



EFFECT OF ETHANOL AND ALKALOID EXTRACT OF *SPIRULINA PLATENSIS* AGAINST DERMATOPHYTE FUNGI

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Abstract

The present study included isolation, diagnosis and purification of *Spirulina platensis* from the Euphrates River in the province of Thi-Qar Province, the algae was cultured and propagated in laboratory by the use of special media Chu10. The alcoholic and alkaloid extracts were prepared for the purpose of conducting qualitative tests of the chemical groups contained in the algae extract and to determine the biological and pharmaceutical efficacy of these compounds against dermatophytes under study, they were isolated from patients coming to Imam Hussein Teaching Hospital and for the period from March to July 2019, and most of the isolated dermatophyte fungi are *Trichophyton rubrum*, *T. interdigitale*, and *T. concentricum*. The results show there is no effect of both alcoholic and alkaloid extracts isolated from *Spirulina platensis* on *Trichophyton rubrum* at concentration 50mg, while it showed efficacy at concentration 100 mg and activity increased at concentration 200mg at P. value (≤ 0.05), while the alcoholic and alkaloid extracts showed an effect on *Trichophyton concentricum*, *Trichophyton interdigitale*, and their activity was increased with increasing the concentration at P. value (≤ 0.05).

Keywords : Dermatophyte, *S. platensis*, Bioactive Compound.

Introduction

Cyanobacteria are potential sources of high value chemicals and pharmaceuticals, *Spirulina* the most potential cyanobacteria used in medicine. *Spirulina* is safe for human consumption as medicine, because it is free of micro-cystin toxin and the long term dietary supplementation of up to 5% of the *Spirulina* may be consumed without evident toxic side effects (Hayes, 2012; Rehab, 2012). It is known to produce intracellular and extracellular metabolites with diverse biological activities such as antifungal, antiviral, and antibacterial activities (Mayer and Rodríguez, 2013).

The ability of seaweeds to produce secondary metabolites of potential interest has been extensively documented. There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antibiotics (antibacterial and antifungal properties), antiviral diseases (Ahn and Michael, 2017).

Dermatophyte fungi or dermatophytes comprise a vast range of filamentous pathogenic fungi including three important genera of *Trichophyton* (*T.*), *Epidermophyton* (*E.*), and *Microsporum* (*M.*), which may lead to superficial infections in both humans and animals-zoonosis. However, *Pityriasis versicolor*, *Saccharomyces cerevisiae*, and *Candida* spp. as opportunistic pathogenic fungi are capable of causing superficial mycotic infections in human beings (Roopa and Biradar, 2015; Islam *et al.*, 2018).

Materials and Method

The isolates of *S. platensis* algae were collected from the Euphrates River in Thi-Qar province during the spring to autumn 2019 period and the method was adopted by (Yang and Wang, 2014). For the purpose of making sure that the isolation is free from organisms contamination, the sample was washed with distilled water and then centrifuged at 3000 rpm for a minute and a half and neglected the leachate, then the process was repeated for twelve times, and the method obtained by was used to verify the purity of the isolation, which included the microscopic examination of the isolates after culturing them on the nutrient agar, then incubated at a temperature 37 °C for 18 hours, then the culture was

examined after repeated the process several times to ensure that it was free of germs and was considered a axenic culture.

Clinical samples of skin infections were collected for different age, and from both sex of patients that clinically diagnosed with Tinea by specializing doctors in dermatology consultant at Al-Hussein Teaching Hospital in the Nasiriya province from the period January 2019 to March -2019 and included 50 samples.

The infected area was treated with 70% ethyl alcohol to reduce bacterial contamination, then small scales were taken from the edge of the ulcers using a sterile scalpel. Hair samples were obtained using sterile forceps, pieces of infected nails were taken with debris that opened the nails after being sterilized, finally the samples were placed in a clean paper bag until they were brought to the laboratory for performed microscopic and cultural tests to diagnose the causative fungus (Islam *et al.*, 2018).

Results

Describe *Spirulina platensis*

In the present study *Spirulina platensis* algae was purified and cultured, it is characterized with a spiral filament structure and irregular in shape and not branched, the threads are spiral narrow single or intertwined with each other and the cell has crossed walls and the length is greater than the width, the apical cells are round and the length between 7-10 microns and 4-6 microns wide, the algae is a dark green color and has a thin cell wall. It is found in freshwater, marine and brackish water, which has a high alkaloid degree up 10.

