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Original Article

Antibacterial effect of a diet pill supplement on the human intestinal bacteria

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Abstract

Introduction: Many people worldwide attempt to lose weight or adopt strategies to control it. Some have resorted to the consumption of commercialized diet pills to achieve this goal. Multiple brands exist without clearly indicating their mechanism of action or adverse effects on human health. This study aims to determine the antibacterial effect of commercial diet pills on members of the intestinal microbiota.

Methodology: Commercialized diet pills were bought from a pharmacy in the North of Lebanon. Broth microdilution test was performed to determine the Minimum Inhibitory Concentrations (MICs) of the aqueous suspension against forty-two isolates distributed into four *Enterobacterales* species. MIC of the digested form was determined against six different strains. GC-MS analysis was performed to elucidate the components of the diet pill compared to the manufacturer's list.

Results: Broth microdilution results revealed that MICs of the diet pill aqueous suspension ranged from 3.9×10^3-9.76×10^2 µg/mL for *Escherichia coli, Enterobacter* spp., and *Proteus* spp. For *Klebsiella* species, MIC of carbapenem-resistant isolates reached 1.95×10^3 µg/mL. The digested form had a significantly lower antibacterial effect compared to the aqueous suspension. GC-MS analysis results corresponded with the list of ingredients provided by the manufacturer.

Conclusions: The results showed significant antibacterial activity of a commercial diet pill on different members of the human intestinal microbiota regardless of their resistance profile. Further work is needed to elucidate the antibacterial effect of the digested components to accurately understand their effect on the intestinal microflora and thus on human health.

Key words: Diet pills; MIC; microbiota; *Enterobacterales*.

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Introduction

Obesity is a worldwide public health concern that may lead to the development of many serious illnesses such as Type 2 diabetes, hypertension, chronic respiratory diseases, heart diseases, strokes, and some types of cancer [1,2]. Lifestyle changes including increasing physical activity and lowering the intake of calories are the ideal way of losing weight [1]. However, since these practices are challenging and need consistent effort and persistence, people tend to seek alternatives. These include inducing vomiting, intermittent fasting, or taking diuretics, laxatives, and weight loss supplements (diet pills) [3-5].

Most diet pills are commercialized as being of natural or herbal origin and free from any chemical additives and hence are safe to be used [6]. A study in Mexico reported that 69.5% of obese/overweight people have used a dietary supplement to support weight reduction during their lifetime [7]. Alonso-Castro *et al.* (2019), claim that the prevalence of self-medication with herbal products to lose body weight ranges from 10% to 98% worldwide [8]. Weight-loss products marketed as dietary supplements are sometimes adulterated or tainted with prescription-drug ingredients or untested pharmaceutically active ingredients that could be harmful [6]. A strong caution against the usage of diet pills in adolescents regardless of their weight was recommended by the American Academy of Pediatrics. The Center for Disease Control and Prevention conducted a study and found that around 23,000 emergency visits/year in the US are attributed to illnesses caused by dietary supplements, with a third of them being adverse effects of weight loss products consumption [9].

Diet and medication are the main factors, besides genetics, that determine the composition and diversity

of the gut microbiota and impact the immune system and metabolic stability. These elements combined affect the overall health of the gut and its influence on the body [10]. Members of the *Enterobacterales* family including *Escherichia coli*, *Klebsiella pneumoniae,* and *Proteus spp.* are natural inhabitants of the human intestinal microbiota [11-13]. *Escherichia coli* (*E. coli)* is among the first bacteria to colonize the human gut at birth. Being a facultative anaerobe, *E. coli* assists in oxygen depletion along the gastrointestinal mucosal surface, creating thereby a hospitable environment for the colonization of other important bacteria such as anaerobes [14]. Moreover, the intestinal microbiota is involved in vital functions inside the human body. These include vitamin synthesis, drug, and nutrient metabolism, suppression of pathogenic organism colonization, and maintenance of the host energy homeostasis [15,16]. Antibiotics can affect the intestinal microbiota by disturbing the competitive exclusion machinery resulting in the growth of pathogens such as *Clostridium difficile* [15]. In addition, antibiotics allow the selection of multi-drug resistant (MDR) organisms such as extended-spectrum beta-lactamases (ESBL) and carbapenem-resistant *Enterobacterales* (CRE) [17]. The aim of this study is to explore the antibacterial activity of a commercialized diet pill on the common inhabitants of the human intestinal microflora. A systematic analysis of the diet pill composition will be also performed to verify its composition.

