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Original Article

STUDY OF THE ANTI-HYPERLIPIDEMIC EFFECT OF THE COMBINED ADMINISTRATION OF THREE NATURAL EXTRACTS IN A POLOXAMER-407 HYPERLIPIDEMIC MODEL AND THEIR LC-ESI-MS/MS^2 AND HPLC PROFILING

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ABSTRACT

Objective: Dyslipidaemia is considered a high-risk factor for inducing atherosclerosis and cardiovascular diseases (CVDs). This study aims to investigate the anti-hyperlipidemic effect of the co-administration of the ethanol extracts of both ginger (root and rhizome) and leek (leaves and bulbs) in addition to the aqueous extract of gum arabic.

Methods: Rats were divided into eight groups: Hyperlipidaemia was induced in rats by a single intraperitoneal injection of Poloxamer 407 (P-407) [1 g/kg], negative control [saline injected], hyperlipidemic control [P-407 injected], positive control [Atorvastatin 70 mg/kg], groups four, five and six received ginger extract (400 mg/kg), leek extract (500 mg/kg) and gum arabic aqueous extract (7.5 g/kg) respectively and groups seven and eight received a co-administration of ginger, leek and gum arabic extracts at doses A and B respectively. Lipid profile was monitored. The profiling of all the tested extracts was performed by LC-ESI/MS and HPLC.

Results: A significant anti-hyperlipidemic activity ($P<0.05$) was seen for group eight among all the tested groups producing ≈54%, 72%, 50% and 72% decrease in the measured parameters total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) respectively. An overall of 56 and 45 compounds were tentatively identified in the ethanol extracts of ginger and leek, respectively. Galactose and arabinose sugars were found to be the major saccharides in gum arabic and glucuronic acid was the major polyuronide part.

Conclusion: the co-administration of a group of natural extracts in the given concentration proved to be more effective than the use of synthetic drugs or the use of a single component.

Keywords: Hyperlipidaemia, Ginger, Gum arabic, Leek, Poloxamer 407

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INTRODUCTION

Dyslipidaemia can be defined as the elevation of cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) serum levels while maintaining low serum levels of high-density lipoprotein cholesterol (HDL-C). This imbalance is considered a high-risk factor for inducing atherosclerosis and CVDs, which are considered the most common cause of death in both western and eastern countries [1]. As per the recommendation of the National Cholesterol Education Program, individuals with blood cholesterol levels above 240 mg/dl are considered hyper-cholesterolemic [2]. Many people achieve healthy levels by eating a balanced diet and through other aspects of their lifestyle including quitting smoking, exercise. However, some require medication to prevent additional health problems.

Traditionally, natural remedies were used to overcome such an imbalance in lipids metabolism and are claimed to be useful in controlling hyperlipidaemia and associated pathologies [3]. According to Hasani-Ranjbar *et al.* [4], About 53 clinical trials were performed to review the efficacy of several natural sources in the management of hyperlipidaemia, twenty-two plants of which were proven to show a significant decrease in total cholesterol and LDL cholesterol. Plants such as Ginger [Zingiber officinale R.], family Zingiberaceae, Leek, Allium ampeloprasum var. porrum (L.) also known as A. porrum, family Alliaceae (Liliaceae), and the edible dried gummy exudate gum arabic are all known to possess anti-hyperlipidemic activities in addition to other diverse biological effects which have been reported for this three species, such as anti-inflammatory, antitumor, antioxidant, hypotensive, prebiotic and ant-diabetic activities [5-10].

Synergy is a process in which the combined effect of some substances cooperates to reach a greater effect than the sum of their separate effects. Previous trials for the use of combined herbal therapy was reported for cancer treatment [11], hyperlipidemia [12] also a combination of herbal and synthetic drugs was under investigation for the treatment of diabetes [13, 14] as a mean to enhance therapeutic effects, reduce side effects and overcome drug resistance.

In the present work, an evaluation of the anti-hyperlipidemic effect of the co-administration of the ethanol extracts of both ginger (root and rhizome) and leek (leaves and bulb) and the aqueous solution of gum arabic versus Atorvastatin in a P-407 induced hyperlipidemia model in rats was performed. Examination of the active constituents of the tested extracts was done using liquid chromatography-electrospray ionization-mass spectroscopy/mass-mass spectroscopy (LC-ESI-MS/MS2) analysis and quantitative high-performance liquid chromatography (HPLC) analysis of the aqueous solution of gum arabic.