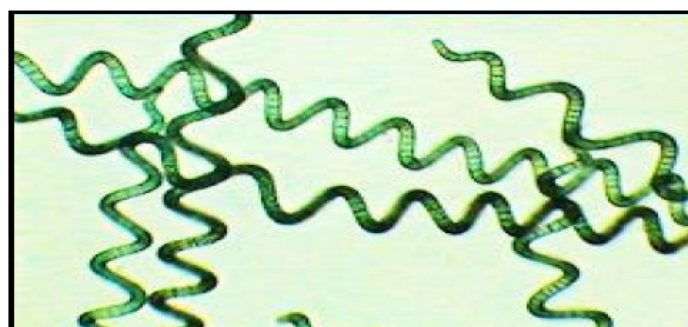


Fig. 1 : *Spirulina platensis* under microscope 40x

Table 1 : Qualitative disclosures for ethanol and alkaloid extract for *S. platensis*

Chemical Groups	Alcoholic & Alkaloid Extract	
	Alcoholic	Alkaloid
Alkaloids	+	+
Peptides	+	-
Proteins	+	-
Carbohydrates	+	-
Phenols	+	-
Soaps	+	-
Tannins	+	-
Glycoside before decomposition	+	-
Glycoside after decomposition	+	-
Terpenoids	+	-
Coumarins	-	-
Flavonoids	-	-

Organic Compounds Extract from *Spirulina platensis* by Alcoholic Extract

Organic compounds were extracts from *Spirulina platensis* algae by GC-Mass technology in laboratories of the Environmental Research Center / Ministry of Science and Technology. The results showed that the alcoholic extract

contained twenty-three organic compounds, each of which constitutes a certain distance. The organic compound that have high effectiveness on the dermatophyte are those that have a higher distance from other organic compounds as shown in the table (2).

Table 2: Organic Compounds Extract from *Spirulina platensis* by Alcoholic Extract

Peak	R. Time	Common Name	Peak Report TIC Area%
1-	2.036	Ethan, Fluoro	65.72
2-	2.939	Acetic Acid	8.87
3-	4.965	Dimethyl Sulfoxide	1.01
4-	18.995	E-2-Tetradecen-1-ol	0.08
5-	20.397	9-Hexadecenoic Acid	0.12
6-	20.626	Hexadecanoic Acid	1.85
7-	21.902	Phytol	3.26
8-	22.169	Gamolenic Acid	1.46
9-	22.372	Ethyl 9,12-hexadecadienoate	2.86
10-	22.661	Octadecanamide	7.55
11-	23.335	8-Methyl-6-nonenamide	0.09
12-	24.111	Methyl (Z)-5,11,14,17-icosatraenoate	2.27
13-	24.346	13-Docosenamide	3.23
14-	24.448	9-Octadecenamide	0.33
15-	24.532	17-Octadecynoic Acid	0.14
16-	25.220	Hexadecanoic Acid	0.16
17-	25.698	1,2-Benzenedicarboxylic Acid, diisooctyl ester	0.12
18-	25.791	Tetracyco[5.3.1.1(2,6).0(4,9)] dodeccan-3-one	0.09
19-	26.786	Sulfurous Acid	0.12
20-	26.887	7-Tetradecenal,(Z)-	0.17
21-	28.395	Eicosane, 7-hexyl	0.10
22-	29.341	Heptadecane, 2,6,10,14-tetramethyl	0.04
23-	30.467	Tetracosane, 11-decyl	0.36
			100%

Organic Compounds Extract from *Spirulina platensis* by Alkaloid Extract

Organic compounds were extracts from *Spirulina platensis* algae by GC-Mass technology in laboratories of the Environmental Research Center / Ministry of Science and Technology. The results showed that the alkaloid extract

contained fourteen organic compounds, each of which constitutes a certain distance. The organic compound that have high effectiveness on the dermatophyte are those that have a higher distance from other organic compounds as shown in the table (3).

Table 3 : Organic Compounds Extract from *Spirulina platensis* by Alkaloid Extract

Peak	R. Time	Common Name	Peak Report TIC Area%
1-	2.035	Ethane, Fluoro	98.41
2-	2.611	Acetic Acid	0.07
3-	18.216	Lauroyl Peroxide	0.07
4-	18.513	7-Oxabicycol[4.1.0] heptan	0.03
5-	19.001	Hexadecanal	0.04
6-	19.897	2-(2-Diethylaminoethylamino)ethanol	0.03
7-	20.180	13-Tetradec-11-yn-1-ol	0.04
8-	20.432	n-Hexadecanoic Acid	0.46
9-	20.603	Ethyl Tridecanoate	0.07
10-	21.865	Phytol	0.18
11-	22.217	Oleic Acid	0.44
12-	22.360	12-Methyl-E,E,13- Octadecadien-1-ol	0.09
13-	22.551	Nonadecanamide	0.02
14-	25.688	1,2-Benzenedicarboxylic Acid	0.05
			100%

Active Organic compounds Extracts from *S. platensis* by Alcoholic and Alkaloid Extract

The table represents the mass spectrometry of organic compounds that formed a high percentage and isolated from

the *Spirulina platensis* by alcoholic and alkaloid extract which is believed to have pharmacological and biological activity against dermatophyte under study.

