

MOLECULAR AND CHEMICAL CHARACTERIZATION OF COMMERCIAL HERBAL SLIMMING PRODUCTS IN EGYPT

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Keywords: Herbal slimming products; DNA Metabarcoding; GC/MS, and pharmaceutical adulteration.

Consumers increasingly turn to herbal and nutraceutical products as perceived safe alternatives to conventional medicines, yet many such products are poorly regulated and can pose serious health risks. Slimming supplements are a particularly vulnerable category: they are frequently promoted with unverified efficacy claims and may be poorly standardized, inaccurately labeled, or deliberately adulterated to enhance perceived activity or sales (Nounou *et al.*, 2018). The global nutraceutical market has expanded rapidly, driven by claims of health promotion and disease-risk reduction, but inconsistent regulation in many regions has facilitated the inclusion of undeclared pharmaceutical compounds and variable product quality (Gulati and Ottaway, 2006; Andlauer and Fürst, 2002, Haneef *et al.*, 2013).

Obesity is a major non-communicable chronic metabolic disorder and a leading public-health challenge of the 21st century (Bray *et al.*, 2017). It arises from abnormal or excessive accumulation of adipose tissue that impairs health and increases risk for hypertension, type 2 diabetes, cardiovascular disease, stroke,

several cancers, non-alcoholic fatty liver disease, and sexual dysfunction (Bagchi and Preuss, 2007 and Musaiger and Al-Hazzaa, 2012). The global rise in overweight and obesity is largely attributed to increased consumption of energy-dense, nutrient-poor diets and reduced physical activity associated with urbanization and sedentary lifestyles (World Health Organization, 2000; Tabassum and Nasar, 2014). Demand for rapid weight-loss solutions has thus fueled consumer uptake of herbal slimming products, despite limited evidence for many formulations.

Safety concerns are well documented. Several studies have detected banned or undeclared pharmaceuticals—most notably sibutramine, withdrawn from many markets because of cardiovascular risk—within commercial weight-loss supplements (James *et al.*, 2010 and UNODC, 2016). Other reported adulterants, such as phenolphthalein and high-dose stimulants (including caffeine), carry risks ranging from insomnia and mood disturbances to cardiac events and carcinogenicity (Rebiere *et al.*, 2012 and Khazan *et al.*, 2014). In addition to

chemical adulteration, microbiological contamination represents an important but underappreciated hazard for botanical products. Contamination can originate from raw materials, processing environments, handling, or storage—factors amplified in complex multi-ingredient formulations—and may permit the presence of pathogenic bacteria or high microbial loads that compromise safety (Tournas and Katsoudas, 2008 and Raclariu *et al.*, 2018).

Robust quality assessment therefore requires integrated chemical and microbiological analyses. Chemical screening can reveal undeclared pharmaceuticals and clarify ingredient composition, while microbiological methods detect viable contaminants and characterize microbial communities. In this study, we evaluated selected commercial herbal slimming products distributed in Egypt for chemical authenticity and microbiological safety. We applied gas chromatography–mass spectrometry (GC–MS) for chemical profiling and adulterant screening, culture-based microbiology for viable counts and pathogen isolation, quantitative PCR (qPCR) for targeted microbial detection, and 16S rRNA metabarcoding to profile bacterial communities. Our aim was to develop and demonstrate a comprehensive evaluation system to assess safety, authenticity relative to label claims, and pharmaceutical quality of herbal slimming products in the Egyptian market.

MATERIALS AND METHODS

Materials:: Five commercial parapharmaceutical slimming products were obtained from various local sources in Egypt: Chromax capsule (PPS1), Turbo Slim (PPS2), Fat Fighter (PPS3), Slim Master (PPS4), and *Garcinia cambogia* (PPS5). The main sources included pharmacies, diet clinics, online stores, television shopping, and other undeclared vendors; some products were locally manufactured, whereas others were imported. All solvents and chemicals used in the analyses were of analytical grade.

Chromax capsules were labeled to contain *Garcinia cambogia* fruit extract and chromium picolinate. Turbo Slim and Fat Fighter capsules were labeled as blends of natural extracts, primarily green tea, *Garcinia cambogia*, and green coffee. Slim Master capsules were labeled to contain mainly green tea extract and *Garcinia cambogia*. *Garcinia cambogia* (PPS5) was marketed as a single-ingredient herbal slimming product based on *Garcinia cambogia* extract.