MATERIALS AND METHODS

Chemicals and reagents

Ator® (Atorvastatin) 40 mg tablets (EIPICO Company [Egyptian international pharmaceutical industries company–Egypt). Tablets were crushed into powder, dissolved in distilled water (120 mg/25 ml). Poloxamer 407, [Sigma-Aldrich Chemical Co. The USA]. P-407 was prepared by dissolving 8 g/100 ml saline for injection and then refrigerated overnight to facilitate its dissolution [15]. 70% ethanol (El Gomhoria Company).

Plant materials

Both dried entire ginger root and rhizome [*Zingiber officinale* R.] and Gum Arabic (*Acacia senegal*) were purchased from local Harazz commercial stores of Medicinal Plants, Cairo-Egypt. Leek (*Allium porrum*) plant leaves and bulbs were collected from Nahia region,

Giza city, Egypt during May and June 2015. All used plants were botanically identified and authenticated by Dr. Abdul Halim Abdul Majali Mohammed, Head of Flora Research and Plant Classification department, Agricultural Research Centre. Three voucher specimens (*Zof*-152/2017 for ginger, *Apo*-153/2017 for leek and *Gar*-154/2017 for gum) were deposited in the herbarium of Pharmacognosy department, Faculty of Pharmacy, Helwan University.

Extracts preparation

One Kg of dried ginger (root and rhizome) and one Kg of leaves and bulbs of leek were air-dried and ground to powder using a clean mill and mortar. They were separately macerated in (2LX3) 70% ethanol and left to stand 72 h with shaking until complete extraction. After filtration, the alcoholic extracts were concentrated under reduced pressure at 45 °C. The yields of the alcoholic extracts were 12.7%, 28.8% for ginger and leek, respectively. Aqueous solution of gum arabic was freshly prepared by dissolving 15 g of gum arabic in 50 ml distilled water to obtain 30% solution.

LC/MS analysis

LC/MS analysis was performed on triple stage quadruple mass spectrometer, TSQ Quantum Access MAX, Thermo Scientific, New York, USA, equipped with electrospray ionization (ESI) operated in the positive ionization mode (60 ev). Identification of the content of the extract was carried on the Accela U-HPLC system, which was composed of Accela 1250 quaternary pump and Accela open autosampler, New York, USA (operated at 25 oC). Hypersil Gold column (C18 bonded ultrapure silica-based column) 50 mm × 2.0 mm (1.9 µm), Thermo-scientific, New York, USA. Isocratic elution using fresh prepared Acetonitrile (A): 0.2% formic acid aqueous solution (B) (90:10) at room temperature. Flow rate (250 µl/min). X-calibur software version 2.2 was used to control all parameters of UPLC and MS and analysis of the obtained data.

Sugar analysis after hydrolysis of gum arabic by HPLC

Galactose: arabinose: rhamnose ratios in gum arabic extract was determined using HPLC. Aliquots of 0.03-0.05 g of the gum were weighed out accurately into tarred 15 cm^3 stoppered Pyrex test tubes and 10 cm^3 of 4% w/w sulphuric acid added to each. The tubes were placed in a water bath at 100 °C for 4 h and were then reweighed and made up to the original weight by the addition of distilled water. The solutions were neutralized by adding 2 g $BaCO_3$ and shaking overnight. The filtered (0.45 urn) hydrolysates were analysed using the Perkin-Elmer Series 10 HPLC system equipped with a 25 x 0.46 cm SS Amino column (phase separations). The sample (60 µdm) was injected onto the column using 80:20 acetonitrile: water eluent at 22 °C and at a flow rate of 0.8 cm^3/min. The retention times of the monosaccharide components were monitored using a Millipore Waters differential refractometer R401 and the proportions of each were determined by integration of the peaks. All analytes were compared with injected standards separately and calculated according to the following equation:

Animals

Forty Sprague-Dawley Albino adult male rats weighing 130-180 g, purchased from the animal house of National Research Centre, were used. The animals were housed in standard metal cages in an air-conditioned room at 22±3 °C, 55±5% humidity for 12 h [light and dark cycles were maintained]. The animals were left for an initial adaptation period of 7 d and were supplied with a standard pellet diet and water ad libitum. The experiment was carried for 4 d. All animal procedures were performed in accordance with the recommendation of the national institute of health guide for care and use of laboratory animals NIH guide, publication No 85-23: (revised1985) US department of health, education and welfare, specific national laws were applied and all experiments were examined by the appropriate ethics committees, approval No: 2017/FPHU150.

