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Comparative Microbial Assessment of Local and Commercial *Spirulina platessis* Samples

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ABSTRACT

Present study is concerning with the assessment of putative bacterial contamination in *Spirulina*, to assert the further studies and pose strict monitoring to get pure supplements. Our study, aim was to assess any bacterial and molds contamination of local and commercial ''*Spirulina*'' supplements available in Jeddah region market. During the study, cyanobacteria "*Spirulina*" was dried and commercially available *Spirulina* were subjected to serial dilution 10-1, 10-2, 10-3 and 10-4 and spread on media. The result revealed that only 10-1 produced colonies on culture Plates. In addition, when they were cultured on specified media e.g., chocolate blood agar, Eosin methylene blue, MacConkey agar, Mannitol salt agar, Sabouraud Dextrose Agar and Salmonella-Shigella agar no pathogenic bacterial colony was found. This study concludes that both local and international *Spirulina* supplement is clear from any pathogenic bacteria and mold.

KEY WORDS: *SPIRULINA*, MICROBIOLOGICAL ANALYSIS, PATHOGENIC MICROORGANISMS.

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INTRODUCTION

Raw materials in biological system can be contaminated with an excessive number of bacteria, fungi and algae due to the medium conditions in which they grow. Today, wastewater treatments needs to filter out the nutrients such as phosphorus and nitrogen that could be hazardous to the environment if not removed from water. The phosphorus and nitrogen contaminations lead to severe hazard to ecosystem if water containg these contaminants mingle into the rivers and stream. Now a days, heavy metals contamination became a global issue due to their high toxicity, non-biodegradability and carcinogenicity. The heavy metals are added up in food chain and get accumulated in human tissues and cause toxicity and carcinogenicity. Early researches conducted on heavy metals removal have shown the inactive biomass play an essential role on this process including biological growth conditions. A filamentous cyanobacterium including bacteria and fungi have the potential to fix such these contaminated materials. (Davis et al., 2003, Gao et al., 2009, Ruiz-Marin et al., 2010, Sud et al., 2008).

Nowadays, 800 million of people all over the world are suffering from malnutrition; 200 million are children and this cause a serious problem in public health (Anon, 2006). In the 21st century, depending on local natural sources particularly microalgae is the best food for future, greatly valuable for bioactive compounds and relatively cheap. The most important genus is *Spirulina* (Arthrospira), which has a considerable micro and macronutrients content (Soni et al., 2017), rich in protein, betacarotene, vitamins and minerals (Ores et al., 2016, Matos et al., 2017). Previous works on *Spirulina* have shown that this microalgae mainly s. platensis can improve the immunity system of organism and promote calcium absorption (Shabana et al., 2017). Moreover, analysis of s. platensis from various product have shown that it's contain up to 70 % protein, carbohydrates, essential amino acids, essential fatty acids such as palmitic acid, linolenic acid and linoleic acid (Lupatini et al., 2016), pigments like chlorophyll a, phycocyanin, carotenes and some minerals. As well as, it has a huge health benefits as antibacterial and antiviral activities, antioxidant, antiinflammatory, anticancer, also it works against obesity, malnutrition, diabetes, anemia and heavy metal toxicity, it has been used as feed additives in many animal species and in waste water treatment (Matos et al., 2017). The genus *Spirulina* is a planktonic photosynthetic cyanobacterium can also fix CO2 therefore reducing carbon dioxide emission (Meng et al., 2009), it was also shown that *Spirulina* seems to be a safe food as shown by Al-Dhabi 2013.

Recently, industrial countries for example China considered as the biggest *Spirulina* manufacturer all over the world (Li et al., 1997), due to an incredible achievements this kind of industry has more attention in China and increased rapidly. The International Energy Agency (IEA) declared that over 50 % of the worldwide of 10,000 tons annually is produced in China (Huo et la., 2011), which generates about $570 million (Yun-Ming et al., 2011).In addition, different organization such as the United Nations World, World Health Organization and UNESCO confirmed that the microalgae S. platensis is the most valuable food for tomorrow. On the other hand, NASA and

Table 1. Total aerobic viable count

Dilution	24 H CFUs/ml		48H CFUs/ml		EU standard	WHO
	Commercial sample	Local sample	Commercial sample	**Local sample**		
1:10	11±0.5	8 ±0.5	10 ±1	8 ±1		
1100	0	0	0	0	1.0×10^5	1.0×10^5
1:1000	0	0	0	0		
1:10000	0	0	0	0		

European Space Agency used it in long-term space mission in space.

MATERIALS AND METHODS

Preparation of water extracts of *Spirulina:* Samples were collected from internet distributors in Jeddah region, Saudi Arabia in 2019 (national and commercial powders). Based on the product label of the commercial sample the manufacturing company: California Gold Nutrition and the original country is India. For the sample preparation, 10 g was emulsified with that of 90 ml peptone H2O. The samples were frequently agitated in water bath for 15 min at 45 °C.

Microbiological analysis: Colony forming method was used for the assessment of microbial load. For this reason, the samples were diluted with distilled water. For dilution, One ml of sample was transfer to 9 ml of distilled water. Furthermore serially diluted with that of 9 ml distilled water. Then these diluted samples were transfer to total plate agar dishes. The plates were incubated for 48 hours at 35±2°C to determine the total plate counts for bacteria. In addition, a numbers of different selective media were used to detect pathogenic bacteria (APHA, 2005). Determination of pathogenic microorganisms: Samples were serially diluted and then transfer to blood agar plates. The plates were incubated for 24 hours at 35±2°C. After incubation, count the colonies on each of the plates and make a subculture the growth from blood agar to different selective media (ATCC, 1984).