Extraction of capsule contents

Capsule contents were removed, air-dried at room temperature, and then ground to a fine powder. The powders were homogenized and each homogenized sample was macerated in a stoppered container with 50 mL of 85% methanol or hexane at room temperature for 3 days for conventional extraction, following Al-Daihan *et al.* (2013). The extracts were filtered and concentrated under reduced

pressure at 40 °C using a rotary evaporator to obtain crude extracts (g), which were stored at 4 °C until analysis. Visual inspection indicated non-uniformity in capsule content, color, and physicochemical appearance across products.

Gas chromatography–mass spectrometry (GC–MS) analysis

Chemical profiling of crude extracts was performed using a Thermo Scientific TSQ gas chromatograph–mass spectrometer with a TG–5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The oven temperature program was: initial hold at 50 °C, ramp at 5 °C/min to 250 °C with a 2 min hold, then ramp to 300 °C at 30 °C/min. The injector temperature was maintained at 270 °C, and helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A solvent delay of 4 min was applied, and 1 µL of diluted sample was injected automatically in split mode using an AS3000 autosampler. Electron ionization mass spectra were acquired at 70 eV over m/z 50–650 in full-scan mode. The ion source and transfer-line temperatures were set at 200 °C and 280 °C, respectively. Detected components were identified by comparison of their mass spectra with the WILEY 09 and NIST14 mass spectral databases.

Microbial isolation (culture-based methods)

To determine microbial contamination in the investigated herbal slimming products, samples from each

formulation (PPS1–PPS5) were subjected to conventional microbiological isolation. Aliquots of each sample were cultured on Dichloran Rose Bengal Chloramphenicol (DRBC) agar for fungal isolation (King *et al.*, 1979) and ISP2 agar for actinobacteria and other bacteria (Shirling and Gottlieb, 1966). Plates were incubated under appropriate conditions and inspected for microbial growth. Distinct colonies from each product were subcultured and maintained on nutrient slants and used for subsequent molecular analyses.

Molecular characterization

Relative quantification of bacterial load using qPCR

Bacterial colony suspensions prepared directly from slant-cultured isolates were used as template DNA for quantitative PCR (qPCR). Universal bacterial 16S rRNA gene primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were employed. Reactions were performed in triplicate using TransStart® Green qPCR SuperMix (TransGen Biotech, Beijing, China) in a final volume of 20 µL containing 100 ng template DNA, 0.5 µL of each primer (10 µM), and 10 µL SYBR Green Master Mix. The qPCR cycling program was: initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 20 s, and 72 °C for 30 s. Melting curve analysis was conducted from 55 to 95 °C with 0.5 °C increments every 10 s. CT values obtained for each sample were compared with those of the

negative control (sterilization control), and relative quantification (RQ) values were calculated as $2^{-\Delta Ct}$ (Schmittgen and Livak, 2008). This measure was used as an index of relative bacterial representation among products rather than absolute quantification.

16S rRNA metabarcoding

DNA extraction, library preparation, sequencing and quality control

DNA extraction and next-generation sequencing were performed by MacroGen Inc. (South Korea). DNA was extracted from the five herbal slimming supplement samples (Chromax capsule, Turbo Slim, Fat Fighter, Slim Master, and *Garcinia cambogia*) and subjected to quality control. Samples passing QC were processed for library construction. The V3–V4 region of the prokaryotic 16S rRNA gene was amplified using KAPA HiFi HotStart ReadyMix and primers Bakt_341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt_805R (5'-GACTACHVGGGTATCTAATCC-3').

Libraries were prepared using the Nextera XT DNA Library Preparation Kit and sequenced on an Illumina MiSeq 3000 platform. MiSeq Control Software v2.2 (Illumina, USA) was used for system control and base calling, and raw data were converted into FASTQ format using bcl2fastq v1.8.4 (Illumina, USA).

Adapter sequences were removed using Scythe (v0.994) and Sickel, and reads shorter than 36 bp were discarded.

Paired-end FASTQ files were merged using BBMerge at a normal merge rate and further filtered to remove low-quality bases ($Q < 30$) and short reads (< 50 bp) using BBduk tools within Geneious Prime (Kearse *et al.*, 2012). The resulting compressed, clean, merged, and filtered FASTQ files were used for downstream bioinformatics analysis.