Experimental design and induction of hyperlipidemia

Rats were randomly divided into 8 groups, each comprising 8 rats. All rats were subjected to a six hours fasting. Animals of the first group were kept as negative control injected intraperitoneal [i. p.] with saline. Rats of the remaining seven groups were given 1g/kgbw (bodyweight) of Poloxamer-407 to induce hyperlipidaemia in a single dose on day one [14]. Group 2 was kept as hyperlipidemic control. Two hours later, group three were given atorvastatin (70 mg/kgbw) [16] and was considered as positive control. Similarly, group 4, 5 and 6 were administered 400 mg/kgbw of the ethanol extract of ginger [17], 500 mg/kgbw of the ethanol extract of leek [18] and 7.5 g/kgbw of the aqueous solution of gum arabic [19] respectively. As for group 7, it was co-administered dose A of the tested extracts, (ethanol extract of ginger (200 mg/kgbw), leek (250 mg/kgbw) and 30% aqueous solution of gum arabic 3.75 g/kgbw). While group eight was co-administered dose B (ethanol extract of ginger 400 mg/kgbw, leek 500 mg/kgbw and 30% aqueous solution of gum Arabic 7.5 g/kgbw). This was repeated once daily for 3 consecutive days by oral gavage [16].

Collection of blood samples

At the end of the experimental period (4 d) and after 12 h of fasting, the rats were anesthetized with isoflurane and oxygen then sacrificed. Blood was collected from the hepatic portal vein in centrifuge tubes and were incubated at room temperature for 30 min. Serum was obtained by centrifugation of blood samples [4000 rpm/15 min]. All samples were stored at-20 °C until further analysis. Handling procedures were conducted in accordance with the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication no. 85-23, revised 1985).

Biochemical analysis

Serum total cholesterol and triglycerides were estimated by enzymatic methods of CHOD-PAP and GPO-Trinder method [20]. Estimation of (HDL-C) was done by precipitation method [17]. All parameters were analysed by Spectrophotometric with quantitative diagnostic commercial kits. Serum concentrations of very low-density lipoprotein-cholesterol (VLDL-C) and Low-density lipoprotein-Cholesterol (LDL-C) were calculated using Friedewald's formula [21].

Statistical analysis

Data were expressed as means±standard error of means [SEM]. Assessment of the results was performed using a one-way analysis of variance [ANOVA] followed by Dunnett as post hoc test using SPSS program, version 16. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Anti-hyperlipidemic effect

The concept of the use of a herbal combination to treat several diseases was the subject of many studies throughout the years [21-23], in the majority of which the combined administration of crude plants or their extracts proved to be beneficial in their intended action having the added value of being of natural sources and minimal side effects when compared to synthetic medications. Results illustrated in (table 1) and (fig. 1) showed that administration of P-407 induced significant elevation ($P<0.05$) in serum levels of TC by ≈ 4 folds, TG by ≈ 8 folds, HDL-C by ≈ 1.7 fold, LDL-C levels by ≈ 6 folds and VLDL-C levels by ≈ 8 folds as compared to a negative control group. The results also proved that group 8 showed a more effective anti-hyperlipidemic effect than group 7 and all the individually administered extracts. Administration of dose B of the tested combination (group 8) showed a significant ($P<0.05$) decrease in TG and LDL-C when compared to both a positive control and the anti-hyperlipidemic control group. It led to a more effective decrease in the lipid profile in all the measured parameters ranging from 10 to 15% more than that caused by atorvastatin reference. In addition to a significant difference seen in the TC and LDL level compared to the hyperlipidemic model. Although the administration of each component individually showed a promising anti-hyperlipidemic effect especially in case of TG and VLDL, the co-administered doses in group 8 surpassed the effect of each component delivered individually causing 54%, 72.5%, 51.2% and 72.5% reduction in TC, TG, LDL-C and VLDL-C levels respectively when compared to a positive control group. Moreover, TG, VLDL-C levels of this group were significantly decreased by 25.9 % and 25.9 %, respectively surpassing the effect of atorvastatin used as a positive standard drug. This may be explained by a synergistic effect of all the included components acting by a multi mechanism of action, including decreased intestinal fat absorption and/or decreased lipid

