Bull. Pharm. Sci., Assiut University, Vol. 27, Part 1, June 2004, pp. 79-85.

NATURAL HAIR RECIPE

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في هذا البحث تم اجراء بعض الدراسات العملية على احدى المستحضرات من اصل طبيعى والمستخدمة فى علاج سقوط الشعر وذلك لفصل المواد الفعالة بها وتقويمها بيولوجياً واجراء بعض التعديلات عليها لزيادة تأثيرها المعالج. واسفرت الدراسة على ان فاعلية المستحضر ناتجة عن الأحماض الدهنية بالميتك، ستريك، اوليك ولينالوليك وباضافة مادة الكانثاريدين ومادة البيلوكاربين لهذه الاحماض تم زيادة فعاليتهم مع ملاحظة ان فاعلية الوصفة المعدلة بأضافة الكانثاريدين كانت أكثر من الوصفة المعدلة بأضافة البيلوكارين.

Biological evaluation of natural preparations used for treatment of hair loss revealed that the saponifiable fraction was the active fraction. The main constituents of this fraction were palmitic, stearic saturated fatty acids and oleic and linoleic unsaturated fatty acids.

Certain modifications were done to increase the activity by addition of Cantharidin or Pilocarpine to the most active subfraction of saponifiable matter. The superior effect on treating hair loss was shown with the formulation containing cantharidin than that which contain pilocarpine.

INTRODUCTION

Hair is of great concern by females and males and they tend to modify it by coloring, wigs and cutting. Different types of hair may be produced by different kinds of follicles, and the type of hair produced in any particular follicle can be changed with age or under the influence of hormones.[1,2] Hair loss is a problem to many females and males, some treatments to cure this case were suggested by many research workers,[3-7] which included some shampoos and folk recipes of natural origin.

Fatty acids play an important role for healthy hair.[8,9] Essential fatty acids deficiencies are characterized by skin lesions particularly on cheeks, headache and low blood pressure.[10] Hair loss is an important feature of essential fatty acids deficiency.[11]

Essential fatty acids are important in oxygen transfer and control of nutrient passage through cell membranes.[12] Fatty acids, specially stearic acid, showing hair damage protecting and excellent hair conditioning effects,[13] fatty acids. Also improve the quality of hair.[14]

There are a lot of natural plants rich in oils were commonly used for hair treatment.[15] On previous study, we studied the biological activity of the most widely used Egyptian preparation.[16] Continuing the work we designed this work to promote some knowledge on one of the most biologically active preparation in an attempt to isolate biologically active constituents and prepare a safe effective recipes for treatment of hair.

EXPERIMENTAL

Materials

1- Herbal sample

The examined sample was provided from National Research Center, as a yellow colored liquid in brown glass bottles (100 ml). The sample composed of a mixture of equal amount of olive oil, almond oil, chamomile oil, peanut oil, nigella oil and carrot seed oil.

2- Cantharidin and pilocarpine

Cantharidin and pilocarpine powders (E. Merck, Germany).

Received in 8/3/2004 & Accepted in 22/5/2004

3- Materials for chromatographic Studies

a- Silica gel G60 for thin layer chromatography (E. Merck, Germany).
b- Silica gel for column chromatography (Sigma, USA).
c- Silica gel ready made plates (Merck).
d- Aluminum sheets precoated with silica gel G-60, 0.2 mm thick (Merck).
e- Solvent Systems:
   1- Petroleum ether: ether (80:20 v/v).
   2- Toluene: ethyl acetate (70:30 v/v).
   3- Benzene: ethyl acetate (80:20 v/v)
   4- Benzene: ethyl acetate (93:7 v/v).
f- Spray reagent: Ethanol sulphuric acid spray reagent (20%).
g- Authentic materials for HPLC were provided from the central laboratory, Faculty of Agriculture, Cairo University.

4- Materials for clinical studies

Recipes

a- Volatile oil, saponifiable & unsaponifiable matter formulations were prepared by dissolving 0.32 g, 5.2 g & 0.64 g of each fraction in 100ml olive oil, respectively.
b- Formulations prepared from fraction A to fraction H, were prepared by dissolving 0.08 g of fraction A, 0.416 g of fraction B, 1.104 g fraction C, 0.28 g of fraction D, 0.104 g of fraction E, 0.256 g of fraction F, 0.504 g of fraction G and 0.184 g of fraction H in 100ml olive oil.
c- Modified recipe I (with cantharidin) prepared by dissolving 1.104 g of fraction C and 1 g cantharidin in 100 ml olive oil.
d- Modified recipe II (with pilocarpine) prepared by dissolving 1.104 g of fraction C & 4 g pilocarpine in 100 ml olive oil.

