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Дахма. Н
Студент
2 курс, магистратура, Альбаас университет, факультет «Естественных наук»
Аль Хаддад. И
Доктор университета , Альбаас университет, факультет «Естественных наук»
Россия, г. Москва
Nermin Dahma
Master student
Department of Biology
Faculty of Sciences Al-Baath University Homs, Russia, Moscow
Emad AL Haddad
Department of Biology
Faculty of Sciences Al-Baath University Homs, Russia, Moscow

MICROBIOLOGICAL QUALITY AND SAFETY OF DIETARY SLIMMING SUPPLEMENTS SOLD IN SYRIA

Annotation: *Nowadays the consumption of dietary slimming supplements has increased because people can easily get them from pharmacies without a prescription or buy them on line. However, there is a need to explore the quality of these products as there are few studies related to this area. Therefore, the objective of this study was to determine the microbiological quality and safety of dietary supplements in the Syrian local markets. In this study, 13 samples of dietary slimming supplements were collected from Syrian markets and were evaluated for microbial contamination depending on the United States Pharmacopoeia(USP) microbial limit test for enumeration and identification. Our findings demonstrated that the analyzed products exceeded the acceptable USP limits for Total Aerobic Microbial Count (TAMC) and Total Yeast and*

Mold Count (TYMC) by 100% and 85% respectively. This indicates that all these products failed to pass the Microbial bioburden tests. Isolation and identification of microbial contamination showed that 77% of the samples were contaminated with pathogenic microorganisms. In conclusion, There is a need to raise the awareness on the dangerous effects of these products on consumers' health as well as the quality of the products.

Key Words: *Dietary supplements, Microbiological quality, fungal contamination, bacterial contamination.*

МИКРОБИОЛОГИЧЕСКОЕ КАЧЕСТВО И БЕЗОПАСНОСТЬ ПРОДОВОЛЬСТВЕННЫХ ПРЕПАРАТОВ, ПРОДАННЫХ В СИРИИ

Аннотация: *В настоящее время потребление диетических добавок для похудения увеличилось, потому что люди могут легко получить их в аптеке без рецепта или купить их онлайн. Тем не менее, существует необходимость изучить качество этих продуктов, так как мало исследований, связанных с этой областью. Поэтому целью данного исследования было определить микробиологическое качество и безопасность пищевых добавок на сирийских местных рынках. В этом исследовании 13 образцов пищевых добавок для похудения были собраны на сирийских рынках и оценены на микробное загрязнение в зависимости от микробного предельного теста Фармакопеи США (USP) для подсчета и идентификации. Наши результаты показали, что анализируемые продукты превышали допустимые пределы USP для общего количества аэробных микроорганизмов (TAMC) и общего количества дрожжей и плесени (TYMC) на 100% и 85% соответственно. Это указывает на то, что все эти продукты не прошли испытания на микробную биологическую нагрузку. Выделение и идентификация микробного загрязнения показали, что 77% образцов были загрязнены патогенными микроорганизмами. В заключение, необходимо повысить осведомленность об опасном воздействии этих продуктов на здоровье потребителей, а также на качество продуктов.*

Ключевые слова: *Биологически активные добавки, Микробиологическое качество, грибковое загрязнение, бактериальное загрязнение.*

1. Introduction:

Obesity is a chronic metabolic disease characterized by an increase of body fat storage has become one of the leading causes of disability and death. There is no need to say that losing weight is becoming an important goal everyone wants to reach in order to keep healthy and fit. As a result the popularity of dietary slimming supplements as an attractive and easy option for losing weight has increased especially because of individuals' misconception that these products are safe [1,c.35][2,c.539].

Dietary supplements are classified by pharmacopeia as non sterile preparations. Although sterility is not a requirement in official compendia for these products but, they are required to pass tests for the absence of certain specified microorganisms and microbial bioburden tests [3,c.443].

The presence of microbes in dietary supplements not only makes them hazardous on human health, but may also change their physical, chemical, and organoleptic properties, alter the contents of active ingredients, or convert them to toxic products. Degraded quality of these products can result in severe consumer dissatisfaction, which can ultimately lead to undesirable financial loss to the manufacturers [4,c.1]. Solid dosage forms like capsules are prone to microbial contamination. The absence of obvious signs of their microbial spoilage or degradation is the most serious problem. Therefore, there is a need to evaluate the microbiological quality of these products constantly [5,c.1701].

