0	0	0	0	0	0
***Strepto. fecalis*: 16x 10^6 /ml**	16000	Hg	900	230	Hg	0	0	1540	0	0
	1600	1430	0	0	24	0	0	0	0	0
	160	14	0	0	0	0	0	0	0	0
***Fh* seeds extract**										
***Staphylo. aureus*: 5.5x10^6 /ml**	5500	1300	Hg	100	280	Hg	17	98	Hg	0
	550	545	250	20	250	40	16	10	0	0
	55	20	5	0	6	0	0	5	0	0
***Strepto. fecalis*: 16x10^6 /ml**	16000	Hg	Hg	1500	Hg	Hg	620	Hg	Hg	158
	1600	1600	1300	540	1452	1240	20	1400	1230	0
	160	153	81	0	80	32	0	40	35	0
***Fh* resin extract**										
***Staphylo. aureus*: 5.5x10^6 /ml**	5500	63	39	42	53	38	40	44	36	11
	550	26	21	18	27	30	3	3	0	0
	55	6	4	0	2	0	0	0	0	0
***Strepto. fecalis*: 16x10^6 /ml**	16000	Hg	Hg	100	Hg	Hg	17	Hg	Hg	0
	1600	1550	1100	200	1300	1400	0	1000	600	0
	160	158	85	12	98	60	0	89	35	0

Hg: High growth

- ***Staphylococcus aureus.***

***** Inhibition by (*Fh*) roots essential oil (Table 2).

- A total inhibition of 550 bacteria /ml and 5500 bacteria /ml was first reached with 5µl/ml and 10µl/ml respectively of roots oil extract concentration after 24 hours of contact.
- No growth was registered (*i.e.* 5500 bacteria/ml were inhibited) from the first 10minutes with 50µl/ml roots oil extract concentration.

Roots oil extract showed a considerable effect on *Staphylococcus aureus* growth since the first 10 minutes of contact as it is shown by the very important reduction in bacterial counts when comparing with the initial ones obtained without any addition of extract. The effect was dose dependent; a growing effect was registered after 10' of contact at 10^{-3} bacterial

concentration when the dose was increased; same for the other bacterial concentrations at the various times of contact.

***** Inhibition by (*Fh*) seeds essential oil (Table 2)

- With 50µl/ml seed oil extract concentration a total inhibition of 550 bacteria /ml started after 1 hour and 5500 bacteria /ml after18 hours of contact.
- At 10^{-3} bacterial dilution a bacteriostatic effect was noticed after 1 hour of contact. Re-growth in bacterial concentration was registered in comparison to what was obtained after 10' of contact with 5µl/ml, 10µl/ml and 50µl/ml.

The effect of (*Fh*) seed oil extract on *Staphylococcus aureus g*rowth was also dose dependent and started since the first 10 minutes of contact.

***** Inhibition by (*Fh*) resin extract (Table 2)

- Total inhibition of 55 bacteria /ml was first reached after 24 hours of contact with 5µl/ml, and 550 bacteria/ml were completely inhibited with 50µl/ml of (*Fh*) resin extract.
- A bacteriostatic effect of the (*Fh*) resin was also registered at 10^{-3} bacterial concentration.

The effect of (*Fh*) resin extract on *Staphylococcus aureus g*rowth was nearly not affected by the increase in added dose, but it started after the first 10 minutes of contact.

- ***Streptococcus fecalis***

***** Inhibition by (*Fh*) roots essential oil (Table 2)

- With 5µl/ml and 10µl/ml roots oil extract concentration, 1600 and 16000 bacteria/ml were respectively inhibited after 1 hour of contact.
- With 50 µl/ml roots oil extract concentration, a total inhibition was first reached after 1 hour of contact with 16000 bacteria /ml.

The effect of *(Fh) roots* oil extract on *Streptococcus fecalis g*rowth is also dose dependent.

***** Inhibition by (*Fh*) seeds essential oil (Table 2)

- Total inhibition was first reached after 24 hours of contact of 160 bacteria /ml with 5 and 10µl/ml seed oil extract concentration; and 1600 bacteria /ml with 50µl/ml grains oil extract concentration.