biosynthesis and/or enhanced cholesterol elimination of ginger [17, 24]. In addition to the effect of sulphur amino acid compounds known to lower all the measured markers in the lipid profile [25]. The viscosity of the fermentable dietary fibers in gum arabic also contributes by producing an acidic PH during its fermentation process in the intestine to promote lipid excretion in stool [26]. Based on these results, such combination can be used alone as prophylactic management to hyperlipidemia as phytopharmaceutical, or as a booster to known anti-hyperlipidemic drugs, Where treating dyslipidaemia requires lifelong use of anti-hyperlipidemic drugs.

Table 1: Effect of different extracts of edible parts of ginger, leek and GA and their combinations versus atorvastatin on lipid pattern in P-407 induced hyperlipidemic rats

Groups Parameter	Negative control	Hyperlipidemic control	Atorvastatin	Ginger	leek	Gum arabic	Dose A	Dose B
TC	90.8±3.5	390.3±24.2	227.5±13.9^{ab}	230.4±18.4^{ab}	245.3±24.1^{ab}	238.2±27.3^{ab}	277.7±6.4^{ab}	179.3±13.6^{ab}
TG	65.1±5.5	509.4±34.9	189.1±11.6^{ab}	156.5±14.8^{ab}	176.8±19.2^{ab}	169.9±8.8^{ab}	208±8.5 ^{ab}	140.1±11.4^{abc}
HDL-C	42.8±1.9	73.3±3.9	52.2±4.6^{b}	69.5±3.2^{ac}	75.8±3.6^{ac}	85.2±4.7^{abc}	42±4.8 ^{b}	45.7±3.6^{b}
LDL-C	35±3.2	215.1±21.4	137.5±9^{ab}	129.6±15.2^{ab}	134.1±22.6^{ab}	119±28.9^{ab}	194.1±8.9^{ac}	105.6±9.5^{ab}
VLDL-C	13±1.1	101.8±6.9	37.8±2.3^{ab}	31.3±2.9^{ab}	35.3±3.8^{ab}	33.9±1.8^{ab}	41.6±1.7^{ab}	28±2.3^{abc}

TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, VLDL-C: Very low-density lipoprotein-Cholesterol, all parameters were measured in (mg/dl) Results are expressed as mean±SEM N=8 animals in each group, [a]Significant difference from the negative control group at P<0.05, [b]Significant difference from the hyperlipidemic control group at P<0.05, [c]Significant difference from Atorvastatin standard Control group at P<0.05

Table 2: Tentative identification of components detected in *Zingiber officinale* R. extract using LC/MS analysis