Methods

Diet pill description

A commercialized diet pill supplement was purchased from a pharmacy in the North of Lebanon. The pill was composed of a dry powder enclosed within a capsule. According to the manufacturer, based in Lebanon, this pill contains acai berry extract, resveratrol, *Garcinia cambogia* extract, green coffee beans extract, green tea extract, ginger, and dandelion.

Bacterial strains

A total of 42 isolates including ten *Escherichia coli*, eleven *Klebsiella* spp., eleven *Proteus* spp., and ten *Enterobacter* spp. were isolated from the feces of several Lebanese healthy donors and patients (UOB Bacterial collection). Bacteria were previously cultured on selective (Mac Conckey agar) and non-selective (Blood agar and Mueller Hinton agar) media for isolation and identification and preserved in 40% glycerol aliquots at -80 °C for further testing.

Bacterial subculture

Using a sterile loop, a volume of 10 µL was taken from each aliquot and streaked using the four-quadrant technique on a Blood agar and Mueller Hinton agar plates to obtain well-isolated pure colonies. Plates were then incubated overnight at 37 °C, to be used the next day.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of collected strains was performed using the Kirby-Bauer disc diffusion method [18]. Twelve antibiotics were used: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cefuroxime (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), ertapenem (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), and trimethoprim-sulfamethoxazole (25 µg). According to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2017, the diameter zones of inhibition were interpreted [19]. Phenotypic detection of extended-spectrum beta-lactamase (ESBL) production was done using the double disk synergy test by laying a disk of amoxicillin-clavulanic acid on the surface of Muleller Hinton agar surrounded by ceftazidime, cefepime and aztreonam disks (at 3 cm distance center to center). A positive test was considered when observing a "keyhole effect". The detection of plasmidic AmpC beta-lactamase (pAmpC) was performed using the Cefoxitin disk test, as described previously [20]. Isolates that lack resistance to beta-lactam antibiotics via the three major mechanisms of resistance (ESBL, pAmpC, carbapenem resistance) and are susceptible to ciprofloxacin or trimethoprim-sulfamethoxazole are considered as sensitive isolates.

Diet pill in vitro digestion procedure

The antibacterial activity of the diet pill's metabolites was evaluated through an *in vitro* digestion method that is a simulation of *in vivo* digestion. This procedure was performed as described by Sabah *et al.* (2021) [21] with modifications from Ryan *et al.* (2010) [22]. Briefly, the diet pill powder was first treated with α-amylase to mimic oral digestion, followed by treatment with porcine pepsin adjusted to pH 2 for gastric digestion. Bile salts and pancreatin were then added to the solution before adjusting the pH to 7.4. The products were then tested for antibacterial activity.

Minimum inhibitory concentration determination

The minimum inhibitory concentration was determined using the broth micro-dilution technique as

per the CLSI guidelines 2017 [19]. Aqueous suspension of the diet pill powder and digested products "P-Final" were serially diluted in broth and a bacterial inoculum was then added. The concentration in the first tube for the diet pill aqueous suspension was 3.1×10^4 µg/mL; whereas for the digested product, the concentration in the first tube was 6.24×10^4 µg/ml. A positive control containing Brain Heart Infusion broth and bacteria and a negative control containing only the broth was also used. The microdilution tubes were then placed in the incubator at 37 °C for 24 hours. The MIC was considered equivalent to the concentration of the product in the first tube that does not show any turbidity (associated to the lowest concentration of the product able to inhibit bacterial growth). Turbidity was assessed by the comparison of the different tubes with the positive and negative controls for growth. Statistically, the values of MIC_{50} and MIC_{90} were calculated using the 50th and 90th percentile, respectively. These values represent the MIC at which ≥ 50% and ≥ 90% of the bacterial species are inhibited, respectively.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

All analyzed products were solid powders encapsulated, and each capsule was emptied. Every 100

Table 1. Resistance profiles of bacterial isolates.