Seed oil extract had nearly no effect, at all of the bacterial dilutions, at the first 10' of contact with the three bacterial concentrations used, but it showed dose dependent effect.

***** Inhibition by (*Fh*) resin extract (Table 2)

- With 5µl/ml resin concentration, no effect at all were shown at the first 10' of contact with all bacterial concentration used

- With 10µl/ml resin concentration, 1600 bacteria/ml were inhibited after 24 hours of contact but a re- growth was noticed in bacterial count after one hour of contact.
- With 50µl/ml resin concentration, 16000 bacteria/ml were inhibited after 24 hours of contact.

Results of inhibition tests of gram negative bacteria

- ***Salmonella typhi***

***** Inhibition by (*Fh*) roots essential oil (Table 3)

- Total inhibition was reached after 24 hours of contact of 2400 and 24000 bacteria /ml with 5µl/ml and 10µl/ml respectively.
- Total inhibition was first reached after 1 hour of contact of 240 and 24000 bacteria /ml with 5 and 50µl/ml respectively.

The effect of (*Fh*) roots oil extract on *Salmonella typhi* growth was dose dependent: a growing effect was registered after 10 minutes of contact at all bacterial concentration when the dose was increased and at the various times of contact.

Roots oil extract showed a considerable effect on *Salmonella typhi* growth as it is shown by the very important reduction in bacterial counts.

***** Inhibition by (*Fh*) seeds essential oil (Table 3)

- With 5µl/ml seed oil extract concentration, 240 bacteria/ml were inhibited after 1 hour of contact.
- With 10µl/ml and 50µl/ml seed oil extract concentration, 2400 and 24000 bacteria/ml were inhibited after 24 hours of contact.

The effect of (*Fh*) seed oil extract on *Salmonella typhi g*rowth was dose dependent: a growing effect was registered after 10' of contact at all bacterial concentration when the dose was increased and at the various times of contact.

***** Inhibition by (*Fh*) resin extract (Table 3)

- After 1 hour of contact, 24000 germs were inhibited by 10 and 50µl/ml resin extract, and after 24 h. of contact 2400 germs were inhibited by 5µl/ml of resin extract.

Bacterial count has been drastically decreased after one hour of contact with the different resin concentration used, and the effect of resin extract is dose dependent because the bacterial count decreased accordingly.

- ***Escherichia coli***

***** Inhibition by (*Fh*) roots essential oil (Table 3)

- With 5µl/ml and 10µl/ml roots oil extract concentration, the only effect registered was a bacteriostatic one; in fact the bacterial count was first diminished after the first 10' of contact, then it started to increase once again.
- After 24 and 1hour of contact with 50µl/ml roots oil extract concentration, 15300 and 1530 bacteria/ml were inhibited respectively.

Once again the effect of (*Fh*) roots oil essential oil on *Escherichia coli* growth was dose dependent.

***** Inhibition by (*Fh*) seeds essential oil (Table 3)

- 5µl/ml of seed oil extract had no effect at all, the first 10' of contact as it is shown by the bacterial counts registered at all of the bacterial dilutions.
- With 10µl/ml grain essential oil concentration, total inhibition of 153 bacteria /ml was first reached after 24 hours of contact.
- With 50µl/ml seed oil essential oil concentration, Total inhibition of 15300 bacteria /ml started after 24 hours of contact. The effect of (*Fh*) grains oil on *Escherichia coli* growth is dose dependent.

***** Inhibition by (*Fh*) resin extract (Table 3)

- Total inhibition was first reached after 24 hours of contact: 153, 1530 and 15300 bacteria/ml were inhibited with 5µl/ml, 10µl/ml and 50µl/ml respectively.
- The resin extract at the first 10' of contact had no effect at all, as it was shown by the bacterial counts registered at all of the bacterial dilutions.

At 10^{-3} bacterial dilution resin extract had a bacteriostatic effect, in fact a re-growth in *Escherichia coli* count after 24 hours of contact was registered.