Bioinformatics pipeline

Sequence data were analyzed using the SILVAngs online pipeline based on the SILVA rRNA database (Quast *et al.*, 2012). Each read was aligned against the SILVA SSU rRNA database using the SILVA Incremental Aligner (SINA v1.2.10; Pruesse *et al.*, 2012). Short reads and those with more than 2% ambiguous bases or excessive homopolymers were discarded. Remaining identical reads were dereplicated, and unique reads were clustered into operational taxonomic units (OTUs) using cd-hit-est v3.1.2 (Li and Godzik, 2006) in accurate mode, ignoring overhangs and applying identity criteria of 1.00 and 0.98.

For each OTU, a representative read was classified by local BLASTn against the non-redundant SILVA SSURef dataset (release 132) using standard parameters (Camacho *et al.*, 2009). All reads were then mapped to OTU reference sequences to obtain quantitative OTU counts per sample using BLASTn features. Sequences with no or weak BLAST hits, defined as those for which "(% sequence identity+% alignment coverage)/2" did not exceed the

value of 93 were retained but considered unclassified or unassigned.

RESULTS AND DISCUSSION

GC–MS Characterization of Herbal Slimming Products

GC–MS analysis revealed multiple bioactive constituents in the investigated herbal slimming products, with pronounced differences in chemical profiles between products and between methanolic and hexanic extracts. The most striking finding was that sibutramine was the predominant constituent in most analyzed samples, with relative abundances ranging from 33.5% in the methanolic extract of Chromax to 100% in both methanolic and hexanic extracts of Slim Master (Fig. 1A). The widespread occurrence of sibutramine across seven of the ten extracts indicates extensive pharmaceutical adulteration and agrees with previous reports identifying sibutramine as one of the most frequently detected undeclared synthetic compounds in herbal weight-loss supplements (Mathon *et al.*, 2014 and Shekari *et al.*, 2018 and Vaclavik *et al.*, 2014).

Sibutramine was originally approved as an anti-obesity medication because of its ability to inhibit serotonin and norepinephrine reuptake, thereby suppressing appetite and promoting energy expenditure. However, results from the Sibutramine Cardiovascular Outcomes Trial (SCOUT) demonstrated an increased risk of cardiovascular events among

susceptible individuals, leading to its withdrawal from major international markets including the United States and the European Union (James *et al.*, 2010). The exceptionally high abundance of sibutramine detected in several products—particularly Slim Master, in which it constituted nearly the entire chromatographic profile—strongly suggests intentional addition rather than accidental contamination. Similar intentional adulteration has been documented in counterfeit and adulterated slimming supplements, where undeclared pharmaceutical compounds are added to enhance perceived efficacy and accelerate weight loss (Vaclavik *et al.*, 2014). Such practices represent a serious public health concern because consumers may unknowingly ingest potent pharmacological agents without appropriate medical supervision, increasing the risk of adverse cardiovascular, neurological, and psychiatric effects.

In addition to sibutramine, several naturally occurring phytochemicals were identified in the products. Unsaturated fatty acids, including linoleic acid (ω -6), α -linolenic acid (ω -3), and oleic acid (ω -9), were detected in both methanolic and hexanic extracts, consistent with their widespread occurrence in plant oils and botanical materials. Their low to moderate relative abundance suggests that they derive from the declared herbal ingredients rather than acting as principal weight-loss agents. Chamazulene, a characteristic constituent of chamomile, has been

reported to possess antioxidant and anti-inflammatory activities, whereas panaxydol is recognized as one of the major bioactive constituents of *Panax* species (McKay and Blumberg, 2006 and Christensen, 2009). Nevertheless, the generally low abundance of these compounds (typically below 10%) indicates that they are unlikely to account for the strong slimming claims associated with the investigated products.

Comparison between extraction solvents revealed clear differences in compound recovery. Methanolic extracts preferentially recovered relatively polar constituents, including sibutramine and other nitrogen-containing compounds, whereas hexanic extracts were enriched with non-polar constituents such as fatty acids, long-chain hydrocarbons, and wax-like compounds. These findings reflect the influence of solvent polarity on solubility and highlight the importance of applying complementary extraction approaches to achieve comprehensive chemical characterization of complex herbal formulations.

