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Original Articles

Antibacterial activity of *Spirulina* Hexane Extract

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Abstract:

Four different concentrations (0.3, 1.5, 3, and 15 g/ml) of *spirulina* hexane extracts were used to study the effects on different pathogens by well diffusion method. Two Gram positive (*Streptococcus mutans* and *Micrococcus luteus)* and four Gram negative (*Pseudomonas aeruginosa*, *Sphingomonas paucimobilis, Roseomonas gilardii,* and *Alcaligenes faecalis)* were used as test organisms. All the bacteria in current study were susceptible, but the higher inhibition zone was (21mm) with *Pseudomonas aeruginosa* in the concentration (15 g/ml) while the less inhibition zone was (11 mm) with *Streptococcus mutans* in the concentration (0.3 g/ml) and no inhibitory effect was found against *Sphingomonas paucimobilis* in the concentration (0.3 g/ml).

Key word: *spirulina,* hexane extracts

Introduction:

Algae are a source of food on Earth and a sign of potential changes in the aquatic environment (Prata *etal*., 2019), in addition to the efficient roll in bioremediation with domestic water by excreting different materials and the nitrogen fixation in various habitats (Abed *etal*., 2021; Abdulrahman *etal*., 2019). Spirulina is a tiny blue-green alga that exists in both fresh and salt water and has looks like of a spiral coil (Sivakumar & Santhanam, 2011). Due to their considerable amount of protein (35–65%) and nutritional value, some of them have been eaten by humans for a long time (El-Baz *etal*., 2013). Algal bioactivities include anti-oxidant, anti-diabetic, anti-inflammatory and immunomodulatory properties (Torres *etal*.,2017) because the existence of phenolic compounds, triterpenoids,steroids, flavonoids, and saponins(Afriani& Setyaningsih, 2019).On the other hand, Phycocyanobilin, polyphenols, carotenoids, chlorophyll, and other substances that have demonstrated in vitro antibacterial action, along with its high concentration of amino acids and short peptides, make it an uncommon resource for antimicrobials(Amaro *etal*., 2011). Bacterial infection has led to serious health issues all around the world. The current treatment strategy of using antibiotics has a number of disadvantages and leads to the bacterial resistance phenomena, meanwhile, antimicrobial resistance problems are a result of a progressive reduction in antibiotic discovery and development as well as the evolution of drug resistance in numerous human infections. Therefore, finding a natural antibacterial that is less dangerous and likely to cause adverse effects is urgently needed (Hidhayati *etal*., 2022; Hutchings *etal*., 2019). The aim of this study was to investigate the antibacterial activity of hexane extract of spirulina with different concentrations against gram-

positive and gram-negative pathogens in an attempt to find natural alternatives to antibiotics against bacteria.

Materials and methods:

Preparation of *spirulina* samples:

The *spirulina* collected from of the Euphrates River in the middle of Iraq. Washed from mud and dirt by tap water then dried at room temperature. Using an electric grinder turned to powder and saved in dry container, so it was ready for extraction as it had been lyophilized (Prescott *et al*., 1996).

Preparation of *spirulina* extract:

Using the hexane as non-polar solvent for extraction process by adding (5 gram) of *spirulina* powder to (250 ml) of solvent, stirred for 24 hours on magnetic stirrer then moved to Soxhlet apparatus with a reflux for 16 hours. Finally, filtered and concentrated at (50 C˚) to get a solid material that weights (1.6 g). Using DMSO that added to D.W. four concentrations were prepared (Al-Aarajy, 2012).

Used bacterial isolates:

Six bacterial species are used in this study, which are obtained from Al- Ameen laboratory in Al-Najaf Al-Ashraf city where the isolation, purification and identification process done as listed in the table1:

Table 1. bacterial isolates used in current study and their response to gram dye:

Bacterial isolates	Gram dye
Streptococcus mutans	G^+
Pseudomonas aeruginosa	G^-
Sphingomonas paucimobilis	G^-
Micrococcus luteus	G^+
Roseomonas gilardii	G^-
Alcaligenes faecalis	G^-

Preparation of culture media:

According to the instruction on the container, weight the powder media, dissolved in the suitable volume of D.W. then stirred then heated until completely dissolving. Finely sterilized by autoclave for 15 minutes, 15 lbs, and 121C˚. Take it out to cool for a while then pour into petri plates in sterilized conditions, keep them in refrigerator until use.