Peak no.	R. T* (min)	(-)ESI-MS (*m/z*)	(+)ESI-MS (*m/z*)	MWt**	Identified compounds	Ref.
1	0.74	N. D	203	202	α-Curcumene	[29]
3	6.81	N. D	153	152	Neral	[29]
4	7.84	195	N. D	196	Zingerol	[30]
5	8.38	377	N. D	378	12-Gingerol	[31]
6	8.51	N. D	197	196	3-(3',4'-Dihydroxy-5'-methoxyphenyl)-1-propanal	[30]
7	10.52	389	N. D	390	5-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone	[32]
8	10.81	389	N. D	390	3-acetoxy-5-hydroxy-1,7-bis(3,4-dihydroxyphenyl)heptane	[32]
9	11.49	405	N. D	406	3,5-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane	[32]
10	11.74	285	287	286	Kaempferol	[33-35]
11	11.97	N. D	137	136	β-Phellandrene	[29]
12	12.85	357	N. D	358	Matairesinol	[36]
14	13.71	431	N. D	432	3,5-diacetoxy-1,7-bis(3,4-dihydroxyphenyl)heptane	[32]
15	13.89	505	N. D	506	3,5-diacetoxy-7-(3,4-dihydroxy-5-methoxyphenyl)-1-(4-hydroxy-3,5-dimethoxyphenyl)heptane	[32]
16	14.09	355	379	356	1,7-bis-(4'-Hydroxy-3'-methoxyphenyl)-4-heptene-3-one	[37]
17	14.76	401	N. D	402	2E-Geranial acetal of 4-Gingerdiol	[30]
18		445	N. D	446	3,5-diacetoxy-7-(3,4-dihydroxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)heptane	[32]
19	15.28	293	317	294	6-Gingerol	[30]
20	15.79	359.3	N. D	360	12-Shogaol	[31]
21	15.92	459	N. D	460	meso and (3S,5S)-3,5-Diacetoxy-1,7-bis-(4'-hydroxy-3'-methoxyphenyl)heptane	[30]
25	16.78	N. D	219	218	(±)-(E)-Nuciferol	[29]
26	16.88	375	N. D	376	12-Gingerdione	[30]
28	17.68	275	277	276	6-Shogaol	[37, 38]
33	18.69	387	N. D	388	1,7-bis-(4'-Hydroxy-3'-methoxyphenyl)-5-methoxyheptan-3-one	[30]
34	18.95	N. D	403	402	12-gingediol	[38]
35	19.43	289	291	290	Methyl 6-shogaol	[37]
36	19.76	N. D	319	318	Methyl (E)-8-shogaol	[30]
37	19.88	N. D	335	334	10-Paradol	[30]
38	20.25	265	N. D	266	4-gingerol	[38]
39	20.34	349	N. D	350	10-gingerol	[38]
40	20.60	N. D	327	326	Methyl Icosanoate	[36]
41	20.72	N. D	373	372	1,7-bis-(4'-Hydroxy-3'-methoxyphenyl)-3,5-heptadione	[30]
42	21.09	295	N. D	296	6-gingerdiol	[37, 38]
43	21.26	415	N. D	416	1-(4'-Hydroxy-3'-methoxyphenyl)-2-nonadecen-1-one	[30]
44	21.26	N. D	205	204	α-Zingiberene	[29]
46	21.62	N. D	205	204	β-sesquiphellandrene	[29]
49	22.11	N. D	387	386	1-(4'-Hydroxy-3',5'-dimethoxyphenyl)-7-(4'-hydroxy-3'-methoxyphenyl)-4-hepten-3-one	[30]
51	22.51	N. D	333	332	10-Shogaol	[30]
52	22.69	293	N. D	294	1-Hydroxy-6-paradol	[30]
60	24.16	277	279	278	6-Paradol	[37]
61	24.28	N. D	377	376	3,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane	[32]
63	24.61	N. D	205	204	α-Farnesene	[29]
64	25.01	N. D	353	352	10-Gingerdiol	[37]
65	25.19	279	N. D	280	6-Dihydroparadol	[37]
66	25.28	N. D	353	352	5-Acetoxy-7-gingerdiol	[37]
67	25.58	N. D	309	308	Methyl 6-gingerol	[30]
71	26.26	N. D	409	408	Diacetoxy-8-gingerdiol	[30]
73	26.50	N. D	409	408	10-Gingerdiol, cyclic methyl ortho ester	[30]

76	26.88	N. D	307	306	8-Paradol	[30]
79	27.35	N. D	381	380	Diacetoxy-6-gingerdiol	[37]
80	28.06	N. D	309	308	Acetoxy-4-gingerol	[30]
81	28.34	N. D	437	436	3,5-dihydroxy-1,7-bis (4-hydroxy-3,5-dimethoxyphenyl)heptane	[32]
82	28.48	N. D	297	296	6-Gingerdiol	[37]
84	28.79	N. D	311	310	5-Acetoxy-4-gingerdiol	[30]
85	29.10	N. D	371	370	dihydrocurcumin	[32]
93	30.89	N. D	223	222	β-Selinenol (β-Eudesmol)	[29]
95	31.12	N. D	223	222	α-Trans-sesquicyclogeraniol	[29]

**MWt. = molecular weight, *RT= retention time

LC-ESI-MS/MS analysis

An overall of 56 compounds was tentatively identified in the ethanol extracts of ginger (root and rhizome) (table 2). Four of the major constituents were subjected to further MS^2 fragmentation (table 3), showing the characteristic fragmentation pattern of gingerol related compounds with a base peak at *m/z* 137 correspondings to the benzylic cleavage product associated with the 4'-hydroxy-3'-methoxy benzyl cation in all four compounds. 6-gingerol being the major constituent at *m/z* 295 $[M+H]^+$.