	AMP	AUG	CXM	FOX	CTX	CAZ	FEP	AZT	ERT	IMP	CIP	SXT	Phenotype
E. coli-1	R	S	R	S	R	R	R	R	S	S	R	R	ESBL/QR/STR
E. coli-2	R	R	R	R	R	R	S	R	S	S	R	S	pAmpC/ESBL/QR
E. coli-3	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
E. coli-4	R	S	R	S	R	S	S	S	S	S	R	S	ESBL/QR
E. coli-5	R	S	R	S	R	S	S	S	S	S	R	R	ESBL/QR/STR
E. coli-6	R	R	R	R	R	R	R	R	S	S	S	R	pAmpC/ESBL/STR
E. coli-7	S	S	S	S	S	S	S	S	S	S	S	R	STR
E. coli-8	R	R	R	S	S	S	S	S	S	S	S	S	Sensitive
E. coli-9	R	R	R	S	R	R	R	R	S	S	R	R	ESBL/QR/STR
E. coli-10	R	R	S	S	S	S	S	S	S	S	S	S	Sensitive
K. pneumonia-1	R	R	R	R	R	R	R	S	R	R	R	R	CRE/QR/STR
K. pneumonia-2	R	S	R	S	R	S	S	R	S	S	S	R	ESBL/STR
K. pneumonia-3	R	R	R	R	R	R	R	R	R	R	R	R	CRE/QR/STR
K. pneumonia-4	R	R	R	R	R	R	R	R	R	R	S	S	CRE
K. pneumonia-5	R	R	R	R	R	R	R	R	R	R	R	R	CRE/QR/STR
K. pneumonia-6	R	S	R	S	R	R	R	R	S	S	S	S	ESBL
K. pneumonia-7	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
K. pneumonia-8	R	R	R	R	R	R	R	R	S	S	S	S	ESBL/pAmpC
K. oxytoca-1	R	S	S	S	S	S	S	S	S	S	S	S	Sensitive
K. oxytoca-2	R	S	S	S	S	S	S	S	S	S	S	S	Sensitive
K. oxytoca-3	R	R	R	R	R	R	S	R	S	S	S	R	pAmpC/STR
Proteus spp-1	R	S	S	S	S	S	S	S	S	S	R	S	QR
Proteus spp-2	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
Proteus spp-3	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
Proteus spp-4	R	S	R	S	S	S	S	S	S	S	R	R	QR/STR
Proteus spp-5	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
Proteus spp-6	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
P. mirabilis-1	R	S	R	S	S	S	S	S	S	S	R	R	QR/STR
P. mirabilis-2	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
P. mirabilis-3	S	S	S	S	S	S	S	S	S	S	S	S	Sensitive
P. mirabilis-4	S	S	S	S	S	S	S	S	S	S	S	R	STR
P. vulgaris	R	R	R	S	S	R	S	S	S	S	R	S	QR
E. cloacae-1	R	R	S	R	S	S	S	S	S	S	S	S	Sensitive
E. cloacae-2	R	R	R	R	R	S	S	S	S	S	R	S	pAmpC/QR
E. cloacae-3	R	R	R	R	R	R	R	R	S	S	S	S	ESBL/pAmpC
E. cloacae-4	R	R	R	R	R	R	R	R	S	S	S	R	pAmpC
E. cloacae-5	R	R	S	R	S	S	S	S	S	S	S	R	STR
E. cloacae-6	R	R	R	R	R	R	S	R	S	S	S	R	pAmpC/STR
E. cloacae-7	R	R	R	R	R	R	S	R	S	S	S	S	pAmpC
E. asburiae	R	R	S	R	S	S	S	S	S	S	R	R	QR/STR
E. aerogenes	R	R	R	R	R	R	R	R	R	R	R	R	CRE/QR/STR
Enterobacter spp	R	R	S	R	S	S	S	S	S	S	R	R	QR/STR

AMP: ampicillin; AUG: amoxicillin-clavulanic acid; CXM: cefuroxime; FOX: cefoxitin; CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; AZT: aztreonam; ERT: ertapenem; IMP: imipenem; CIP: ciprofloxacin; SXT: trimethoprim-sulfametoxazole; QR: quinolones resistant; STR: sulfonamide trimethoprim resistant; R: resistant; S: susceptible.