Among all investigated samples, *Garcinia* (PPS5) represented a notable exception. The hexanic extract of *Garcinia* was the only sample in which sibutramine was not detected. Instead, this extract was characterized by a predominance of aliphatic alcohols, particularly 2-decen-1-ol (32.8%), together with several unidentified compounds. *Garcinia* species are widely marketed as natural weight-loss supplements due to the presence of

hydroxycitric acid, a compound proposed to influence lipid metabolism and appetite regulation (Onakpoya *et al.*, 2011). The absence of detectable sibutramine may therefore indicate a comparatively more authentic formulation than the other investigated products. However, the presence of multiple unidentified chromatographic peaks underscores the limitations of spectral library-based identification and suggests that complementary analytical techniques, such as LC-MS/MS or high-resolution mass spectrometry, could provide improved structural elucidation of unknown constituents.

Overall, the GC-MS findings demonstrate substantial variation in the chemical composition of the analyzed herbal slimming products and provide compelling evidence of widespread pharmaceutical adulteration. The predominance of sibutramine in most samples suggests that the reported slimming effects of these products are likely attributable to undeclared synthetic drug content rather than the naturally occurring phytochemicals detected. These results raise significant concerns regarding product authenticity, regulatory compliance, and consumer safety and underscore the necessity for routine chemical surveillance of herbal weight-loss supplements.

Microbiological evaluation (culture-based)

Culture-based microbiological analysis on DRBC agar and ISP2 agar

revealed microbial growth from several products, with colonies displaying different sizes, shapes, and colors, indicative of diverse microbial populations (Fig. 2). Subsequent molecular characterization assigned many of these isolates to phyla such as *Pseudomonadota* and *Actinomycetota*. In contrast, no colonies were observed for products 4 and 5 on the tested media, which may reflect either low levels of contamination or the use of culture conditions and media that were not optimal for the microorganisms present in these products. The numbers of colonies and corresponding CFU values for each product, dilution, and medium are summarized in Table (1).

Quantification and identification of microbial load

Relative quantification by qPCR

To determine whether microbial loading was present in the examined herbal slimming products, bacterial isolates were recovered from each sample (PPS1–PPS5) through microbiological isolation and maintained on nutrient slants. Genomic DNA was extracted from the cultured isolates and subjected to qPCR analysis targeting the universal bacterial 16S rRNA gene. Relative bacterial load was estimated using a comparative Ct approach, whereby Ct values obtained from each sample were evaluated relative to the negative (sterilization) control and expressed as relative quantification ($RQ = 2^{-\Delta Ct}$). RQ values demonstrated clear variation in relative bacterial load among the examined

samples (Fig. 3). Sample S2 yielded the highest RQ value (≈ 24.5), indicating a substantially elevated bacterial burden relative to the calibrator, whereas S1 showed an intermediate microbial load ($RQ \approx 9.8$). The remaining samples, S3, S4, and S5, displayed progressively attenuated RQ values (≈ 1.9 , 1.0 , and 0.7 , respectively), with S4 approximating the calibrator baseline and S5 falling marginally below it.

These findings provided an initial comparative assessment of bacterial presence and supported the selection of samples for subsequent 16S rRNA metabarcoding analysis to enable more detailed taxonomic characterization of the associated bacterial communities. Variation in microbial load among herbal products has frequently been associated with differences in raw material quality, harvesting practices, manufacturing processes, storage conditions, and post-production handling (Tournas *et al.*, 2008 and Kunle *et al.*, 2012). The elevated bacterial load observed in S2 may therefore reflect increased microbial colonization of plant materials or greater exposure to environmental contamination during production and storage. Although qPCR offers a sensitive measure of bacterial abundance, it does not reveal the taxonomic identity of the microorganisms present. Therefore, 16S rRNA metabarcoding was employed to characterize the bacterial communities associated with each product and to provide a comprehensive assessment of microbial diversity.

16S rRNA metabarcoding analysis

The metabarcoding analysis yielded 1,005 sequences, 95.22% of which were successfully classified, indicating high-quality taxonomic assignment and reliable microbial community profiling. Sequence lengths ranged from 154 to 365 bp, with an average length of 274 bp, and a total of 247 operational taxonomic units (OTUs) were identified across all samples, demonstrating substantial bacterial diversity within the analyzed products. Taxonomic profiling revealed that the microbial communities comprised several bacterial phyla, including *Pseudomonadota*, *Actinomycetota*, *Bacillota*, *Bacteroidota*, *Chloroflexota*, *Cyanobacteriota*, *Verrucomicrobiota*, *Planctomycetota*, and *Nitrospirota*. Considerable variation in microbial richness and relative abundance was observed among samples, indicating heterogeneous microbial compositions likely resulting from differences in plant ingredients, environmental exposure, and manufacturing conditions. Similar variability has been reported in previous investigations of herbal medicinal products and botanical supplements, where microbial communities differed substantially among products and production batches (Raclariu *et al.*, 2018).

