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Assessment of antibacterial activity of *Spirulina platensis* cultivated on camel urine medium, in vitro

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Abstract

This research sheds new light on the evolution of microalgae behaviour, specifically their interaction and adaptation strategies to atypical media. The present study was conducted to evaluate the antibacterial activity of *Spirulina platensis* cultivated in a medium made from camel urine at concentrations of 1:1, 1:2, 1:3 and 1:4 (v:v) during periods (7, 28, and 42 day). The test was performed by the disc diffusion method, against five types of human pathogenic bacteria [*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 25923), Methicillin-Resistant *Staphylococcus aureus* and *Proteus* spp]. Results showed that *S. platensis* grew and adapted well in most camel urine media, especially in T2 medium with biomass (5.1 g/L), chlorophyll a and b (2.4 and 1.2µg/ml), carbohydrates 40.0% (w/w), and total protein (24µg/ml). In contrast, the bacterial susceptibility test's results were unexpected. The maximum rate of pathogen-bacteria inactivation was attained in the last phase following 42 days. In detail, Significant activity was observed for *S. platensis* extracts cultivated in low-concentration camel urine medium (T4) against all bacterial species compared to *S. platensis* extracts naturally cultivated in standard medium. *Staphylococcus aureus* was the most sensitive species of bacteria to *S. platensis* extracts with an inhibition zone of 5.0 cm.

Keywords: Antibacterial, *Spirulina platensis*, Camel urine, Bio-control

1. Introduction

Cultivation of microalgae in photobioreactors has become a sustainable as well as viable way to deal with several environmental and energy-related issues [1]. In recent years, extensive research has been on the combined processes of pollutant removal, and lipid production through cultivating microalgae in domestic wastewater [2], [3]. Ancient folklore references the therapeutic use of camel

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urine, with its use in medicine dating back to Avicenna's The Canon of Medicine, written in the 10th century [4], [5]. Based on recent preliminary research, camel urine has active compounds, absent from human or cow urine [6]. Its composition includes purine bases such as hypoxanthine, along with sodium, potassium, creatinine, urea, uric acid, and phosphates [7]. In theory, urine precipitates could provide adequate amounts of phosphorus, calcium, and magnesium, to serve as an inexpensive media, especially with the addition of iron, EDTA, and inorganic carbon from the outside [8]. However, little is known about how metabolism affects microalgal growth in such media, the extent of production and accumulation of toxins, and its potential effect on photosynthetic pigments [9]. Other compounds formed from algae as secondary metabolites are likely to be very beneficial, particularly in the biological control of microbes [10]. Due to increasing reports of bacterial resistance to drugs and antibiotics, as well as the need for new materials or tactics to combat these microorganisms, microalgae have been considered as one of the potential solutions to this problem [11], [12]. After a throughout literature review, we have yet to find any attempts to cultivate cyanobacteria in camel urine media and explore its adaptability.

Therefore, this study aimed to evaluate the growth performance of *Spirulina platensis* in different concentrations of camel urine media and assess its antimicrobial activity against several human pathogens in vitro.

2. Materials and Methods

The strain *Spirulina platensis* was obtained from the Algae Bank, Department of Biology, Omar Al-Mukhtar University, Libya. The authors created a medium based mainly on camel urine to cultivate *S. platensis* as a simulation of the design of Zarrouk's Medium [13].

2.1. The procedure of cultivation

Samples of urine from camels were collected from their pens in the eastern Libyan district of Martouba region. It was brought to the lab for a standard urine analysis, and confirmed to be microbe-free. The nutrient medium for growing *S. platensis* was created by adding rising concentrations of urine to sterile water, following the ratio of the five nutritional conditions:

- T0: Zarrouk's Medium (control).
- T1: Urine camel + sterile water (1:1) (v/v).
- T2: Urine camel + sterile water (1:2) (v/v)
- T3:. Urine camel + sterile water (1:3) (v/v).
- T4: Urine camel + sterile water (1:4) (v/v).