Table 2. MIC of a commercial diet pill on *Enterobacterales* isolates used in this study.

Species	Resistance	MIC µg/ml	MIC 90	Species	Resistance	MIC µg/ml	MIC 90	Species	Resistance	MIC µg/ml	MIC 90	Species	Resistance	MIC µg/ml	MIC 90
E. coli-1	E/QR/STR	1.95×10^3		*K. pneumoniae*-1	CRE/QR/STR	1.95×10^3		*Proteus spp.*-1	QR	1.95×10^3		*E. cloacae*-1	S	1.95×10^3	
E. coli-2	E/pA/QR	1.95×10^3		*K. pneumoniae*-2	E/STR	1.95×10^3		*Proteus spp.*-2	S	9.76×10^2		*E. cloacae*-2	Pa/QR	1.95×10^3	
E. coli-3		3.90×10^3		*K. pneumoniae*-3	CRE/QR/STR	1.95×10^3		*Proteus spp.*-3		9.76×10^2		*E. cloacae*-3	E/Pa	3.90×10^3	
E. coli-4	E/QR	3.90×10^3		*K. pneumoniae*-4	CRE	1.95×10^3		*Proteus spp.*-4	QR/STR	9.76×10^2		*E. cloacae*-4	pA	3.90×10^3	
E. coli-5	E/QR/STR	3.90×10^3	3.90×10^3	*K. pneumoniae*-5	CRE/QR/STR	1.95×10^3	1.95×10^3	*Proteus spp.*-5		9.76×10^2	1.95×10^3	*E. cloacae*-5	STR	3.90×10^3	3.90×10^3
E. coli-6	E/pA/STR	4.88×10^2		*K. pneumoniae*-6	E	2.44×10^2		*Proteus spp.*-6	S	9.76×10^2		*E. cloacae*-6	pA/STR	4.88×10^2	
E. coli-7	STR	4.88×10^2		*K. pneumoniae*-7	S	4.88×10^2		*P. mirabilis*-1	QR/STR	1.95×10^3		*E. cloacae*-7	pA	9.76×10^2	
E. coli-8	S	9.76×10^2		*K. pneumoniae*-8	E/Pa	9.76×10^2		*P. mirabilis*-2	S	9.76×10^2		*E. asburiae*	QR/STR	1.95×10^3	
E. coli-9	E/QR/STR	9.76×10^2		*K. oxytoca*-1	S	4.88×10^2		*P. mirabilis*-3	S	9.76×10^2		*E. aerogenes*	CRE/QR/STR	1.95×10^3	
E. coli-10	S	9.76×10^2		*K. oxytoca*-2	S	4.88×10^2		*P. mirabilis*-4	STR	9.76×10^2		*Enterobacter spp.*	QR/STR	3.90×10^3	
				K. oxytoca-3	pA/STR	9.76×10^2		*P. vulgaris*	QR	1.95×10^3					

E: ESBL; pA: pAmpC; E/pA: ESBL/pAmpC; CRE: carbapenem resistant *Enterobacteriaceae*; QR: quinolones resistant; STR: sulfonamide trimethoprim resistant; S: sensitive.

mg of fine powder was mixed with 1 mL of absolute methanol. Samples were mixed thoroughly by vortexing, followed by 15 minutes of sonication and 5 minutes of centrifugation at 4000 rpm. The supernatant was collected and filtered by 0.2 µm membrane filters for GC-MS analysis. GC-MS system consists in 6890 GC, 7000 TripQuade from Agilent, capillary column DB5-MS (30m × 0.25 mm id × 0.25 µm film thickness). A 1 µL injection using splitless mode was performed on a 280 C injector port with helium flow at 1 mL/min. The oven ramping temperature was held at 150 °C for 20 min, then increased at 10 °C/min to 280 °C and left for 15 minutes. The screening was performed on selected ion monitoring mode at m/z 58, 72, 114 (sibutramine), while identification was done on full scan mode (40-500 a.m.u). The spectra obtained for the compounds were compared to the spectra of known compounds, using the NIST Library [23].