Patients

This study included female volunteers, their ages from 25-40 years old and their socioeconomic level and health habits were almost similar. Clinical examination was done by the aid of Dr. H. El-Nazer, professor of dermatology, national research centre, for all volunteers to exclude diseased cases (persons suffering from diseases affecting the hair such as scalp infection, alopecia or any internal diseases related to hair fall such as endocrinal diseases, hepatic diseases, renal diseases or anemia.

Apparatus

1- Essential oil hydrodistillation apparatus.
2- Apparatus for GC analysis.
   Hewlett Packard G.C 5890 series II plus FID, capillary column of 0.32 µm internal diameter & 0.30 µm film thickness, 0.4 µl sample size, (60-200°) oven temp., program (rate) 3°/min, 150° inject. port temp., 1ml/min helium carrier gas flow rate & 100:1 split ratio.
3- Apparatus for GC/MS analysis:
   Hewlett Packard GC/MS 5890 series II plus with mass selective detector (MSD), 1 ml sample size, (40°-200°) oven temp., program (rate) 2°/min, 150° inj. port temp., 1ml/min helium carrier gas flow rate, 100:1 split ratio, mass ionization voltage 70 ev. and 1800 electron multiplier voltage.
4- Apparatus for HPLC:
   Shimadzu, Sc I-10A vp, UV-Vis detector (200 nm), solvent methanol: water (1:1), temp. 80°, flow rate 0.5, volume of injection 20 µl.

Techniques

1- TLC.[17]
2- GLC coupled with mass spectroscopy.

Methods

1- Preparation of essential oils

The sample under investigation were subjected to hydrodistillation method according to E.P. (1984).[18] The volatile fraction was subjected to CLC analysis and biological investigation.

2- Unsaponifiable and saponifiable fraction

Sample was saponified by refluxing with 10% alcoholic potassium hydroxide for 5 hours acc. to E.P[18] method.

The saponifiable and unsaponifiable matters were subjected to biological investigation and analyzed using gas liquid chromatography.

3- Chromatographic fractionation of the biologically active fraction

The saponifiable fraction (65 g) was applied on a silica gel column (250 g) and eluted gradually with hexane, benzene, benzene: chloroform, chloroform, chloroform:

methanol with increasing polarity (10%). The collected fractions (100 ml) were monitored by TLC and similar fractions were combined together giving eight subfractions (A-H).

Each subfraction from A to H was subjected to GC-MS analysis and to biological study.

4- Increasing biological activity by certain modification

Cantharidin and pilocarpine are the most powerful natural constituents used in treating hair loss.

Two modified preparations were done by adding 1% of cantharidin[4] and 4% pilocarpine[19] to the most effective subfraction (fraction C).

5- Biological Study

The clinical study were done in NRC, Dokky, Egypt. All the tested fractions included in this study as well as olive oil (the solvent used for sample preparation) were topically applied on the scalp of female volunteers for 6 weeks and each group consist of twenty four females volunteers this study is divided into three experiment. In the first experiment six groups were included [control, diseased, V.O treated group, saponifiable fraction treated group and olive oil(solvent treated group].In the second experiment eight subgroups were included for eight sub fractions of the most effective fraction in the first experiment(A-H). In the third experiment two subgroups were included (fraction C+cantharidin and fraction C+pilocarpin). Four samples were collected from each of the tested groups according to the method described by Kessels et al.[20,21] The first sample was collected before applying the preparation to the patients scalp and considered as the zero time of the experiment. The second sample was collected two week later, the third sample was collected four weeks after the zero time and the last sample was collected after six weeks from the zero time. For the first 4 weeks the tested preparations were topically applied twice per week, while for the last two weeks applied once per week. All the calculation and data presentation for the results of present study were carried out by the use of the Software Microsoft Excel for Arabic edition (2000), Microsoft Corporation, USA.