This study relied on nutrients and elements found mainly in camel urine. Forty-two days were spent cultivating *S. platensis* in a glass tank, with a fluorescent light source running on a daily cycle of 14 hours of light and 10 hours of darkness during the cultivation phase. To lower the pH to 9.5, air pumps and NaOH were added. Over a six-day period, 20 ml of culture was sampled daily for further measurement. Using a previously established standard absorbance curve at 670 nm versus dry mass, the inoculum cell concentration (about 1.00 g/L) was ascertained. 10 ml of *S. platensis* growth medium was extracted for each test medium at 7, 14, 21, 28, 35 and 42 days. The extract was then centrifuged for 15 minutes at 3500 rpm after being filtered, pulverized, and combined with methanol. Several metabolic measurements were carried out by monitoring the rates of *S. platensis* cultivation. All the experiments were performed in triplicate.

2.2. Algal tests

2.2.1. Biomass productivity:

For all the cultures, biomass productivity was computed gravimetrically, according to the equation [14].

2.2.2. Determination of Chlorophyll (a and b) Pigments:

Using a spectrophotometer, the absorbance was measured at 470, 652.4, and 665.2 nm against blank methanol. The algorithm and chlorophyll extraction were carried out following the procedure [15].

2.2.3. Determination of total carbohydrates

The anthrone technique was used to determine the quantity of monosaccharides in hydrolysates following each pretreatment, according to Waghmare et al. 2016 [16]. Using glucose as the standard and water that is pure as a blank, the standard curve was constructed using the optical density values.

2.2.4. Determination of total Proteins:

The algae extract was placed in 10 ml of hot water and left for 2h. After cooling, the water extract was centrifuged and the supernatant was decanted and supplemented to a specified volume with distilled water and extinction was measured at 700 nm, according to the method adopted by [17]. A calibration curve was constructed using egg albumin and the data were expressed as µg albumin mg^{-1} dry weight.

2.3. Antibacterial susceptibility test

2.3.1. Bacterial strain

The microbial strains are identified strains and were obtained from Laboratories

- The MRSA strain used in the current study was con-firmed in-house clinical isolate from an Egypt hospital, (Al-Saraya Hospital).
- *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145) and *Staphylococcus aureus* (ATCC 25923).
- *Proteus* spp.: was provided by the bacterial collection, Department of Biology, Omar Al-Mukhtar University.

2.3.2. Spirulina platensis extracts Preparation

This experiment was divided into three phases: the seventh, twenty-eighth, and forty-second days, which correspond to the start of growth, mid-cultivation and harvest phase. The algal cells were harvested by centrifugation at 3000 rpm for 10 minutes, for all media. After drying, crude was extracted using acetone. Then, 10 g of dry powder of *Spirulina platensis* from each media was added to 20 ml of acetone, on a vibratory shaker at 35 ° C. After 24 hours the solution was centrifuged at 3000 rpm for 15 min, and the liquid solvent phase evaporated using a rotary evaporator maintained at 50 °C to obtain a dry powder.

2.3.3. Antibacterial Assay

The test was conducted using the Kirby-Bauer method. Pure bacterial colonies were taken with a loop and transferred to test tubes containing 0.1 ml of bacterial inoculum suspension. A sterile cotton swab in an L-shape was then used to spread the bacteria evenly on Mueller-Hinton agar plates. After 15

minutes, the discs of the extract were placed on the agar, and the plates were incubated at 37°C for 24 hours. The results were evaluated by measuring the diameter of the inhibition zones [18].

2.4. Statistical Analysis

All statistical analyses were performed using SPSS version 24 (Statistical Package for the Social Sciences)– which varies in culture conditions and biomass composition between all Media with Tukey's test. Statistical significance was set at $p < 0.05$ for all the analyses.

3. Results and Discussions

3.1. Algal Tests

Biomass was measured on days 7, 14, 21, 28, 35, and 42. The control culture medium showed statistically significant differences at ($P<0.05$), achieving the maximum biomass output of 5.9 g/L. The study's initial phases saw the rapid adaption of *Spirulina platensis* across all media. Up to the 28th day, the biomass production curve decreases for all media, including the control treatment. Based on the information in Figure 1. *S. platensis* can rapidly adapt to camel urine conditions in all types of media, especially in urine media (T2), which recorded a maximum biomass production of 5.1 g/L. The urine medium (T1) produced 3.3 g/L of biomass and came in second. In contrast, the biomass of two urine media (T3 and T4) decreased noticeably to 2.7 and 2.4 g/L. In general, the study's steepest decline is shown in the final day of cultivation.