Table 4 : Active Organic compounds Extracts from *Spirulina platensis* by Alcoholic Extract

Peak	R. Time	P. R. Area	Comp Name	M. Weight	Chemical Structure
1	2.036	65.72	Ethan, Fluoro	48 KD	C ₂ H ₅ F

Activity of organic compound Extracted from Algae on Dermatophyte Fungi

(i) Activity of organic compound Extracted from *S. platensis* on *T. rubrum*

The results of the present study showed that the alcoholic and alkaloid extract that isolated from algae have a biological and pharmaceutical activity on *T. rubrum*. The results also showed there are a very strong correlation between the extract concentration and the inhibition diameter on fungal culture, and the effectiveness increased by increasing the concentration of the extract and within a significant level as in the table (5).

(ii) Activity of organic compound Extracted from *S. platensis* on *T. interdigital*

The results of the present study showed that the alcoholic and alkaloid extract that isolated from algae have a

biological and pharmaceutical activity on *T. interdigital*. The results also showed there are a very strong correlation between the extract concentration and the inhibition diameter on fungal culture, and the effectiveness increased by increasing the concentration of the extract and within a significant level as in the table (6).

(iii) Activity of organic compound Extracted from *S. platensis* on *T. concentricum*

The results of the present study showed that the alcoholic and alkaloid extract that isolated from algae have a biological and pharmaceutical activity on *T. concentricum*. The results also showed there are a very strong correlation between the extract concentration and the inhibition diameter on fungal culture, and the effectiveness increased by increasing the concentration of the extract and within a significant level as in the table (7).

Table 5 : Activity of organic compound on *T. rubrum*

Fungi	Alcoholic Extract	CON%	Zone of Inhibition / CM ²
<i>T. rubrum</i>		50	0
		100	17
		200	21
R= 1.0		P. Value (0.00)	
Fungi	Alkaloid Extract	CON%	Zone of Inhibition / CM ²
<i>T. rubrum</i>		50	0
		100	16
		200	20
R= 1.0		P. Value (0.00)	

Table 6 : Activity of organic compound on *T. interdigital*

Alcoholic Extract		CON%	Zone of Inhibition / CM ²
Fungi	<i>T. interdigital</i>	50	18
		100	20
		200	22
R= 1.0		P. Value (0.00)	
Alkaloid Extract		CON%	Zone of Inhibition / CM ²
Fungi	<i>T. interdigital</i>	50	19
		100	20
		200	30
R= 1.0		P. Value (0.00)	

Table 7 : Activity of organic compound on *T. concentricum*

Alcoholic Extract		CON%	Zone of Inhibition / CM ²
Fungi	<i>T. concentricum</i>	50	0
		100	23
		200	30
R= 1.0		P. Value (0.00)	
Alkaloid Extract		CON%	Zone of Inhibition / CM ²
Fungi	<i>T. concentricum</i>	50	0
		100	14
		200	20
R= 1.0		P. Value (0.00)	

Discussion

Importance of Isolated Algae

Spirulina platensis algae in current study were isolated from the local aquatic environments being the most suitable environments for green algae, especially algae under study (Christaki *et al.*, 2013). The high content of substances with biological and pharmaceutical activities of these algae encouraged the isolation of these organic compounds from them and extracted by alcoholic and alkaloid methods and purified and then uses to know their medical importance and therapeutic capacity in various fields, especially in the treatment of dermatological infection caused by fungal (Raja, 2013).

The algae in the current study contains a large number of photosynthetic dyes and auxiliary dyes, especially carotenoids and xanthophylls, as well as dye vicocyanin attributed to most of the antioxidant properties possessed by these algae, as well as the nutritional value found in these algae, it is used as a dietary supplement in many countries because it contains 55-75% proteins, 12-25% sugars, 18 % essential amino acids, and salts, minerals and vitamins (Brandt, 2015). Other reasons that lead to isolating *Spirulina platensis* algae being non-toxic algae and easy to collect and deal with compared to some types of bluish green algae and green algae (Breuer *et al.*, 2012). Several international and local studies have been confirmed the medicinal important of a number of algae species, especially those species, including a study (Harith, 2012; Khalaf and Amery, 2017; Alsereah, 2018).