RESULTS AND DISCUSSION

GLC of the volatile constituents Table (1), revealed that cineol, thymoquinone and chamazulene were the major constituents. While GLC analysis of saponifiable fraction and unsaponifiable matters of the examined preparation Tables (2,3) revealed that the saponifiable fraction contain pentadecanoic, linoleic, nonanoic and palmitic acids as the major constituents. Octacosane and eicosane were the major constituents in unsaponifiable fraction. Biological investigation of volatile, saponifiable and unsaponifiable matters (Table 4, Fig. 1) indicated that the saponifiable fraction was the effective one in treating hair fall. These results were agreed with the literature review which indicated that, fatty acids showing hair damage protection, excellent hair conditioning effects,[13] hair growth stimulant[11] and hence, hair loss is an important feature of essential fatty acids deficiency.[10]

Table 1: GLC of the volatile oil fraction

Identified compounds	Relative Retention Time (Rrt)	Concentration (%)
α -pinene	0.36	5.77
Camhene	0.805	0.793
Cineol	1	17.711
α -terpinene	1.435	3.000
Sabinene	1.820	0.103
Myrcene	2.085	0..034
Borneol	2.985	0.781
Thymoquinone	4.315	15.897
Gurjuene	5.131	0.013
Thymol	5.976	2.891
Geranyl acetate	6.736	1..579
α -bisabolol	7.111	1.464
Chamazulene	8.116	16.001
Carotol	9.466	2.016

Table 2: GLC of fatty acid methyl esters

Identified compounds	Rrt	Concentration (%)
n-heptanoic (7)	0.26	1.867
Caprylic (8)	0.41	3.982
Nonanoic (9)	0.59	14.487
Undecylenic (11:1)	0.709	5.581
Pentadecanoic (15:0)	1	24.667
Palmitic (16:0)	1..53	11.218
Palmitoleic (16:1)	1.60	4.054
Stearic (18:0)	1.77	3.807
Oleic (18:1)	1.92	9.292
Linoleic (18:2)	2.2	17.654

Table 3: GLC of the unsaponifiable matters

Identified Compounds	Rrt	Concentration (%)
Dodecne (12)	0.135	0.116
Tetradecane (14)	0.196	0.151
Hexadecane (16)	0.245	0.047
Heptadecane (17)	0.315	0.233
Octadecane (18)	0.378	0.242
Nonadecane (19)	0.434	0.183
Eicosane (20)	0.549	27.305
Heneicosane (21)	0.609	0.167
Docosane (22)	0.663	0.103
Tricosane (23)	0.724	0.581
Hexacosane (26)	0.922	0.788
Octacosane (28)	1	64.938
Triacontane (30)	1.356	0.222
Campesterol	1.823	1.336

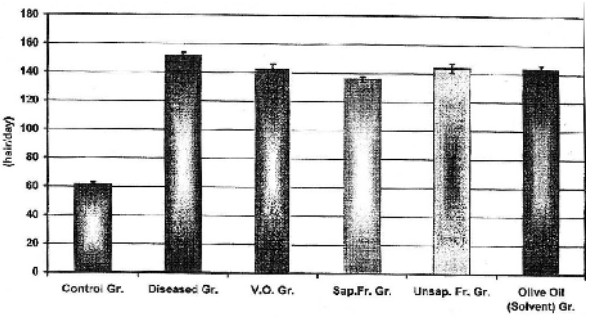

Fig. 1: Change in the six studied groups after 6 weeks treatment (Mean ± SE).

Table 4: The descriptive statistics and the changes of the six studied groups after 6 weeks treatment (hair/day).

	Control Gr.	Diseased Gr.	V.O. Gr.	Sap. Fr. Gr.	Unsap. Fr. Gr.	Olive oil (solvent) Gr.
Mean	61.458	151.375	143.357	135.667	143.833	143.417
S.E.	1.537	2.469	2.733	1.931	3.110	2.407
Median	61.000	149.000	143.000	136.500	145.500	144.000
S.D.	7.530	12.097	13.390	9.462	15.236	11.791
Range	25	38	50	33	67	42
Minimum	47	136	117	119	112	122
Maximum	72	174	167	152	179	164
Sum	1475	3633	3441	3256	3452	3442
Count	24	24	24	24	24	24
Sig. from control t value		at $p \leq 0.001$ 30.915	at $p \leq 0.001$ 26.124	at $p \leq 0.001$ 30.064	at $p \leq 0.001$ 23.745	at $p \leq 0.001$ 28.699
Sig. from diseased Gr. t value			at $p \leq 0.001$ 2.172	at $p \leq 0.005$ 5.011	at $p \leq 0.05$ 1.899	at $p \leq 0.05$ 2.308
Sig. from V.O. Gr. t value				at $p \leq 0.05$ 2.303	NS 0.111	Ns 0.111
Sig. from Sap.Fr.Gr. t value					at $p \leq 0.05$ 2.231	at $p \leq 0.01$ 2.511
Sig. from Unsap.Fr.Gr. t value						NS 0.106