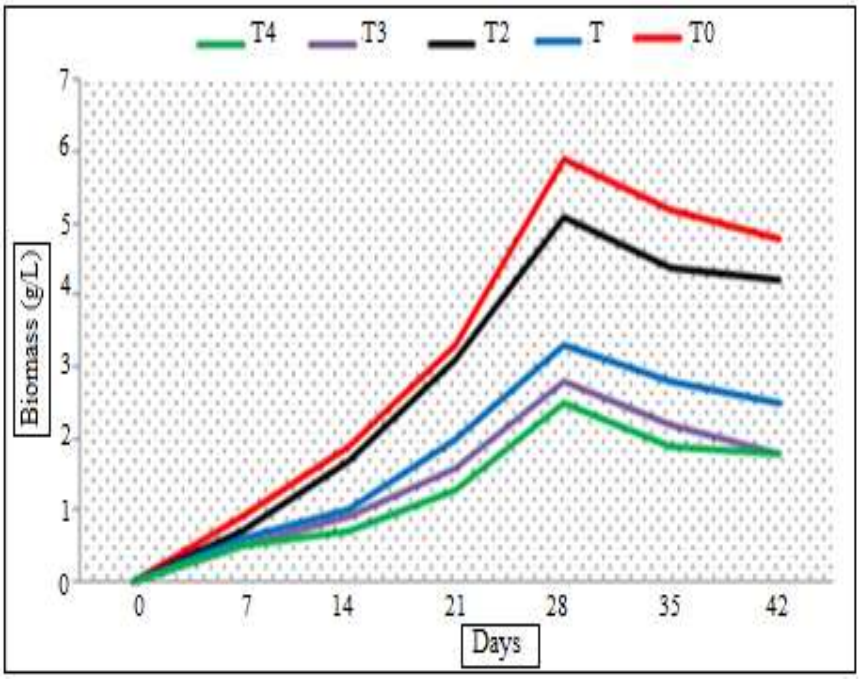

Figure 1. Biomass concentration curve to the culture of *S. platensis* of 42 days.

As demonstrated in Figure 2, the pigment content in all test media was significantly changed, reaching a maximum on the 28th day of all treatments by T1 (1.9), T2 (2.4), T3 (0.9) and T4 (1.1µg/ml) vs. 3.6µg/ml of the control). Similar to these results, all media's chlorophyll-b content decreased, especially at the end stages. Nevertheless, it was observed that, in contrast to what was recorded for all

indicators, the maximum values for chlorophyll-b were on day 35 for the majority of camel urine media, as illustrated in Figure 3.

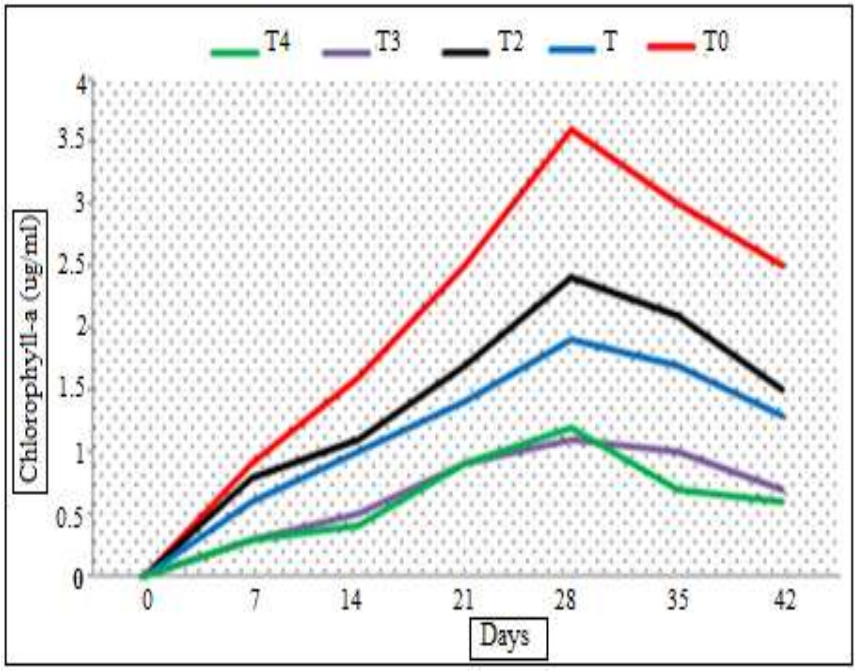

Figure 2. Chlorophyll-a curve to the culture of *S. platensis* of 42 days.