- ***Pseudomonas aeruginosa***

***** Inhibition by (*Fh*) roots essential oil (Table 3)

- With 5, 10 and 50µl/ml roots oil extract concentration, total inhibition was reached after 24 hours of contact at 10^{-5}, 10^{-4} and 10^{-3} bacterial concentration. (*i.e.* 33, 330 and 3300 bacteria/ml were inhibited respectively). The effect of (*Fh*) roots oil extract on *Pseudomonas aeruginosa* growth was dose dependent. Roots oil extract had nearly no effect on the bacterial. Ddilutions, at the first 10' of contact, with the three concentrations used same for after one hour of contact.

***** Inhibition by (*Fh*) seeds essential oil (Table 3)

- With 5 and 10µl/ml seed oil extract, 33 bacteria /ml were inhibited after 24 hours of contact.
- 5 and 10µl/ml of seed oil extract had no effect at all at the first 10' of contact, nor after one hour of contact.
- With 50µl/ml seed oil extract, 3300 bacteria/ml were inhibited after 24 hours of contact.

***** Inhibition by (*Fh*) resin extract (Table 3)

- Total inhibition was first reached after 1hour of contact of 330 bacteria /ml with 5µl/ml resin.

Total inhibition was first reached after 24 hours of contact of 33000 bacteria /ml with 10µl/ml resin concentration.

TABLE 3

Number of Gram Negative Bacteria Remaining after Inhibition Test

Time	**0'**	**10'**	**1h**	**24h**	**10'**	**1h**	**24h**	**10'**	**1h**	**24h**
Concentration of E.O.		**5µl/ml**			**10µl/ml**			**50µl/ml**		
***Fh* roots extract**										
***Salmonella typhi*: 24x10^6 /ml**	24000	500	420	120	430	389	0	224	0	0
	2400	18	13	0	17	10	0	0	0	0
	240	3	0	0	0	0	0	0	0	0
***E. coli*: 15.3x10^6 /ml**	15300	Hg	Hg	Hg	Hg	83	1440	1000	2	0
	1530	1040	1566	Hg	824	24	6120	45	0	0
	153	156	306	Hg	127	81	100	23	0	0
***Pseudo. aeruginosa*: 33 x 10^6 /ml**	3300	Hg	Hg	78	Hg	Hg	56	Hg	Hg	0
	330	380	300	89	360	264	0	310	230	0
	33	34	30	0	31	29	0	28	30	0
***Fh* seeds extract**										
***Salmonella typhi*: 24x10^6 /ml**	5500	1300	Hg	100	280	Hg	17	98	Hg	0
	550	545	250	20	250	40	16	10	0	0
	55	20	5	0	6	0	0	5	0	0
***E. coli*: 15.3x10^6 /ml**	16000	Hg	Hg	1500	Hg	Hg	620	Hg	Hg	158
	1600	1600	1300	540	1452	1240	20	1400	1230	0
	160	153	81	0	80	32	0	40	35	0
***Pseudo. aeruginosa*: 33 x 10^6 /ml**	3300	Hg	Hg	100	Hg	Hg	98	Hg	Hg	0
	330	349	332	27	330	300	16	295	123	0
	33	29	20	0	24	0	0	19	0	0
***Fh* resin extract**										
***Salmonella typhi*: 24 x 10^6 /ml**	24000	Hg	420	112	Hg	0	0	Hg	0	0
	2400	255	23	0	198	0	0	196	0	0
	240	24	5	0	22	0	0	22	0	0
***E. coli*: 15.3x10^6 /ml**	15300	Hg	Hg	Hg	Hg	760	Hg	Hg	700	0
	1530	1550	1430	9	1300	1295	0	1210	0	0
	153	145	120	0	128	110	0	110	98	0
***Pseudo. aeruginosa*: 33 x 10^6 /ml**	33000	Hg	Hg	68	Hg	Hg	0	Hg	1000	0
	3300	345	300	9	328	295	0	310	250	0
	330	30	0	0	28	0	0	31	0	0

Hg: High growth

Comparison between (*Fh*)'s various extracts anti-microbial activity and the antibiogram with usual antibiotic discs after 24 hours of contact

The resistance, as well as the sensibility of the studied bacterial strains, towards the antibiotics is all registered in Table 4. The Ciprofloxacin (Cip) showed the highest efficiency, among the other antibiotics used, against the studied germs except for *Streptococcus fecalis* where the highest efficiency was for the Ampicillin.