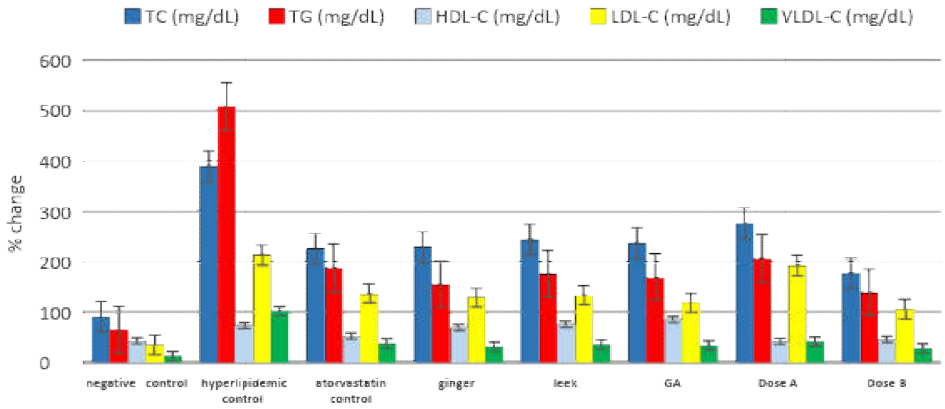

Fig. 1: Percentage change of TC, TG, HDL-C, LDL-C and VLDL-C for all tested samples against atorvastatin control group in P-407 induced hyperlipidemic rats, Dose A =ethanol extract of ginger 200 mg/kg bw, leek 250 mg/kg bw and 30% aqueous solution of gum arabic 3.75 g/kg bw, Dose B= ethanol extract of ginger 400 mg/kg bw, leek 500 mg/kg bw and 30% aqueous solution of gum Arabic 7.5 g/kg bw, Results are expressed as mean±SEM n=8

Table 3: MS^2 fragmentation pattern of four major constituents of *Zingiber officinale* R

Cpd No	Peak No.	Name	(-)ESI-MS(*m/z*)	(+)ESI-MS(*m/z*)	MWt	Fragmentation *m/z*	Reference or/Mass bank N0
1	19	6-Gingerol	293	295	294	194, 177, 277, 137	[27] ID: CO000213
2	28	6-Shogaol	275	277	276	137,159,179	[27]
3	51	10-Shogaol	N. D	333	332	137,123	[27]
4	65	6-Dihydroparadol	279	N. D	280	279, 138	[28]

MWt. = molecular weight, ND= not detected, cpd= compound

Table 4: Tentative identification of components detected *Allium ampeloprasum* var. porrum (L.) using LC/MS analysis

Peak no.	R. T* (min)	(-)ESI-MS (m/z)	(+)ESI-MS (m/z)	MWt**	Compounds Identified	Ref.
1	0.75	N. D	175	174	Arginine	[39]
2	0.83	149	N. D	150	Dipropyl disulfide	[40]
3	1.03	150	N. D	151	Methiin	[41]
4	1.35	130	132	130	leucine	[39]
5	2.09	164	166	165	Ethiin	[41]
7	4.12	181	N. D	182	Dipropyl trisulfide	[40]
8	7.20	431	N. D	432	Apigenin-7-glucoside	[40]
9	8.46	N. D	197	196	Hydroferulic acid	[42]
10	8.64	112.9	N. D	114	Diallyl sulfide	[40]
11	9.48	463	N. D	464	quercetin-3-glucoside	[43; 44]
12	9.62	178	N. D	179	propiin	[41]
13	10.32	N. D	284	283	N-*p*-coumaroyltyramine	[45]
14	10.48	447	449	448	Astragaline	[42; 44]
16	11.74	285	287	286	Luteolin	[42; 45]
17	12.13	N. D	181	180	Caffeic acid	[42; 44]