Due to absence of clear regulations and guidelines for quality assessments of these products an increasing demand has made their use a public health issue and has increased the doubt that people may get products with low quality [6,c.4264]. However, there are very limited published information regarding the quality of these products in Syrian markets. Therefore, the objective of this study was to determine the microbiological quality and safety of dietary slimming supplements in Syria .

2. MATERIALS AND METHODS:

Samples of dietary slimming supplements were collected at random from the local market. These samples were divided into 5 categories according to the following symbols : A,B,C,D,E. Each product had 3 different batch numbers except for the sample E which had only one batch number. All pharmaceutical forms were capsules in containers. The samples A,B,C were Locally Manufactured while both samples D and E were internationally manufactured.

The culture media used for the microbiological analysis include: Macconkey agar, Violet red bile glucose agar, Simmon citrate agar, Kligler iron agar, Sabouraud dextrose agar, Tryptone soya agar, Xylose lysine deoxycholate agar, Enterobacteria enrichment broth mossel, Rappaport vassiliadis enrichment broth, Indole broth, Tryptone soya broth. All media were obtained from Merck chemical co. (Darmstadt, Germany) and were prepared according to the manufacturers' instructions.

2.1. Preparation of samples

Sample preparation was conducted according to the United States Pharmacopeia (USP 41) [6]. The outside surfaces of all containers were swabbed with 70% v/v ethanol before being opened. In general, at least 1 g samples were tested for each product and 1:100 sample dilutions of the products in each of sterile Tryptone Soy Broth (TSB).

2.2. Method suitability verification

Method suitability verification using standard test microorganisms was conducted according to the USP. Reduction of growth indicates antimicrobial activity and invalidates this portion of examination, which requires a modification of the procedure.

2.3. Microbial bioburden tests: Total viable aerobic count

After verification, Microbial bioburden tests were conducted according to the guidelines of United States Pharmacopeia USP 41 using the spread plate technique. From the tested sample dilutions, aliquots were transferred onto Tryptone Soy Agar (TSA) plates suitable for the cultivation of bacteria or Sabouraud Dextrose Agar (SDA) plates, suitable for the cultivation of fungi. Triplicate plates of each culture medium were done for each test dilution. At the end of the incubation period, the recovered

colonies from each plate were enumerated and the arithmetic mean count was used for calculating the viable count of the test sample (CFU/g) with each culture medium having from 30 to 300 colonies per plate [7,c.8200].

2.4. Isolation of specified microbial contaminants

10 mL of prepared sample was added to 100 mL of casein soyabean digest broth. The further procedure was dependent on the determination of the absence or limited occurrence of specified microorganism that may be detected:

– Enterobacteriaceae: incubation (2–5 h, 20-25°C), transmission to Enterobacteria enrichment broth- Mossel (incubation 24–48 h, 30-35°C), then transmission on violet red bile glucose agar.

– E. coli :incubation (24 h, 30-35°C) and transmission to Macconkey broth (incubation 48 h, 42-44°C), then transmission on Macconkey agar.

– Salmonella spp: incubation (24 h, 30-35°C) and transmission to Rappaport Vassiliadis (incubation 24 h, 30-35°C) then transmission on Xylose lysine deoxycholate agar [7,c.8200][8].

2.5. Biochemical tests

identification tests were based on characteristic colony growth morphologies and biochemical tests(Indol broth, Kligler iron agar, Simmon citrate agar) in order to confirm the identity and differentiate certain members of the Enterobacteriaceae.

3. Results and DISCUSSION

Results indicated that all samples ,except one, do not inhibit the multiplication of indicator microorganisms under the test conditions. Its antimicrobial property was eliminated using dilution method in Tryptone soya broth (TSB) prior to conducting bioburden tests.

Total Aerobic Microbial Count (TAMC)and Total Yeast and Mold Count (TYMC):

The acceptance criteria of pharmaceuticals should be strictly maintained according to the recommended specifications given by the pharmacopeia, Based on USP pharmacopoeia (USP 41). The total aerobic microbial count(TAMC) should be under 10^3 (CFU/g) and the total yeast mold count(TYMC) should not exceed 10^2

(CFU/g) within the finished products of oral non-aqueous preparations using Tryptone soy agar, Sabouraud dextrose agar culture media .
100% of the analyzed products were above 10^4 colony-forming units (CFU/g) exceeded the (TAMC) acceptable USP limit and 85% of the analyzed products were above 10^2 colony forming units (CFU/g) exceeded the (TYMC) acceptable USP limit .Our findings indicate that all of the products failed to pass the Microbial bioburden tests.