TABLE 4

Count of Germs that Were Inhibited by Various Antibiotics Discs and *Fh* Extracts

Germs / ml Antibiotics	**Concen-tration**	***Staphylo aureus* 550/ml**	***Strepto. fecalis* 1600/ml**	***Salmo typhi* 240/ml**	***E. coli* 1530/ml**	***Pseudo. aeruginosa* 330/ml**
Gentamycin	(10µg)	5	69	22	101	13
Kanamycin	(30µg)		13	22	101	6
Chloramphenicol	(30µg)	41	167	30	213	0
Nalidixic acid	(30µg)	0	0	22	163	7
Ciprofloxacin	(5µg)	41	87	38	230	55
Ampicillin	(10µg)	30	194	26	92	0
Streptomycin	(10µg)	24	0	5	75	3
***Fh* essential oil Root**	5 µl/ml	550	1600	240	1530	141
	10 µl/ml	550	1600	240	1530	330
	50 µl/ml	550	1600	240	1530	330
***Fh* essential oil Seeds**	5 µl/ml	530	1060	235	1476	303
	10 µl/ml	530	1580	240	918	314
	50 µl/ml	550	1600	240	1530	330
***Fh* essential oil Resin**	5 µl/ml	530	1400	240	1521	320
	10 µl/ml	530	1600	240	1492	330
	50 µl/ml	550	1600	240	1530	330

The Minimum Inhibition Concentration (MIC), in µl/ml, was recorded as the lowest concentration that prevented visible growth. All tests were conducted at 10^{-4} dilution of the bacterial cultures.

- ***Staphylococcus aureus***: The highest efficiency among the anti-microbial discs used was equal for the Ciprofloxacin 5µg and for the Chloramphenicol 30µg where 41 germs out of 550 were killed. Whereas the lowest concentration of seed oil extract applied (5µl/ml) killed 532 germs; nearly 13 times of what was killed in the case of Ciprofloxacin (same dose) and nearly 78 times of what was killed in the case of Chloramphenicol. Hence, it is obvious that the antibacterial activity of *Ferula hermonis* strongly exceeds that of usual antibiotics.

- ***Sreptococcus fecalis***: The highest efficiency among the anti-microbial discs used was for the Ampicilin 10µg where 194 germs out of 1600 were killed. Whereas at the same dose (10µl/ml) seed oil extract killed 1580 germs; nearly 8 times of what was killed in the case of the considered antibiotic.

- ***Salmonella typhii***: The highest efficiency among the anti-microbial discs used was for the Ciprofloxacin 5µg where 38 germs out of 240 were killed. Whereas the lowest concentration of seed essential oil applied (5µl/ml) killed 235 germs; nearly 6 times of what was killed in the case of Ciprofloxacin.

- ***Escherichia coli***: The highest efficiency among the anti-microbial discs used was for the Ciprofloxacin 5µg where 230 germs out of 1530 were killed. The "lowest efficiency" was the seed essential oil, but 10µl/ml killed 918, which is in effect nearly two times of that of the Ciprioflоxacin. For the root oil and resin extract the efficiency was respectively 6.6 and 6.5 times highest.

- ***Pseudomonas aeruginosa***: The highest efficiency among the anti-microbial discs used was once again for the Ciprofloxacin 5µg where 55 germs out of 330 were killed. Whereas the lowest concentration of root extract (5µl/ml) killed 141 germs. Comparing (*Fh*) extract to Ciprofloxacin, the root, seed and resin extracts were respectively 2.5, 5.5 and 6 times more efficient.

It is obvious that the anti-bacterial activity of *Ferula hermonis* strongly exceeds that of usual antibiotics (Figure 1).