Sample PPS02B exhibited the highest taxonomic richness, containing the largest number of detected bacterial phyla, whereas PPS03C and PPS04D showed comparatively lower diversity. Notably, PPS02B corresponded to the sample that

exhibited the highest bacterial load in the qPCR analysis, suggesting that products with elevated microbial abundance may support more diverse microbial communities. However, microbial abundance and taxonomic diversity are not always directly correlated, as highly abundant communities may sometimes be dominated by a limited number of taxa (Lozupone and Knight, 2008). *Pseudomonadota* represented the dominant phylum in most samples. Members of this phylum are among the most widespread bacteria associated with plant tissues, soil environments, irrigation water, and processed plant-derived products due to their remarkable metabolic versatility and ecological adaptability (Fierer *et al.*, 2007 and Bulgarelli *et al.*, 2013). Their predominance in the analyzed products is therefore consistent with previous studies describing *Pseudomonadota* as a major component of plant-associated microbiomes.

In contrast, *Cyanobacteriota* was markedly enriched in PPS05E, despite the relatively low bacterial load detected by qPCR. Cyanobacterial DNA may originate from environmental exposure during cultivation, irrigation water, or plant-associated microbial communities and can persist in processed materials owing to the stability of DNA molecules (Nawaz *et al.*, 2024). This finding demonstrates that products exhibiting relatively low microbial abundance may nevertheless possess distinct microbial compositions, highlighting the complementary nature of qPCR and metabarcoding approaches. The

recurrent detection of Actinomycetota and Bacillota further supports the presence of soil- and plant-associated microorganisms. Members of these phyla are well known for their ability to withstand environmental stresses through mechanisms such as spore formation and desiccation tolerance, allowing them to persist during drying, transportation, and storage of herbal materials (Goodfellow & Williams, 1983 and Nicholson *et al.*, 2000).

Overall, the combined qPCR and metabarcoding analyses revealed substantial heterogeneity in microbial abundance and community composition among the herbal slimming products investigated. These findings underscore the importance of microbial quality assessment and molecular surveillance to ensure the safety and quality of commercial herbal preparations, particularly those marketed for weight loss.

Conclusion

DNA metabarcoding revealed diverse microbial communities and biological components in the analyzed herbal slimming products. GC–MS profiling identified both natural phytochemicals and, in most samples, the synthetic anorectic drug sibutramine. The high levels of sibutramine detected indicate widespread pharmaceutical adulteration and raise serious safety concerns, given the well-documented cardiovascular and neurological risks associated with this banned medication. Taken together, the complementary chemical and molecular approaches provided a robust assessment

of product authenticity, contamination, and overall quality. These findings underscore the urgent need for stricter quality control measures and more effective regulatory monitoring of herbal slimming products to protect consumers from undeclared and potentially harmful ingredients.

Dot plot showing the distribution, relative abundance, and sequence counts of bacterial phyla detected in samples PPS01–PPS05. Each dot represents an operational taxonomic unit (OTU) assigned to a given phylum, with dot size proportional to sequence counts. Dominant phyla include Pseudomonadota, Actinomycetota, Bacillota, and Bacteroidota, with sample-specific enrichment of Cyanobacteriota in PPS05E, illustrating heterogeneity in microbial community structure among products.

SUMMARY

Herbal slimming products have gained wide consumer interest, yet concerns remain about their authenticity, safety, and quality. This study assessed five commercially available slimming products sold in Egypt using an integrated approach combining GC–MS profiling, microbial isolation, qPCR, and 16S rRNA metabarcoding. GC–MS analysis revealed both plant-derived phytochemicals and undeclared sibutramine in most samples, with relative abundances ranging from 33.5% to 100%, indicating likely pharmaceutical adulteration. Microbial culturing and qPCR showed variable bacterial burdens, while metabarcoding identified diverse bacterial communities

associated mainly with plant- and soil-related taxa. The findings suggest that some commercial herbal slimming products may contain undeclared drugs and microbial contaminants, emphasizing the need for stronger regulation and routine surveillance.

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Table (1): Total number of microbes on both media ISP and DRBC agar.