RESULTS AND DISCUSSIONS

A comparative analysis of commercially available *Spirulina* and locally collected *Spirulina* were carried out. The *Spirulina* sample collected locally from Jeddah region was processed and crushed to powder. The commercially available and locally collected *Spirulina* powder were dissolved in deionized distilled water to get a homogenized mixture. The mixtures were subjected to serial dilution e.g., 10-1, 10-2, 10-3, 10-4. The results (Table 1) revealed that each dilution when spread on plate produced different number of bacterial colonies after 24 h and 48 h of incubation. In addition, 10-1 dilution showed CFU/mL but all the other dilution such as 10-2, 10-3 and 10-4 showed no bacterial colonies.

Growth on different media: Different specified media such as chocolate blood agar, Eosin methylene blue, MacConkey agar, Mannitol salt agar, Sabouraud Dextrose Agar and *Salmonella-Shigella* agar were used to spread the 10-1 dilution of both collected and commercial *Spirulina* powder. The results (Table 2) showed that no colonies were grown in any of the above media. The absence of the colonies on any specified media revealed that there were no pathogenic bacterial or fungi present in the food such as Salmonella and Shigella. EU, European Union Pharmacopeia (Kneifel et al. 2002), WHO, World Health Organization (Belay, 2008). *Spirulina* refers to nutrition rich, cyanobacterial biomass that can be consumed by humans. The *Spirulina* has been investigated be very rich Proteinous supplement. The biomass of *Spirulina* is used

Table 2. Determination of pathogenic organisms

Selective media	Observations		EU standard	WHO
	Commercial sample	Local sample		
chocolate blood agar	No growth	No growth	No data	No data
Eosin methylene blue	No growth	No growth	No data	No data
MacConkey agar	No growth	No growth	No data	No data
Mannitol salt agar	No growth	No growth	No data	No data
Sabouraud Dextrose Agar	No growth	No growth	1.0 x104	1.0 x103
Salmonella-Shigella agar	No growth	No growth	Absent in 10g	Negative

interesting element to overcome the malnutrition especially in the countries where malnutrition and deficiencies in nutritional cause serious problems (Wu and Pond. 1981). It has been an ancient traditional food in many parts of the world and is remain a great source to overcome malnutrition as well as undernutrition in all over the world. In the present study, analysis of *Spirulina* local and commercial was conducted to assess the contamination in both samples. The presence of total bacterial micro-flora was resulted in local and commercial *Spirulina* in the dilution 10-1 only. However, 10-2, 10-3 and 10-4 dilution showed no colonies of the bacteria. The colonies in 10-1 dilution could be due to the violation of hygienic condition at the time of harvest. Unlike present results, other studies conducted on the importance of shelf life and packaging as well as nutritional values showed a significantly high concentration of bacteria which was assumed to be contamination of *Spirulina* samples (Tidjani et al., 2013 Laurencia et al. 2017).

According to the study of Karar et al. (2016), the absence of faecal coliform was due to the improve quality of the samples used in serial dilution followed by culture. Final out comes of the microbiological study should be according to the national and international standards (Belay, 2008; Dillon and Phan, 1993). Optimized standards such as coliform densities and standard plate count are being used to counter mal handling in food industries during food processing (FDA, 1998).In addition, when collected and commercial *Spirulina* suspensions were spread on different media e.g., MacConkey agar, Eosin methylene blue, chocolate blood agar, Mannitol salt agar, Sabouraud Dextrose Agar and *Salmonella-Shigella* no bacterial colonies were found. These results were comparable with the studies conducted on commercially produced Spirulina, which proved absence of pathogenic bacteria e.g., *Shigellae-Salmonella*, or Staphylococci in the *Spirulina* samples (Tidjani et al., 2013).

Some of the studies also show that fungi did not grow in *Spirulina* supplemented samples, and this is possibly due to the high alkalinity the culture medium is an excellent barrier against bacteria, fungi, or algae (Kumar et al., 2011, Butler and Day., 1998).Vermorel et al. (1975) performed a study on microbes associated with *Spirulina* and concluded that presence of the microbes were very rare or non-pathogenic in *Spirulina*. Moreover, alkaline pH of the growth media were also a factor to stop the microbial growth (Chapeland-Leclerc et al., 2005). In short, the *Spirulina* collected locally or commercially were almost free of pathogenic bacteria and could be used as food supplement. It is well known that, food industry required standard methods such as; bacterial plate counts to monitor the food products during processing (FDA, 1998). These methods differed from country to another in a small range). Plate counts of aerobic A. platensis in Sweden, France, California and Japan have been reported (Costa et al. 2003; Vonshak 1997).

Previous work by (Yousef et al., 2014) found that the total bacterial counts for spirulina platensis in closed and open system was between 1.2 x103- 1.4 x104 respectively. Vonshak (1997) as well reported that limits of the standard plate count for *Spirulina* in dry form are < 0.05x106 cfu g-1 in Japan, < 10x106 cfu g-1 in Sweden, < 0.1x106 cfu g-1 in France and < 1 x 106 cfu g in USA. In addition, it has been reported that the level of contamination would be decreased by drying process. Indeed, all obtained data shown in (table 1, 2) regarding the microbial contents were satisfactory according to the European Union and World Health Organization standards.

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