Growth Rate

The rate of growth is a rapid growth criterion, which refers to the rate of division of biomass during a particular unit of time, the *S. platensis* was harvested in the middle of the stationary phase based on the growth curve which was determined based on the absorbance measurement. While the exponential phase is the phase of cell division and multiplication, therefore, this stage needs energy, which led to the demolition of some of the energy-canceling substances such as carbohydrates, proteins and fats, therefore.

The stationary phase is considered the appropriate phase of the harvest as it is the phase of production of chemical compounds rather than demolition, because the microorganism reaches the maximum production of secondary metabolites in the middle of the stationary phase, although it produces such materials before this period sometimes.

The current study greed with the previous study obtained by Wang *et al.* (2010), they study the effect of some organic compound extracted from *Chlorella vulgaris* on patients with lung cancer, and record that the algae was harvested in the stationary phase, the present study has reached the same conclusion that reached by El-Baz *et al.*, (2013), they study the reduction of infectious viral units after treatment with ethanol extract of *Spirulina platensis*, and recorded the chemicals compounds were harvested with the alcoholic extract in the medium of the stationary phase. While the study of Nuhu, (2013), she study activity of alcoholic extract against *Candida* Spp and *Aspergils*, and record the best time to harvested organic compound from culture in the beginning of the stationary phase.

The reason for harvesting algae in the stationary phase, the growth began to decrease due to the consumption of most nutrients in the culture medium and the introduction of some undesirable substances to the medium as a result of metabolic activities and competition was the rest of the growth requirements, in this phase there was a clear reduction in the mass of algae and this refers to the decline phase after which the phase of death began, in which cell death and degradation occurred as a result of the accumulation of undesirable substances from the algae to the medium as well as the depletion of nutrients, as well as the existing algal density, which prevented the arrival of light to all the medium homogeneously, consequently, the culture conditions there for changed as pH, lack of nutrients and accumulation of harmful substances, which was negatively reflected on the growth of algal cells and caused their death, therefore, algal isolation was collected in the middle of the stationary phase between 14-16 days of culture.

Chemical Reagent of the Extract

Chemical reagents are of great importance to detect the nature of active chemical families in the alcoholic and alkaloid extract, especially secondary metabolites compounds, because of its have high medical effectiveness (Imhoff and Labes, 2011).

The results of the present study of *Spirulina platensis* alga extract showed the presence of ethanol, fatty acids, peptides, carbohydrates, flavonoids, glycoside and tannins and did not contain flavonoids, alcoholic and alkaloid extracts are solvents from solvents that extract compounds of biological and pharmaceutical efficacy, especially polar medium,

Cyanobacteria and green algae produce a wide range of biologically active secondary metabolites compounds, Our results agreed with study of (Rawat and Bhargava, 2011), they studied on *Nostoc commune* and isolation and diagnosis a diterpenoid compound know Noscomin that have biological activities such as antifungal, antibacterial, antiviral, anticancer, antitumor, algicide, enzyme inhibiting, immunostimulant, antiplatelet aggregation, cytotoxic and antiplasmodium activities, the results of the current study have reached similar results Raja, (2013), he isolate protein, carbohydrates, and lipids from many algae including *Chlorella* and *Spirulina*, and recorded some materials isolated from algae have proven effective on fungi, bacteria, virus, and as antioxidant.

Spectrometry Gas Chromatography-Mass

One of the most important techniques used in the isolation and diagnosis of secondary metabolites compounds from alcoholic and alkaloid from cyanobacteria and green algae and this is confirmed by a study (Hayes, 2012), The following materials have been isolated and proven effective on skin fungi, Ethyl Tridecanoate, Phytol, Gamolenic Acid, Hydroxylamine, O-methyl, Ethan and Fluoro and their derivatives, which is due to the biological and pharmaceutical activities on skin fungi, which have been observed in the present study and this is confirmed by a study of Cox and Abu-Ghannam, (2010), they study activity of bioactive compounds isolate from *Spirulina platensis* against pathogenic bacteria, and recorded the organic compound isolated from *Spirulina platensis* by alcoholic extract such as phytol (2-hexadecen-1-ol,3,7,11,15-tetramethyl) and their

derivative neophytadiene have antimicrobial activity usually attributed to long-chain unsaturated fatty acids ($C_{10}H_{20}O$, $C_{16}-C_{20}$).