Product no.	Culture media	Conc.	No. of colonies	Total no. of microbes(CFU)
1	ISP	10^{-1}	2	20
1	ISP	10^{-2}	-	-
1	DRBC	10^{-1}	-	-
1	DRBC	10^{-2}	-	-
2	ISP	10^{-1}	7	70
2	ISP	10^{-2}	2	200
2	DRBC	10^{-1}	2	20
2	DRBC	10^{-2}	-	-
3	ISP	10^{-1}	22	220
3	ISP	10^{-2}	20	2000
3	DRBC	10^{-1}	8	80
3	DRBC	10^{-2}	-	-
4	ISP	10^{-1}	-	-
4	ISP	10^{-2}	-	-
4	DRBC	10^{-1}	1	10
4	DRBC	10^{-2}	-	-
5	ISP	10^{-1}	-	-
5	ISP	10^{-2}	-	-
5	DRBC	10^{-1}	-	-
5	DRBC	10^{-2}	-	-

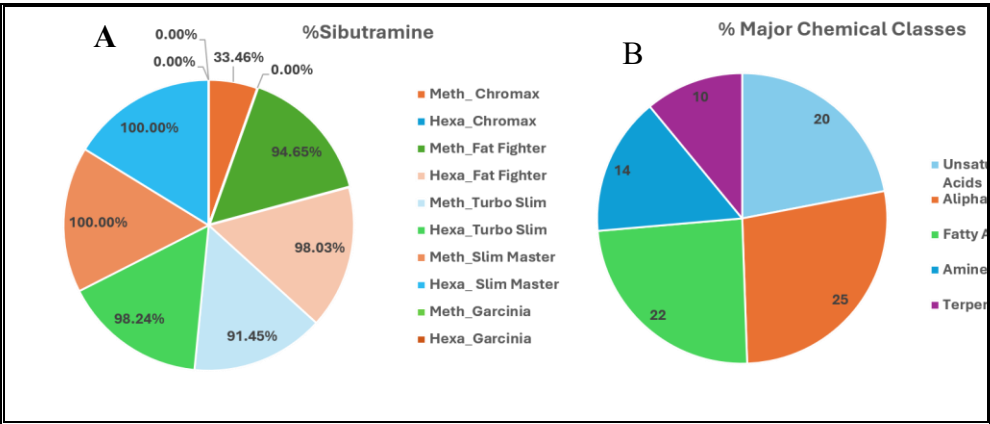


Fig. (1). GC–MS characterization of herbal slimming products.

(A) Relative abundance (%) of sibutramine in ten extracts (five methanolic and five hexanic) of commercial herbal weight-loss products (Chromax, Turbo Slim, Fat Fighter, Slim Master, and Garcinia cambogia). Values represent sibutramine peak area expressed as a percentage of the total identified compounds in each chromatogram. (B) Relative abundance of major chemical classes (e.g. synthetic anorectic drugs, fatty acids, terpenoids, other phytochemicals) identified by GC–MS in the same herbal weight-loss products.