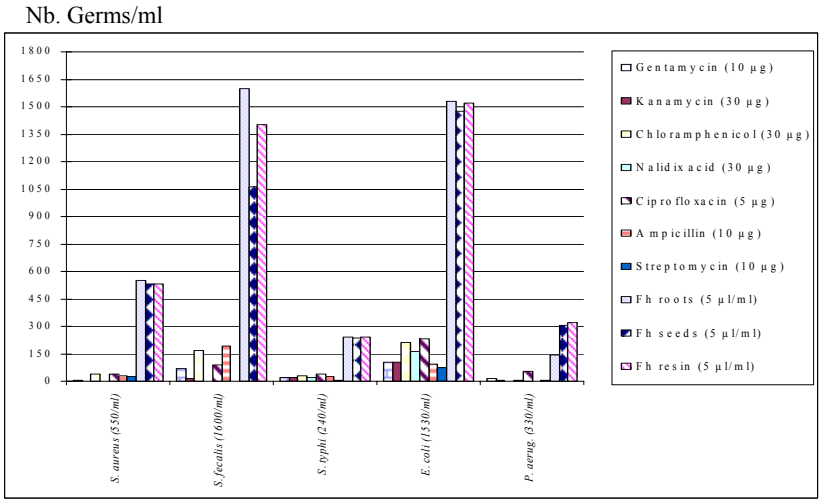

Figure 1. Comparison between the antibacterial activity of the three types of (*F h*) extracts and the antibiotic on gram positive and negative bacteria.

Comparison of the antibacterial activity strength of the three types of (*F h*) extracts (Fig. 2, 3, 4 , 5 & 6).

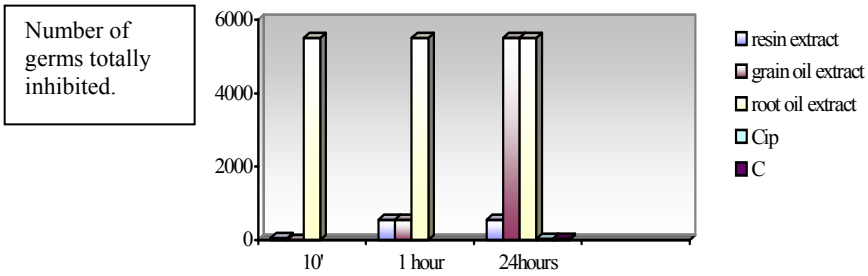

Figure 2. Comparison between the effects of the 3 extracts types at 50 µl/ml concentration on *Staphylococcus aureus* growth.

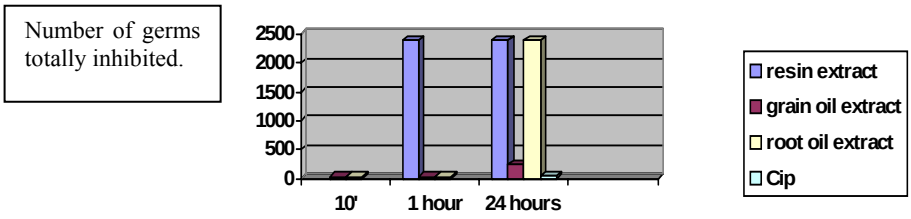

Figure 3. Comparison between the effects of the 3 extracts types at 10 µl/ml concentration on *Streptococcus fecalis* growth.

For gram positive bacteria (Figures 2 and 3) the comparison between the effects of the three types of extracts and that of the most effective antibiotic disc used, showed that the highest effect was for the roots oil extract, where at the three times of application the highest number of germs were inhibited at the three concentrations used. (Total inhibition of 5500 and 16000 germs of *Staphylococcus aureus* and *Streptococcus fecalis* respectively).

For gram negative bacteria (Figures 4, 5 and 6) the comparison between the effects of the three types of extracts and that of the most effective antibiotic disc used, showed that the highest effect was for the resin extract, where at the three times of application the highest number of germs were inhibited at the three concentrations used. (Total inhibition of 2400, 15300 and 3500 germs of *Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa* respectively).