Results

Resistance pattern of collected Enterobacterales isolates

Out of 42 strains, phenotypic testing showed that 13 strains (31%) were sensitive, 5 strains (12%) were carbapenem-resistant and 14 strains (33%) were ESBL and/or ampC beta-lactamase producers. For non-beta lactam antibiotics, 38% and 45% of collected strains were resistant to ciprofloxacin and trimethoprim-sulfamethoxazole, respectively (Table 1).

MIC of the aqueous diet pill suspension

MIC ranging from 1.95E + 03 µg/mL to 9.76E + 02 µg/mL was obtained for all tested species. No correlation between resistance phenotype and MIC, nor MIC significant difference between different species was observed (Table 2). The minimum inhibitory concentration that inhibits 90% of the tested organisms, $MIC_{90,}$ was 3.90E + 03 µg/mL for *E. coli* and *Enterobacter spp.* MIC_{90} was almost reduced by half, 1.95E + 03 µg/mL, for *Klebsiella* and *Proteus spp.*

MIC of the diet pill-digested products

Broth microdilution testing using a digestion product was performed against two strains of *E. coli*, two strains of *Klebsiella spp.,* and one strain of both *Proteus mirabilis* and *Enterobacter cloacae* (Table 3). Compared to the aqueous suspension of the diet pill, digested products showed a significantly lower antibacterial activity against the six strains tested with MIC ranging from 1.95E + 03 µg/mL to 7.80E + 03 µg/mL.

Table 3. MIC of the diet pill digested products on selected isolates of *Enterobacterales*

Species	Resistance	MIC using P-Final µg/mL	MIC using Diet Pill µg/mL
E. coli-4	E/QR	7.80×10^3	3.90E + 03
E. coli-7	STR	7.80×10^3	4.88E + 02
K. pneumoniae-3	CRE/QR/STR	7.80×10^3	1.95E + 03
K. oxytoca-1	S	7.80×10^3	4.88E + 02
P. mirabilis-4	QR/STR	1.95×10^3	9.76E + 02
E. cloacae-7	pA	3.90×10^3	9.76E + 02

E: ESBL; pA= pAmpC; CRE: carbapenem resistant Enterobacteriaceae; QR: quinolones resistant; STR: sulfonamide trimethoprim resistant; S: sensitive.

Gas Chromatography-Mass Spectrometry

Results of the GC-MS analysis are presented in Table 4 and Figure 1. Gingerol, zingerone, and zingiberene that are active components of ginger were found. Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S- (R*, S*)]-, found in green and black tea were also detected. Other constituents common to sunflower plants such as heptacosane were also observed. This could be related to the sunflower "dandelion" ingredient as per the manufacturer's list. Furthermore, several different fatty acids were also detected such as ethyl iso-allocholate and squalene.