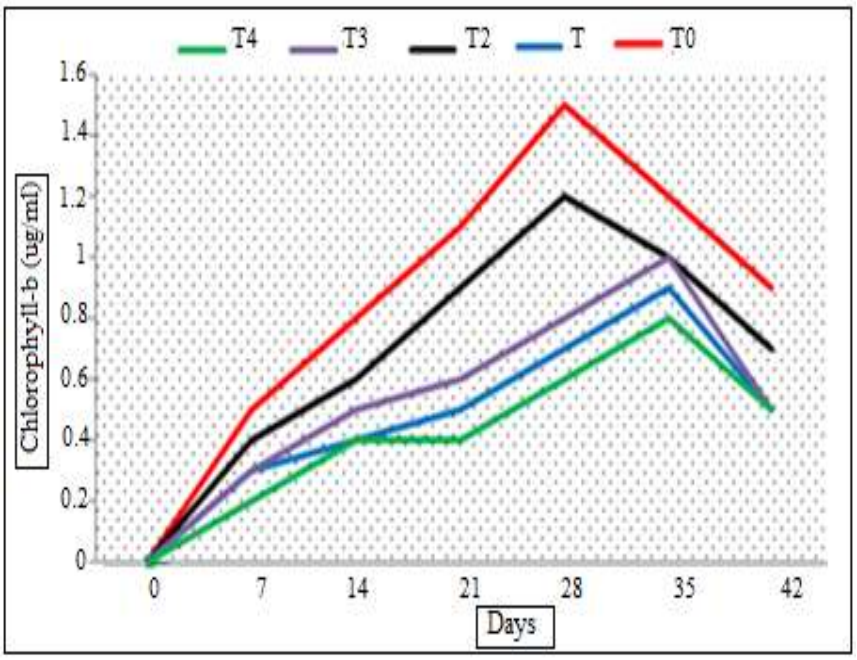

Figure 3. Chlorophyll-b curve to the culture of *S. platensis* of 42 days.

The relative contents of proteins and carbohydrates during the cultivation stage are presented in Figures 4 and 5, respectively. Treatments T0 and T2 of approximately 55 and 40% (w/w) indicate the final phase of the experiment, which is when the maximum carbohydrate rise was obtained. For all media evaluated by T1 (18), T2 (24), T3 (14), and T4 (12µg/ml compared to 37µg/ml of control), the

highest amount of protein was detected in mid-cultivation phase. The 21st Day marked the top of protein synthesis.