19	13.24	285	287	286	kaempferol	[42; 45]
21	18.24	N. D	273	272	Naringenin	[42; 44]
22	18.69	N. D	343	342	sucrose	[39]
24	20.11	N. D	317	316	Isorhamnetin, Rhamnetin	[17]
26	20.39	N. D	257	256	palmitic acid	[40]
27	20.72	N. D	355	354	Chlorogenic acid	[44]
28	21.26	295	297	296	Phytol	[40]
29	21.46	577	N. D	578	Apigenin-7-*O*-neohespiroside	[39]
32	22.44	N. D	537	536	kampherol dervatives	[39]
33	22.61	579	N. D	580	Naringin	[47]
38	23.81	459	N. D	460	(25*R*)-5α-spirostane-3β,6β-diol-2,12-dione (Porrigenin C)	[46, 47]
40	24.02	N. D	225	224	Sinapenic acid	[42, 44]
41	24.16	277	279	278	α-linolenic acid	[48]
42	24.40	N. D	499	498	Kaempferol hexose	[39]
46	25.17	279	N. D	280	linoleic acid	[48]
47	25.44	N. D	303	302	Quercetin	[42; 44]
48	25.54	N. D	303	302	Hesperetin	[42]
52	26.52	N. D	314	313	N-feruloyltyramine	[46]
57	28.29	N. D	271	270	Apigenin	[44]
59	28.58	N. D	285	284	Acacetin	[46]
61	29.02	N. D	536	535	Kaempferol deoxyhexose	[39]
63	29.47	N. D	611	610	Rutin	[44]
64	29.85	N. D	684	683	Kaempferol deoxyhexose	[39]
69	30.60	N. D	505	504	kestose	[39]
70	30.68	N. D	535	534	Kaempferol malonyl hexose	[39]
71	30.79	N. D	603	602	Kaempferol derivative	[39]
73	31.15	197	199	198	Syringic acid	[44]

**MWt. = molecular weight. *RT= retention time

Table 5: MS^2 Fragmentation pattern of four major constituents of *Allium ampeloprasum* var. porrum (L.)

Cpd No.	Peak No.	Name	(-)ESI-MS (*m/z*)	(+)ESI-MS (*m/z*)	MWt	Fragmentation *m/z*	Reference/Mass bank ID No
1	5	Ethiin	164	166	165	28,42,77,79,103,118,120	ID: HMDB0029432
2	11	Quercetin glucoside	463	N. D	464	151,243,255, 271, 299,300,301	ID: PR100677
3	14	astragaline	447	449	448	107,121,153,165,287	PR100243
4	19	kaempferol	285	287	286	107,121,133,153,165, 181,269,287	ID: PB004123

MWt. = molecular weight, ND= not detected, Results of HPLC quantitative analysis of sugars after hydrolysis of the aqueous solution of gum arabic tabulated in (table 6). The major constituents were found to be galactose, arabinose and glucouronic acid.

Table 6: Sugars and uronic acids analysed by HPLC in GA after hydrolysis

Peak no.	Saccharides	RT *(min)	(%) Saccharides after hydrolysis
1	Glucuronic acid	5.141	6.60
2	Stachyose	5.442	0.20
3	Galacturonic	5.624	0.06
4	Sucrose	6.372	0.04
5	Glucose	7.425	0.63
6	Galactose	8.719	19.36
7	Arabinose	10.430	15.76
8	Manitol	14.267	0.006
9	Sorbitol	18.726	0.004

*RT= retention time

As for the leek ethanol extract, 45 compounds were tentatively identified (table 4) and four of its major constituents were further fragmented by MS^2. It is the first record for MS^2 analysis of ethiin detected as the major component in leek extract (table 5). It showed the protonated molecular ion at *m/z* 166 $[M+H]^+$. The characteristic ion fragment at *m/z* 103 was detected due to loss of both -COOH and -NH2 groups and rearranging of the parent protonated molecule.

CONCLUSION

It can be concluded that the co-administration of a group of natural extracts with each other in certain concentrations proved to be more effective than the use of synthetic drugs or the use of a single component. This may be due to the multi-mechanism of action of the extracts and/or they may be having less adverse effects. Such a combination can be used as prophylactic management or as a booster to known anti-hyperlipidemic drugs.

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AUTHORS CONTRIBUTIONS

S. A. Mina and A. M. Abd EL-Maksoud conceived and planned the experiments. H. S. Mohammed and M. A. Fouad carried out the experiments and contributed to sample preparation. S. A. Mina, A. M.

Abd EL-Maksoudand H. S. Mohammed contributed to the interpretation of the results. S. A. Mina took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

CONFLICT OF INTERESTS

Declared none

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