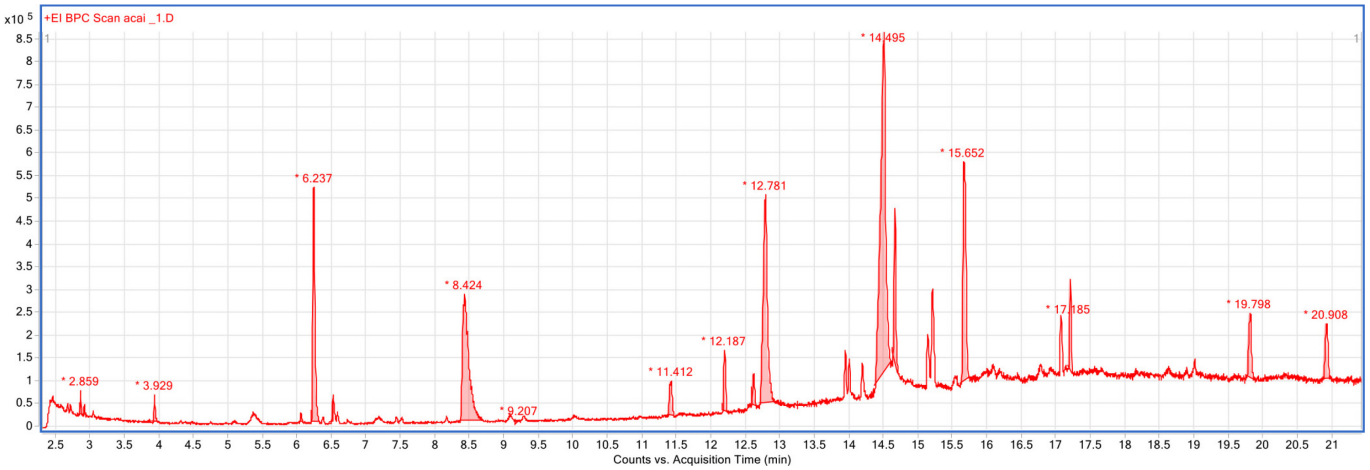
Discussion

The mucosal surface of the human gastrointestinal (GI) tract is about 200–300 m^2 and is colonized by $10^{13–14}$ bacteria of 400 different species and subspecies [24]. An extensive catalog of the functional capacity of the human gut microbiome was recently obtained, where 9,879,896 genes were identified through a combination of 249 newly sequenced and 1,018 published samples [25]. In the same study, Li *et. al* (2014) identified country-specific microbial signatures, suggesting that gut microbiota composition is shaped by environmental factors, such as diet, and possibly host genetics [25]. With the advancement in molecular technology, together with the next generation sequencing, the

Table 4. GC-MS analysis results of the diet pill used in this study.

RT	Area	Area Sum%		
6.24	4035284	3.56	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	
6.371	2559947	2.26	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	Zingiberene
6.521	635892	0.56	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	Bisabolene
6.732	691270	0.61	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	
8.406	4889312	4.31	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	Zingerone
12.606	4543774	4.01	1,2-Benzenedicarboxylic acid, butyl decyl ester	
12.792	3392495	2.99	n-Hexadecanoic acid	
13.935	775180	0.68	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	
13.993	139051	0.12	11-Octadecenoic acid, methyl ester	
14.182	1555394	1.37	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	
14.448	5800326	5.11	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid
14.666	1804579	1.59	Ethyl iso-allocholate	
15.132	1100762	0.97	Cyclohexanone, 2-(2-nitro-2-propenyl)-	
15.201	1791906	1.58	Hesperidin	
15.66	7582404	6.68	Gingerol	
17.218	6.4E + 07	56.62	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	
18.99	2719263	2.4	14β-Pregnane-3,12,15,20-tetrone, 14-hydroxy-	
19.805	3149135	2.78	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	All-trans-Squalene
20.915	2041287	1.8	Heptacosane	

Figure 1. GCMS analysis of the diet pill used in this study.



Y axis represents the intensity of the signal, while the X axis is expressed in minutes that is. when this signal was released.

human microflora continues to be unraveled [26]. Lloyd-Price *et al*. (2017) reported 1631 new metagenomes derived from 265 individuals [27]. In this study, the effect of diet pills on fecal isolates, members of the *Enterobacterales,* and common inhabitants of the intestinal microbiota, were studied. These species are, not only among the most commonly isolated organisms from human infections but are also associated with a high level of antibiotic resistance [17]. It is well known that most urinary tract infections, as well as many blood-borne infections, are generated from the intestinal tract, whether by internal or external transfer from one organ to another. In addition, the presence of Gram-negative bacteria, members of the *Enterobacterales*, in the intestines should be always kept "under control", and this is guaranteed by a healthy microbiota. Their overgrowth is largely associated with a significant loss of "beneficial" flora and might lead to many issues, mainly in specific populations of patients [28]. In this context, it is important to see how their growth will be affected in patients using diet pills as a mean to control their weight.