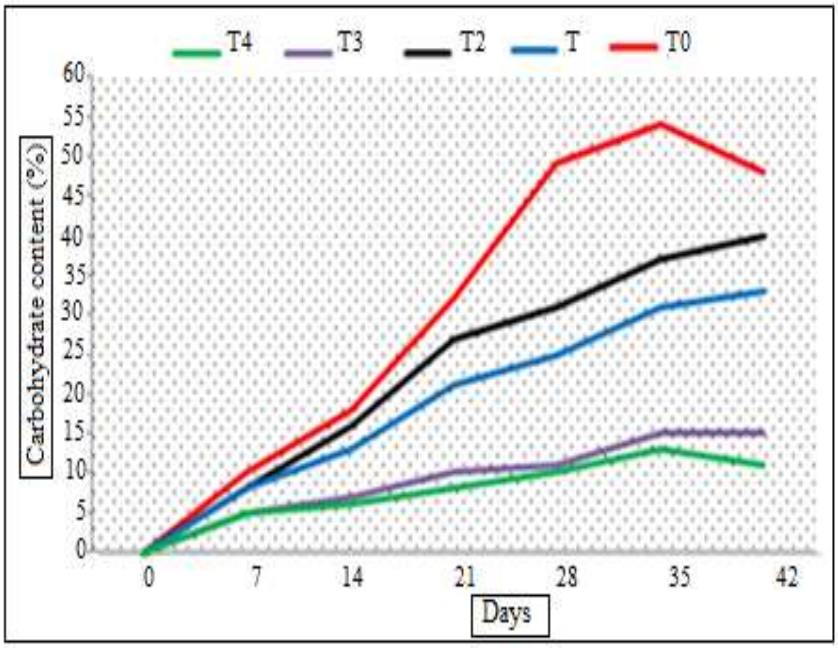

Figure 4. Carbohydrate content curve to the culture of *S. platensis* of 42 days.

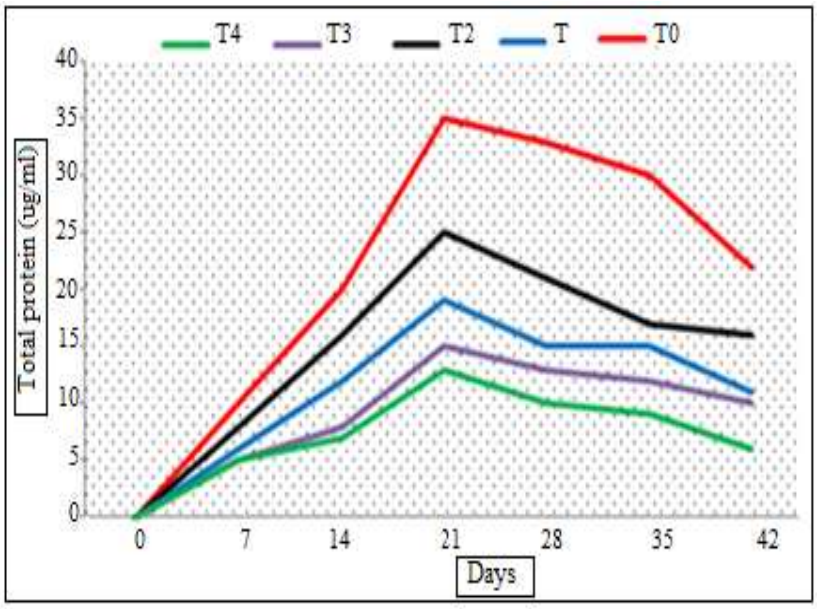

Figure 5. Total protein curve to the culture of *S. platensis* of 42 days.

This experiment was conducted on different cultures of urine camel media, which opened up the possibility of cultivating *S. platensis* on these new media, while meeting other cultivation requirements. This may be due to the high Ca, K, Mg, N and P levels in camel urine [19], [20], indicating the likelihood that animal feces can be used as a new growth medium for microalgae [21]. The results also showed that the Al-Zarouk medium represents the best medium for peak growth of *S. platensis* in all growth indicators. This is consistent with many studies that have tried to find suitable media that is

superior to this standard medium, but to no avail [22], [23]. However, this study presented camel urine (T3) as a medium that achieved somewhat satisfactory results in increasing pigment content, biomass yield, carbohydrate content, and total protein. While the adaptation and growth of S.platensis decreased with increasing camel urine concentration above (1:3 v/v). The result disagrees with [24], who confirmed that *S. platensis* growth rates were enhanced by adding more nitrogen to the culture medium. In addition, the 28th day represented the peak of optimal growth of *S. platensis* for all study media, this explains the decrease in the growth curve in the next stage due to the depletion of the basic elements of the medium, which was confirmed by [25].