Chromatographic fractionation of the saponifiable fraction gave eight fractions (A-H), GC-MS and HPLC analysis of each fraction indicated the presence of n-heptanoic in fraction A and B, caprylic, nonanoic and undecylenic in fraction B, stearic present only in fraction C, while, palmitic, oleic and linoleic occurred in fraction C and D. Palmitic and oleic occurred in fraction E, F and H. While palmitoleic occurred in fraction G and H.

The biological evaluation of these fractions (A to H) showed that fraction C had much more superior effect after 6 weeks of its use, its activity starting from the second week of treatment (Tables 5,6, Figs. 2,3). This fraction contain palmitic, stearic, oleic and linoleic fatty acids.

Table 5: The descriptive statistics and the changes of the control, diseased and Sap. Fr. groups and the 4 subgroups A, B, C, D after 6 weeks treatment (hair/day).

	Control Gr.	Diseased Gr.	Sap.Fr. Gr.	Subgr. A	Subgr. B	Subgr. C	Subgr. D
Mean	61.458	151.375	135.667	135.958	136.542	77.792	107.875
S.E.	1.537	2.469	1.931	2.759	2.950	2.385	2.994
Median	61.000	149.000	136.500	142.000	148.000	92.000	108.000
S.D.	7.530	12.097	9.462	13.518	14.452	11.684	14.665
Range	25	38	33	50	65	43	49
Minimum	47	136	119	108	112	59	83
Maximum	72	174	152	158	177	102	132
Sum	1475	3633	3256	3263	3277	1867	2589
Count	24	24	24	24	24	24	24
Sig. from control t value		at $p \le 0.001$ 30.915	at $p \le$ 0.001 30.064	at $p \le 0.001$ 23.587	at $p \le 0.001$ 22.572	at $p \le 0.001$ 5.757	at $p \le 0.001$ 13.794
Sig. from diseased Gr. t value			at $p \le$ 0.001 5.011	at $p \le 0.005$ 4.163	at $p \le 0.001$ 3.856	at $p \le 0.001$ 21.434	at $p \le 0.001$ 11.210
Sig. from Sap.Fr. Gr. t value				NS 0.087	NS 0.248	at $p \le 0.001$ 18.858	at $p \le 0.001$ 7.801
Sig. from Subgr. A t value					NS 0.144	at $p \le 0.001$ 15.948	at $p \le 0.01$ 6.898
Sig. from Subgr. B t value						at $p \le 0.001$ 15.487	at $p \le 0.001$ 6.821
Sig. from Subgr. C t value							at $p \le 0.001$ 7.860

Table 6: The descriptive statistics and the changes of the control, diseased and Sap. Fr. groups and the 4 subgroups E, F, G, H after 6 weeks treatment (hair/day).

	Control Gr.	Diseased Gr.	Sap.Fr. Gr.	Subgr. E	Subgr. F	Subgr. G	Subgr. H
Mean	61.458	151.375	135.667	134.125	134.792	135.042	92.417
S.E.	1.537	2.469	1.931	2.564	1.794	1.765	2.020
Median	61.000	149.000	136.500	150.000	143.000	124.000	94.500
S.D.	7.530	12.097	9.462	12.561	8.787	8.645	9.895
Range	25	38	33	44	29	28	38
Minimum	47	136	119	108	116	122	72
Maximum	72	174	152	152	145	150	110
Sum	1475	3633	3256	3219	3235	3241	2218
Count	24	24	24	24	24	24	24
Sig. from control t value		at $p \le 0.001$ 30.915	at $p \le$ 0.001 30.064	at $p \le 0.001$ 24.309	at $p \le 0.001$ 31.046	at $p \le 0.001$ 31.444	at $p \le 0.001$ 12.198
Sig. from diseased Gr. t value			at $p \le$ 0.001 5.011	at $p \le 0.005$ 4.846	at $p \le 0.001$ 5.434	at $p \le 0.001$ 5.382	at $p \le 0.001$ 18.482
Sig. from Sap.Fr. Gr. t value				NS 0.480	NS 0.332	NS 0.239	at $p \le 0.001$ 15.476
Sig. from Subgr. A t value					NS 0.213	NS 0.295	at $p \le 0.01$ 12.779
Sig. from Subgr. B t value						NS 0.099	at $p \le 0.001$ 15.687
Sig. from Subgr. C t value							at $p \le 0.001$ 15.893