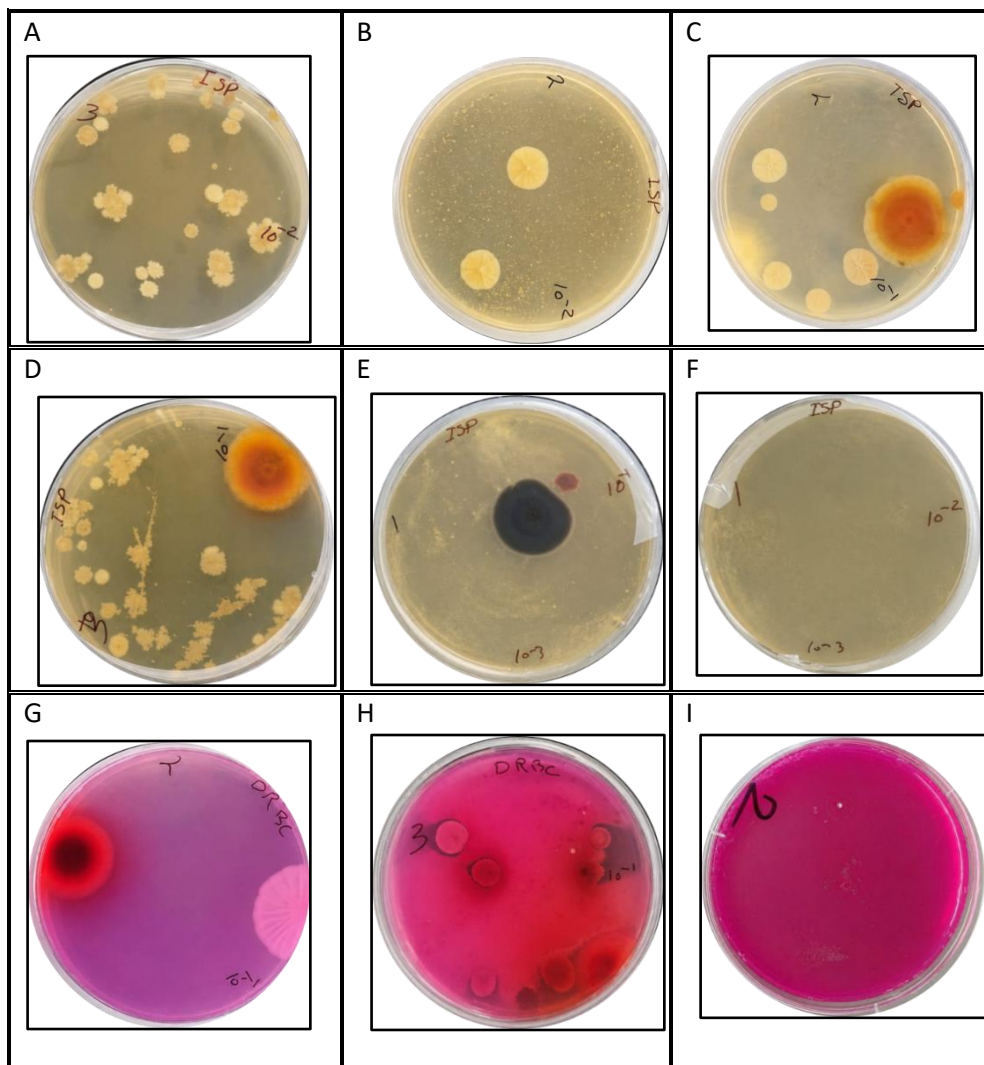


Fig. (2). Culture-based microbial evaluation of parapharmaceutical slimming products.

Representative plates showing colony growth on ISP2 agar from selected products: Chromax capsule (PPS1), Turbo Slim (PPS2), Fat Fighter (PPS3), Slim Master (PPS4), and Garcinia cambogia (PPS5). Colonies differing in size, shape, and color reflect the presence of diverse bacterial taxa, later assigned to phyla including Pseudomonadota and Actinomycetota. No visible growth was observed for some products on the tested media, suggesting low contamination or growth conditions not optimal for the microorganisms present.