Identification of the recovered microbial contaminants was carried out through studying colonies characteristics and number of confirmatory biochemical reactions: 8 samples(62%) were contaminated with Enterobacteriaceae (1 sample contaminated with Escherichia coli , 1 sample contaminated with Enterobacter, 6 samples contaminated with klebsiella), 9 samples (69%) were contaminated with Aspergillus niger (FIGURE 1,FIGURE 2).

Unfortunately, there are few studies on microbial quality of dietary slimming supplements especially in the Middle East. The percentage of samples that didn't comply with the Pharmacopeial specification in the present study is close to that previously reported in a similar research, where an incidence of rejection of 100% was reported [9]. To our knowledge, this is the first reported case of microbiologically contaminated dietary supplements sold in Syria.
The microbiological contamination recorded for products tested in this study may be attributed to several factors: poor bacteriological quality of raw materials especially those of natural origin which have initial microbial levels and their microbiological quality is influenced by (Soil, harvesting, drying, storage and packaging conditions), manufacturing process, poor packaging process, contamination by workers, inferior storage conditions or transportation [5,c.1701][9,c.167].

The contamination of any preparation with gram negative organisms is not desirable and constitutes a public health concern especially the occurrence of Enterobacteriaceae with respect to hygienic practices. This is because Enterococcus genus have the human colon as a natural habitat and its presence in products is a strong indication of fecal contamination [8].

The presence of certain molds is harmful since they produce metabolites that may be toxic to consumers and cause rapid deterioration of the product due to the biodegradation of the different components of formulations arising from the production of toxins [10,c.1].

Ochratoxin A (OTA) a ubiquitous mycotoxin produced by Aspergillus niger isolated in our study is nephrotoxic and is suspected of being the main etiological agent responsible for human Balkan endemic nephropathy (BEN) and associated with urinary tract tumors. Group of researchers reported positive findings for the genotoxicity, teratogenicity and immunotoxicity of OTA [11,c.61]. Generally, the presence of potentially pathogenic opportunistic microbes, cannot be overemphasized, because they may cause a significant deterioration in the health status of patients, particularly in elderly, chronically sick ones, those who are immunologically compromised [9,c.167].

3. **Conclusion**

From this study we can conclude that there are some dietary slimming supplements with low microbiological quality are sold in Syria. Stringent regulatory actions should be employed by the Syrian government to ensure that the good manufacturing practices (GMP) are being accurately maintained in all the local dietary supplements industries in the country because the failure of strict observation of GMP at any stage of production may greatly affect the microbiologic quality of the end products. The dietary supplements microbiological control in accordance with GMP is required at each stage of production, particularly at the stage of the final product prior to release. Quality control of those products is not only important for compliance with standards, but also reduces the risk to the end user . Quality has to be built into the whole process ranging from the selection of raw material to the final product. Also post marketing surveillance should be taken into consideration to detect any problem relating to product quality.