3.2. Susceptibility of microorganism strains used in the assay

Disc diffusion results revealed that most of the extracts were ineffective against the majority of investigated bacteria when *S. platensis* was grown on camel urine medium for seven days, with inhibition zones ranging between (0.5-1 cm) (Table 1). After 28 days of culture cultivation, the antibacterial activity of the same prior treatments was shown (Table 2). Following 28 days of development, there was no discernible increase in the antibacterial activity of *S. platensis* cultures, with the inhibition zone measuring between 0.4 and 1 cm. Once *S. platensis* was harvested and tested for every harmful bacterium, the extracts' antibacterial efficacy varied (Table 3). Low-concentration extracts of *S. platensis* (T4) revealed a higher degree of inhibitory activity than other extracts, with the highest inhibitory capacity against *Staphylococcus aureus* (5.0cm), and moderate activity against *Proutes* spp. at a diameter (of 2.0cm), and Mid-low sensitivity against *E.coli* and *Pseudomonas aeruginosa* at a diameter (of 1.7, and 1.5cm) respectively. Additionally, T3 was effective against all strains of bacteria, the best of which was against *S.aureus* (3 cm), and the least against *P. aeruginosa* and MRSA with the same measure of inhibition (0.5 cm).

Table 1. Antibacterial activity of *S. platensis* extracts against pathogens bacteria after 7das.

Treatment	*E.coli*	*P.aeruginosa*	*S.aureus*	MRSA	*Proteus* spp
C (T0)	-	-	1.0±0.0 b	-	0.9±0.2 a
T1	-	-	1.5±0.4 a	1.0±0.0 a	-
T2	-	-	-	-	-
T3	0.5±0. a	-	0.5±0.2 c	-	-
T4	-	-	-	-	-

Table 2. Antibacterial activity of *S. platensis* extracts against pathogens bacteria after 28days.

Treatment	*E.coli*	*P.aeruginosa*	*S.aureus*	MRSA	*Proteus* spp
C (T0)	0.5±0.1 a	-	1.2±0.2 a	-	1.0±0.0 a
T1	-	-	-	-	0.4±0.0 b
T2	0.6±0.2 a	-	-	-	-
T3	-	-	0.7±0.2 b	-	-
T4	-	-	1.0±0.0 ab	-	1.0±0.2 a

Table 3. Antibacterial activity of *S. platensis* extracts against pathogens bacteria after 42days.

Treatment	*E.coli*	*P.aeruginosa*	*S.aureus*	MRSA	*Proteus* spp
C (T0)	1.0±0.2 b	-	2.5±0.0 bc	-	1.6±0.0 b
T1	-	-	-	-	-
T2	-	-	0.5±0.2 c	-	-
T3	1.0±0.3 b	0.5±0.0 b	3.1±0.3 b	0.5±0.1 b	1.0±0.0 c
T4	1.7±0.2 a	1.5±0.3 a	5.0±0.5 a	1.0±0.0 a	2.0±0.4 a

The adaptation that algae make in harsh or less-than-ideal environments may cause an increase in energy consumption leading to a decrease in photosynthesis. This decrease may lead to the accumulation of intermediate compound intermediates, which are acted upon by subsequent pathways to form secondary metabolites, and thus may play an important role in the production of antibacterial compounds [26]. Based on this, Sensitivity testing and biological control against bacteria achieved good results, as *S. platensis* extracts cultivated on camel urine at a low concentration, represented by treatment (T4), proved effective against most pathogenic bacteria. Although this medium did not show good growth indicators, *S. aureus* was the most sensitive bacteria to these extracts. There is no clear explanation for the results obtained in this study. By reviewing several pieces of literature, we found that when microalgae are exposed to stress, it may lead to the production of non-target toxic compounds [27]. Furthermore, this increased ionic pressure appears to affect the production of secondary metabolites by microalgae, as it's well known that camel urine is highly alkaline, especially due to the presence of high levels of potassium, magnesium, and albumin protein, and low concentrations of uric acid, sodium, and creatine, although, unlike the urine of other animals [28]. This may explain the decrease in growth indicators as evidenced and the lack of consumption of materials available in the medium by *S. platensis* in very high concentrations, and it may be what gave it activity against bacteria in the medium of low-concentration camel urine.

4. Conclusiones

In general, the results of the current study demonstrated that *Spirulina platensis* can be cultivated in camel urine media. The antibacterial activity results also indicated that *S.platensis* metabolites showed good results in inhibiting the growth of human pathogenic bacteria, and that modifications of the pigment compositions and metabolites could provide new avenues for investigating antibacterial substances. This study, the potential for *S.platensis* extracts to function as effective antibacterial agents in a novel setting. However, Further research is recommended for practical application.

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