Antibacterial test:

Using agar well diffusion method, by a sterile loop, a small number of colonies moved to the test tube with 5 ml of D.W. and compared the turbidity with McFarland solution.

With a sterilized cork borer 10 mm five wells per plate was made and filled with 0.2 ml of spirulina hexane extract from different concentrations (0.3, 1.5, 3 , 15) mg/ml respectively and the D.W. as control. This process repeated three replicated each bacterial isolate. then incubated for 24 hours at 37C˚ ,when a clear zone observed it means that active antibacterial extract ,measured the diameter of inhibition zones with high accuracy(Faden, 2018).

Results and discussion:

The antibacterial activity of hexane extracts of *Spirulina* is presented in Table 2 and fig.1. All the concentrations exhibited different degree of antibacterial activity against tested microorganisms and the inhibition zones increase with the increase of concentrations whereas the higher inhibition zone was (21mm) with *Pseudomonas aeruginosa* in the concentration (15 g/ml) while the less inhibition zone was (11 mm) with *Streptococcus mutans* in the concentration (0.3 g/ml) and no response with *Sphingomonas paucimobilis* in the concentration (0.3 g/ml). These results are combatable with (Hetta *etal.*, 2014) that found *Pseudomonas aeruginosa* more susceptible than other organisms in the study, and disagree with (Nayyef & Thalij, 2020) which found *Sphingomonas paucimobilis* was susceptible to water extraction.

Many studies showed that when compared to other solvents, the Spirulina hexane extract

displayed the lowest inhibitory zone against bacterial and fungal pathogens (Usharani *etal*., 2015). Also (Gheda, & Ismail,2020) agreed with this result and explained the variation in inhibition zones with different solvents, could be explained by the various active metabolite compositions dissolved in these extracts. And (Karthika & Muruganandam ,2019) mentioned that bioactive compounds that extracted with methanol were clearly demonstrated than hexane.

On the other hand, the best methods for extracting the antimicrobial chemicals from the microalga were organic solvents with low polarity including hexane, due to the ability to extract the γ-linolenic acid, (Santoyo *etal*., 2006). Since the main rule of solvent extraction is "like dissolves like," each solvent only dissolves substances that have the same or similar polarity, so, alkanes, fatty acids, pigments, terpenoids, and alkaloids, will be extracted into hexane (Tavakoli *etal*., 2021). While (Martins *etal*., 2023) used nanofibers method for extraction the bio active compounds instead of organic solvents because they are unhealthy for the environment, the operator, and the health of customers.

In general, Gram-positive bacteria are easier to reduce or kill than Gram-negative bacteria because of the thickness of the cell wall, meaning that Gram-positive have just one layer, or a monolayer. Contrarily, Gram-negative bacteria have a thin cell wall with three layers (multilayer), which makes it difficult for such substances to be absorbed (Naviner *etal*., 1999).

According to reports, bacteria penetrate the guts of both people and animals via the same mechanisms of adhesion and invasion, and the antimicrobial action of spirulina may be explained by its ability to prevent pathogen motility, invasion, biofilm formation, and quorum sensing (Abd El-Hack etal., 2019; Abdel-Moneim etal., 2020).

Table 2. Antibacterial activity of hexane extracts of Spirulina

Con. / bacteria	0.3 g/ml	1.5 g/ml	3 g/ml	15 g/ml
Streptococcus mutans	11	11	13	14
Pseudomonas aeruginosa	13	16	19	21
Sphingomonas paucimobilis	0	14	17	17
Micrococcus luteus	12	13	17	17
Roseomonas gilardii	13	15	20	20
Alcaligenes faecalis	13	14	18	18