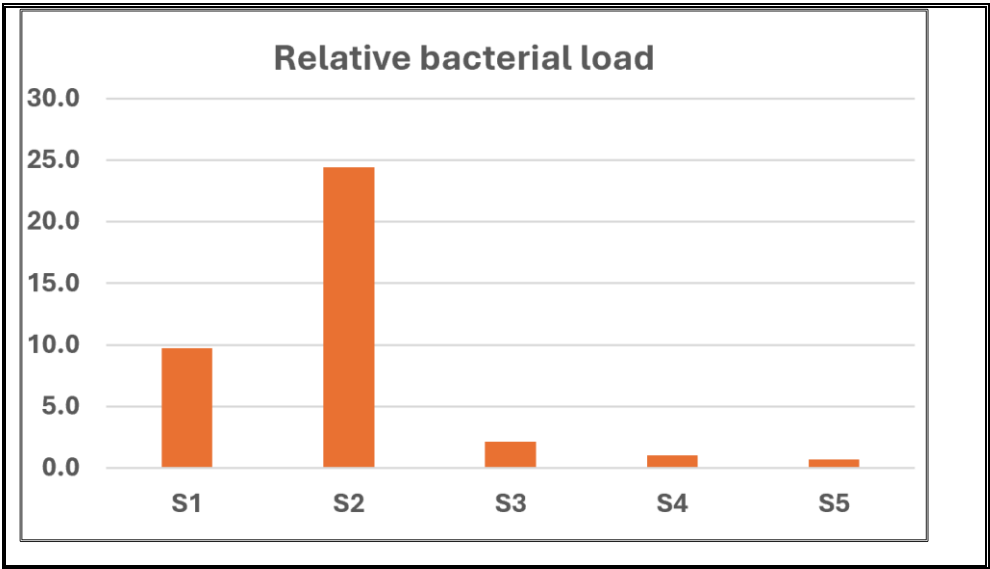


Fig. (3) Relative bacterial load in herbal slimming products estimated by qPCR.Relative quantification (RQ) values for samples S1–S5 based on amplification of the universal bacterial 16S rRNA gene and expressed as $2^{-\Delta Ct}$ relative to the negative (sterilization) control. S2 shows the highest bacterial burden (RQ $\approx$ 24.5), followed by S1 (RQ $\approx$ 9.8), whereas S3, S4, and S5 display lower RQ values ($\approx$ 1.9, 1.0, and 0.7, respectively), indicating substantial variation in microbial load among products.

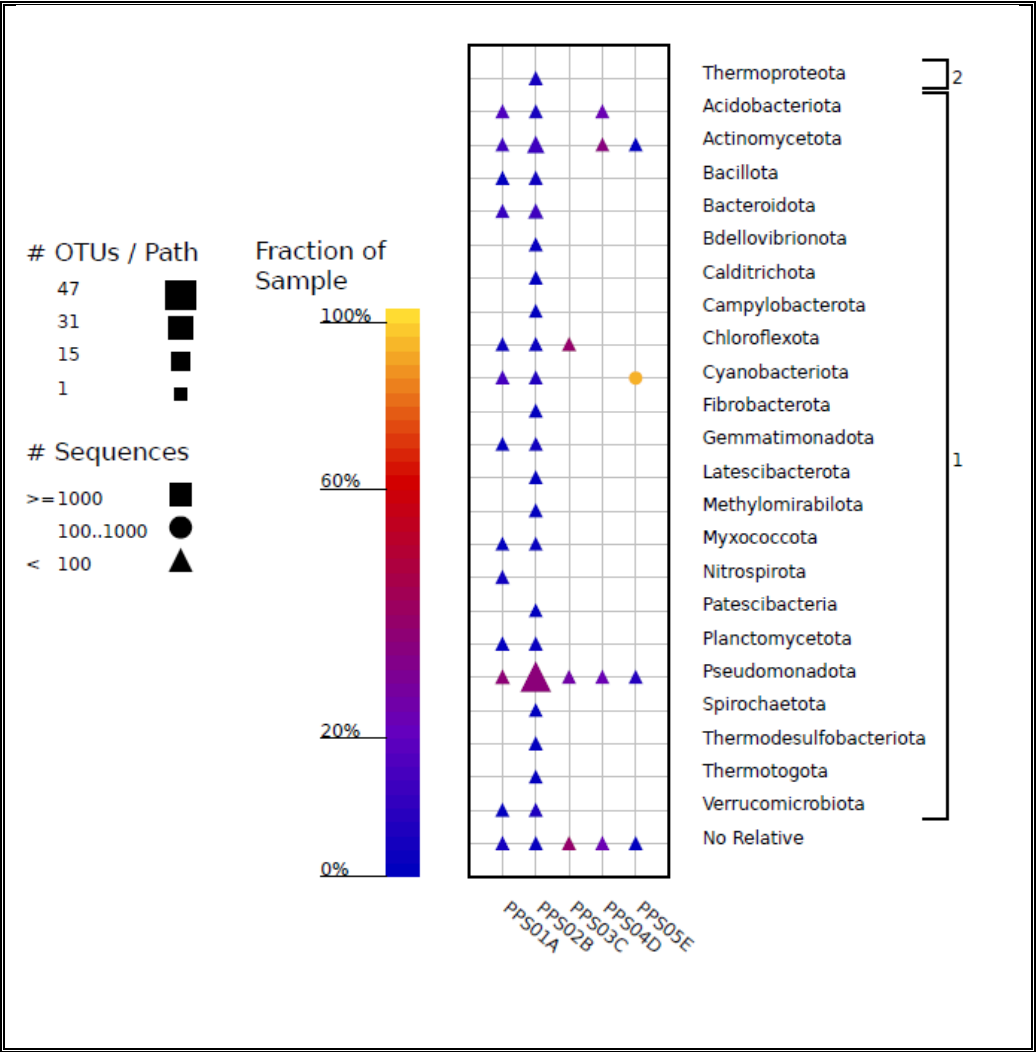


Fig. (4). Taxonomic fingerprint of bacterial phyla in herbal slimming products revealed by 16S rRNA metabarcoding.