In this study, the commercialized diet pill had an antibacterial effect on the four bacterial species tested. Antibiotic susceptibility testing as well as the phenotypic detection of ESBL was done according to the CLSI guidelines. Our findings indicate that there is no correlation between the elevated MICs and high levels of antibiotic resistance in the tested isolates. This result suggests that the mechanism of action by which these pills work is distinct from that of standard antibiotics to which the isolates are resistant. In other words, the diet pills do not work in the same way as the antibiotics that the tested isolates are resistant to, and thus their efficacy does not appear to be affected by the presence of antibiotic resistance. These findings are important for understanding how these pills can be used as a complementary or alternative treatment option to traditional antibiotics in the fight against bacterial infections.

The disturbance of the gut microbiota could arguably be one additional mechanism by which these pills promote weight loss. Backhed *et al*. (2004) demonstrated that mice raised with no microorganisms in their gastrointestinal system were 40% leaner than those that had normal gut microbiota [29]. *Ganoderma lucidum*, a potential anti-obesity medicinal mushroom has been shown to decrease body weight by reducing endotoxin-bearing Proteobacteria and the ratio of Firmicutes/Bacteroidetes; this reduction leads to lowered metabolic endotoxemia while preserving the intestinal barrier integrity [30,31]. The dominant phyla in the human gut microbiota are the firmicutes which are composed of Gram positives. Other dominant phyla include Bacteroidetes, Proteobacteria (Gram-negatives), and Actinobacteria which all together make up over 97% of the gut microbial population [32]. Disrupting this bacterial ecosystem can have adverse effects on human health. Indeed, several studies have linked disturbed gut microbiota to cirrhosis, cancer, cardiovascular, and neurodevelopmental diseases [33]. For example, it is well known that hypertension is among the most common risk factors for cardiovascular diseases. In hypertensive patients, it was found that microbial diversity is significantly decreased and the ratio of Firmicutes/Bacteroidetes is increased [34].

In this study, the diet pill components obtained by GC-MS analysis correspond to the ingredients listed by the diet pill's manufacturer. A high number of different fatty acids were also found in the mixture which we suspect were obtained from the Acai Berry extract and separated into different molecules due to the preparation process. Although with moderate activity, Acai berry extract has been reported to exert an antibacterial effect against isolates of *K. pneumoniae*, *Pseudomonas aeruginosa,* and *P. mirabilis* [35]. The active components of ginger, zingerone, and zingiberene, were also detected by GC-MS. Al-Daihan *et al.* (2013) showed that *Zingeber officinale* (*Z. officinale*), commonly known as ginger has a strong antibacterial activity. The methanolic extracts showed stronger antibacterial activity than the aqueous extracts from these plants. The disk diffusion method results showed that the methanolic extract of *Z. officinale* had antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *E. coli*, and *P. aeruginosa* [36,37]. Another study that evaluated the antibacterial effect of *Zingiber officinale* on several species including *K. pneumoniae*, *P. aeruginosa,* and *E. coli*, found that *Zingiber officinale* has a remarkable antimicrobial activity that is mainly due to napthalenamine, decanal, and alfa-copaene [36]. Moreover, Manjunatha *et al.* (2013) demonstrated that the quinoline derivative of tetrahydro-curcumin (THC) and zingerone both have a high antibacterial effect [38].

Conclusions

Diet pills are continuously marketed without a clear understanding of their mechanism of action which might involve by itself a complexity of mechanisms. The diet pill tested in this study exhibited antibacterial activity on different members of the intestinal microbiota. Other supplements should be also explored for their antibacterial activity, our results could not be

generalized as each diet pill has a different composition. The results of this study only shed light on the possible side effects of these supplements which are often consumed without any medical prescription. Disrupting the gut microbiome can have adverse effects on human health. More studies should be performed on a larger number of intestinal bacteria to clearly understand the effect of these supplements on the human microbiome. Moreover, future work should also study the antibacterial effect of the digested byproducts of diet pills to further understand *in vivo* diet-pill-microbiota interactions.

Authors' Contributions

MH and MO performed all experimental procedures. ID helped in MIC and antibiotic susceptibility testing and writing of the manuscript. RAM and ZD developed the work, were involved in results analysis, interpretation, and manuscript revision. All authors read and approved the final manuscript.

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