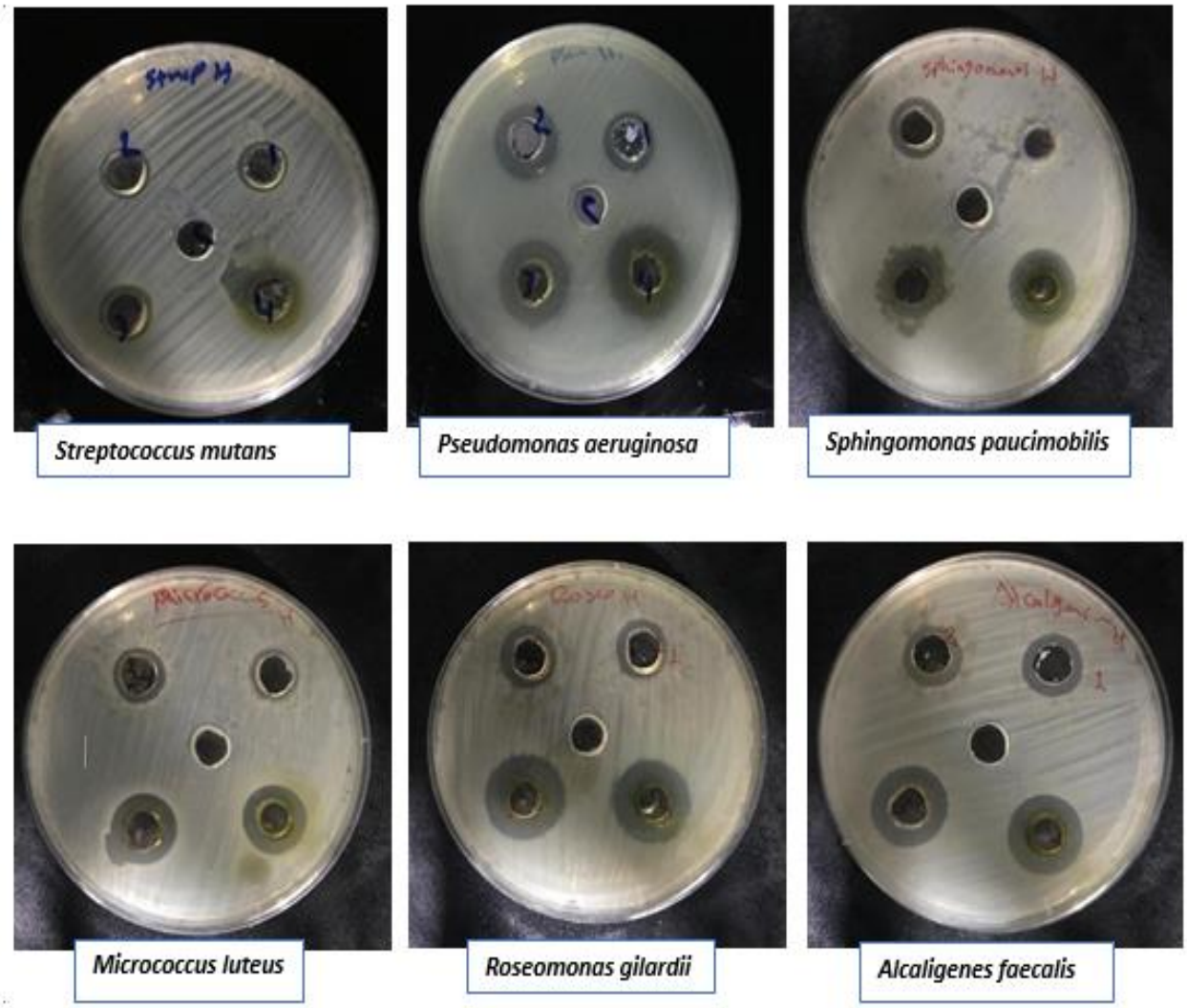

Fig.1 the inhibition zones of *spirulina* hexane extracts against *Streptococcus mutans, Pseudomonas aeruginosa, Sphingomonas paucimobilis, Micrococcus luteus, Roseomonas gilardii,* and *Alcaligenes faecalis*

Conclusions:

The hexane extracts inhibited the growth of the tested microorganisms and the Gram negative are the most affected pathogens. The findings show that *spirulina* has a variety of antibacterial activities that support some of its conventional usage and suggest that it may have use in pharmaceutical sciences.

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