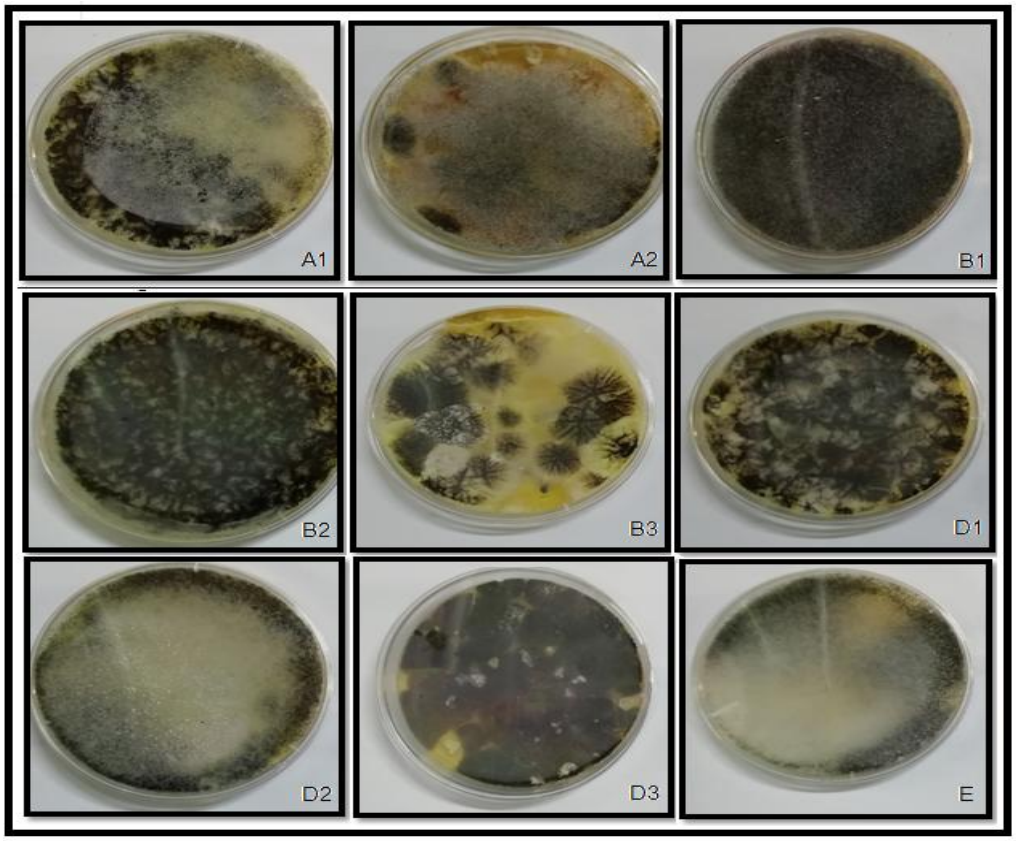

FIGURE 1: Samples contaminated with Aspergillus niger

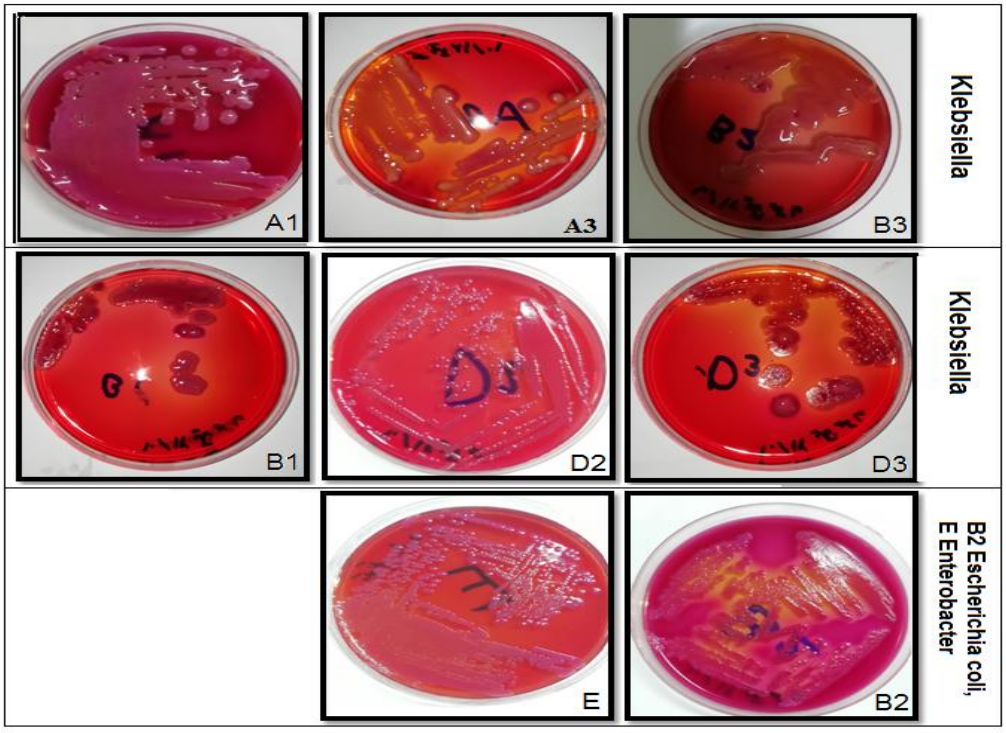

FIGURE 2: Samples contaminated with Enterobacteriaceae

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