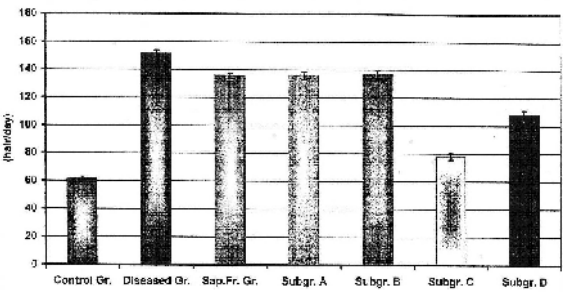

Fig. 2: Change in the control, diseased and Sap.Fr. groups and the 4 subgroups A, B, C, D after 6 weeks treatment (Mean ± SE).

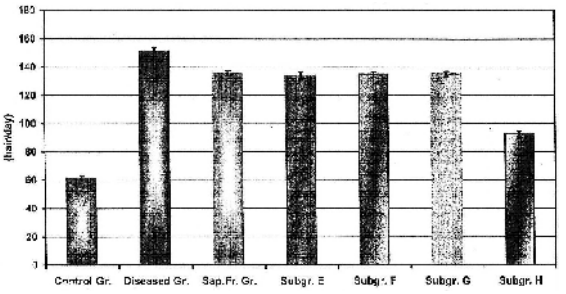

Fig. 3: Change in the control, diseased and Sap.Fr. groups and the 4 subgroups E, F, G, H after 6 weeks treatment (Mean ± SE).

Table 7: The descriptive statistics and the changes of the control, diseased , subgroup C, fraction C, cantharidin and fraction C + pilocarpine after 6 weeks treatment (hair/day).

	Control Gr.	Diseased Gr.	Subgr. C	Fraction C + Cantharidin	Fraction C + Pilocarpine
Mean	61.458	151.375	77.792	65.042	70.167
S.E.	1.537	2.469	2.385	1.319	1.751
Median	61.000	149.000	74.000	66.000	69.000
S.D.	7.530	12.097	11.684	6.464	8.580
Range	25	38	43	25	32
Minimum	47	136	59	52	57
Maximum	72	174	102	77	89
Sum	1475	3633	1867	1561	1684
Count	24	24	24	24	24
Sig. from control t value		at $p \leq 0.001$ 30.915	at $p \leq 0.001$ 5.757	at $p \leq 0.05$ 1.769	at $p \leq 0.001$ 3.737
Sig. from diseased Gr. t value			at $p \leq 0.001$ 21.434	at $p \leq 0.001$ 30.837	at $p \leq 0.001$ 26.825
Sig. from Subgr. C t value				at $p \leq 0.001$ 4.678	at $p \leq 0.001$ 2.577
Sig. from Fraction C + Cantharidin t value					at $p \leq 0.01$ 2.337

These results will agree with the reported data that unsaturated fatty acids were effective in treating hair fall.[7,12] Modifications to the most effective fraction (C) were made by the addition of cantharidin (modified recipe I) and pilocarpine (modified recipe II) which are the most widely used hair tonics. Biological evaluation of the modified recipes I and II investigated that, modified recipes I and II showed a significant decrease in hair fall at the 6th week (Table 7, Fig. 4) when compared to the fraction C, the most superior one is modified recipe I. So we concluded that formulation containing palmitic, stearic, oleic and linoleic fatty acids in addition to cantharidin is a good preparation for treating hair fall.

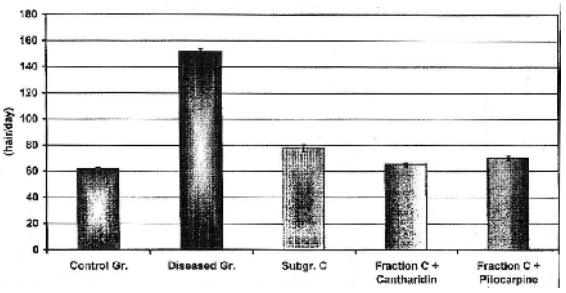

Fig. 4: Change in the control, diseased subgroup C, Fraction C + Cantharidin and Fraction C + Pilocarpine after 6 weeks treatment (Mean ± SE).

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