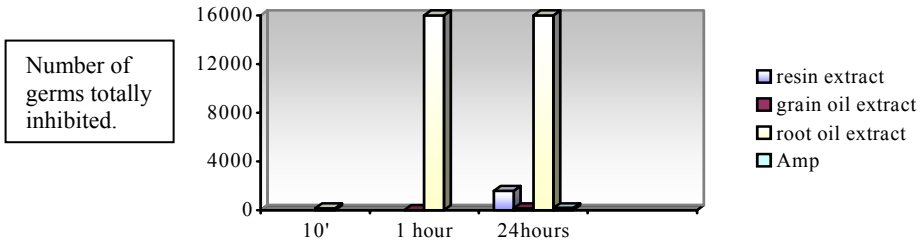

Figure 4. Comparison between the effects of the 3 extracts types at 10 µl/ml concentration on *Salmonella typhi* growth.

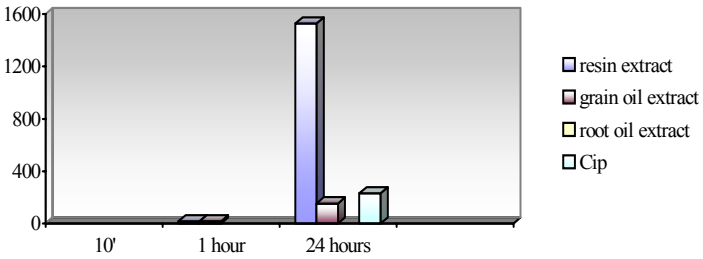

Figure 5. Comparison between the effects of the 3 extracts types at 10 µl/ml concentration on *Escherichia coli* growth.

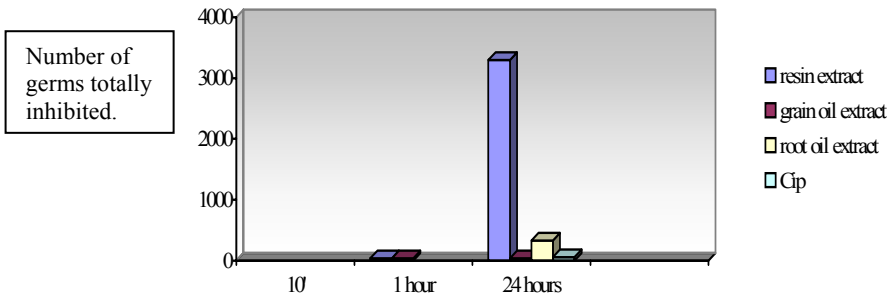

Figure 6. Comparison between the effects of the 3 extracts types at 10 µl/ml concentration on *Pseudo aeruginosa* growth.

The final scope of this study being to establish the most effective combination: extract type- concentration – time of application; where the highest number of inhibited germs is obtained with the lowest dose of extract at the shortest period of time, in this case the most effective combination would be:

Staphylococcus aureus: roots oil extract- 50µl/ml- 10 minutes (total inhibition of 6000 germs)
Streptococcus fecalis: roots oil extract- 10µl/ml- 1 hour (total inhibition of 16000 germs)
Salmonella typhi: resin extract- 10µl/ml- 1 hour (total inhibition of 2400 germs)
Escherichia coli: resin extract- 50µl/ml- 18 hours (total inhibition of 15300 germs)
Pseudomonas aeruginosa: resin extract- 10µl/ml- 18 hours (total inhibition of 3500 germs)

Statistical analysis for the evaluation of the antibacterial activities

* For gram positive bacteria

The multivariate analysis of variance for the influence of 3 sources (roots, seeds and resin *Fh* extracts) on the inhibited number of 2 gram positive bacteria reveals the following major results (Table 2).

- The type of *Fh* extract is significant for *S. aureus* (F= 7.5; P=0.01) and highly significant for *S. fecalis* (F= 18; P=0.001).
- The number of *S. fecalis* inhibited is also highly influenced by the time of inhibition (F= 26; P=0.000)
- The interactive effects (*Fh* extract x concentration; *Fh* extract x time; concentration x time) are not significant.

In this context, the multiple range tests (Duncan Test) reveal the following homogenous subsets:
- For *S. aureus*: 2 homogenous subsets are identified. The first subset (*Fh* roots extract; *Fh* seeds extract) has 2 means ranging from 4.2 to 14.2 cfu.mL^{-1} and the second has one mean 126 cfu.mL^{-1} These results indicate that the inhibition of *S. aureus* by *Fh* extract roots and *Fh* extract resin do not differ significantly.
- For *S. fecalis*: 2 homogenous subsets are also displayed. The first subset, *Fh* roots extract, has one mean 162 cfu.mL^{-1} and the second has 2 means ranging from 795 to 976 cfu.mL^{-1}. These results indicate that the number of *S. fecalis* inhibited by *Fh* extract seeds and *Fh* extract resin are not different from each other.

* For gram negative bacteria

The multivariate analysis of variance for the influence of 3 sources (roots, seeds and resin *Fh* extracts) on the inhibited number of 3 gram negative bacteria reveals the following major results (Table 3).

- The type of *Fh* extract is significant for *Salmonella* (F= 6.4; P=0.02) and is not significant for *E. coli* (F= 1.9; P=0.21) and *P. aeruginosa* (F= 0.7; P=0.50)
- The level of concentration is also significant for *Salmonella* (F= 4.8; P=0.04) and highly significant for *P. aeruginosa* (F= 2.02; P=0.2) but do not influence the *E. coli* (F= 2.02; P=0.2)
- The time of inhibition is highly significant for *P. aeruginosa* (F= 266; P=0.000) and do not influence the others gram negative bacteria.

- The interactive effects (*Fh* extract x concentration ; *Fh* extract x time ; concentration x time) are not significant.

In this perspective, the multiple range tests display 2 homogenous subsets for *Salmonella* and one group for *E. coli* and *P. aeruginosa* in relation of the type of *Fh* extract.

The "Z- TEST" comparative results

The statistical validation based on "the Z- test" for the comparison between the lowest number of germs inhibited by *Fh* essential oils extracts from (root, seeds and resin) and the highest number of germs inhibited by the most efficient antibiotic at a P-value of 5%, reveals the following major results :

Fh root oil extract inhibited 96.4% of 550 germs of *Staphylococcus aureus*, 87.5% of 1600 germs of *Streptococcus fecalis*, 60.0% of 1530 germs of *Escherichia coli*, 98.0% of 240 germs of *Salmonella typhi* and 42.7% of 330 germs of *Pseudomonas aeruginosa*, since the Ciprofloxacin inhibited 7.5% 12.1%, 15.0%, 16.7% and 15.8% of the germs respectively (*i.e* the influence Fh extracts *vs* antibiotics is highly significant, P=0) (Table 4).

CONCLUSION

Ferula hermonis Boiss. the endemic plant of Mount Hermon is a very aromatic one, specially its roots that are known to exhibit exotic smells. In fact the essential oil's profile of *Ferula hermonis* roots was shown to be very rich in components, where most of the essential oils were at the state of traces, whereas there was an obvious predominance of *α-pinene* (38% and 51%) in roots and seed extracts respectively. The evaluation of (*Fh*) extract proves its anti-bacterial properties. Its activity strongly exceeded that of usual antibiotics. The carvacrol, thymol and the sesquiterpen hydrocarbon present in this plant, and previously proven to exhibit such activity (Jay, 1999), (Galal *et al*., 2000), are presumed to be the major components responsible for it in *Ferula hermonis.* And the thorough study of this activity in the case of different extracts of this plant, led to the most probable conclusion that gram negative germs (*Salmonella typhi, Pseudomonas aeruginosa* and *Escherichia coli*) are mostly susceptible to components found in the resin of this plant. While gram positive germs (*Staphylococcus aureus* and *Streptococcus fecalis*) are mostly susceptible to components found in its roots. Hence *Ferula hermonis*, besides its established fame as an aphrodisiac, possesses two major biological activities: an anti-bacterial and probably anti-oxidant. These properties are the result of the presence of some secondary metabolites and for their utilization on an industrial scale.

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