The current study the GC-Mass of the alcoholic extract of *Spirulina platensis* contain twenty three organic compound one of them has a highest percentage and represented by Ethan, Fluoro 65.72%, while the alkaloid extract for *Spirulina platensis* contain fourteen organic compounds one of them has a highest percentage and also represented by Ethan, Fluor 98.41%, which gave the largest percentage of the total area of organic compounds diagnosed by GC- Mass, organic compounds isolated from natural sources that occupy the largest percentage of the total area are compounds to which various biological and pharmaceutical activities (Hayes, 2012).

Dermatophyte and Activity of Organic Compounds Isolated

The present study indicates the efficacy of the alcoholic and alkaloid extract of *S. platensis* on dermatophyte fungi and showed that both alcoholic and alkaloid extract had no effective effect on the *T. rubrum* and *T. concentricum* when the concentration of extracts was 50, while the efficacy was shown on *T. interdigital*, however, the efficacy of the extracts increased on all dermatophytes under study when the concentration of the extract was 100, and the extracts gave the strongest effect on all the fungi when the concentration of the extracts was 200, The present study found that the effectiveness of the extract in inhibiting the growth of dermatophyte fungi has a direct relationship with increased concentration and vice versa. This may be attributed to the increase in the concentration of active substances in the extract by increasing its concentration, which plays an important role in inhibiting the growth of fungi. It can also be explained by increasing the inhibitory capacity of the extract by increasing its concentration to the accumulation of substances outside the cell membrane, which affects the permeability of the cell membrane and the work of transporting enzymes (Roemer *et al.*, 2011).

The efficiency of the alcoholic extract may be due to the organic matter in its raw form, as it has been proven to be more effective compared to the alkaloid extract due to the presence of a mixture of effective compounds that have been isolated from algae and that cooperate with each other in the raw extracts to inhibit the growth of fungi, due to the synergy phenomenon, which gives more effectiveness than an organic compound alone, or the ability of ethyl alcohol to dissolve many active substances dissolved in alcohol and in other polar and non-polar solvents, such as phenols, tannins, and resins, which are responsible for the efficacy of stabilization Fold the growth of fungi.

The results of the present study were also shown among the organic compounds extracted in both alkaloid and alcoholic methods, which proved to be effective against dermatophytes under study is Fluoro with percentage 65.72 and RT 2.036, the study obtained by Rivero *et al.* (2012), they study biosynthesis of 5-fluoro-2-dioxuridine one derivative of Fluoro and used therapeutic agents against malignant tumors, they recorded the efficacy of the synthetic organic compound towards malignant tumors,

while the study of Rames *et al.* (2003), in Tamilnadu, India, they studied manufacturefluorine-6-dyuro-di-hydro-4-

oxo-quinoline-3-carboxyl derivatives as potential antifungal and antibacterial agents, and record all the compounds exhibited significant antibacterial and moderate antifungal activities for *Aspergillus niger*, the reason for the difference between the effectiveness of the organic compound Fluoro between my studies and the previous study may be due to several reasons including different source of Fluoro as well as different fungi that applied to the effectiveness of the organic compound (Ordog, 2000).

The current study also show the activity of Fluconazole against dermatophyte under the study with use Chloramphenicol for inhibit bacterial growth and Cychloheximide for inhibit growth of non-pathogenic fungi, the result show the Fluconazole inhibit the growth of *T. rubrum*, while the Fluconazole don't inhibit growth of another fungi, the results of the current study showed similar results obtained by Wirth and Goldani, (2012), in Italy, they isolated *Rhodoturella* Spp in patients with prostate swelling who use catheters for urination, and recorded the patients were treated with amphotericin B and fluconazole after catheter removal had recovered from fungal infection, also the study obtained by Harwood, (2016), he studied the therapeutic response to *Candida* Spp were isolated from several hospitals to fluconazole, and concluded the 63.2% *Candida* species were resistant to fluconazole therapy while all isolates were sensitive to Filastatin, the variation in the therapeutic response of the same fungus that treated by the same antifungal agent, the difference in inhibition rates is due to the nature of the fungus itself in terms of the disparity in the composition of cell membranes and their thickness and the size of the fungal cells and the difference in the speed of growth between the fungi that have thick walls that are more resistant to the action of active compounds in the extracts because this impedes the penetration of these compounds into the cells to affect them (Ahn and Michael, 2017), likewise, the fungi with relatively large cells are more affected by the action of these compounds compared to the fungi with small cells, and the reason for this is due to the increase in the area exposed to the action of these compounds in the cells of large size, then they penetrate through the cell membrane inside to affect there, as well, the fungi are slow Growth is more sensitive to the action of compounds than those that grow rapidly, due to the fact that these compounds in the case of slow-growing fungi have got enough time to be in direct contact with the cells so that they penetrate into them and affect cellular enzymes.

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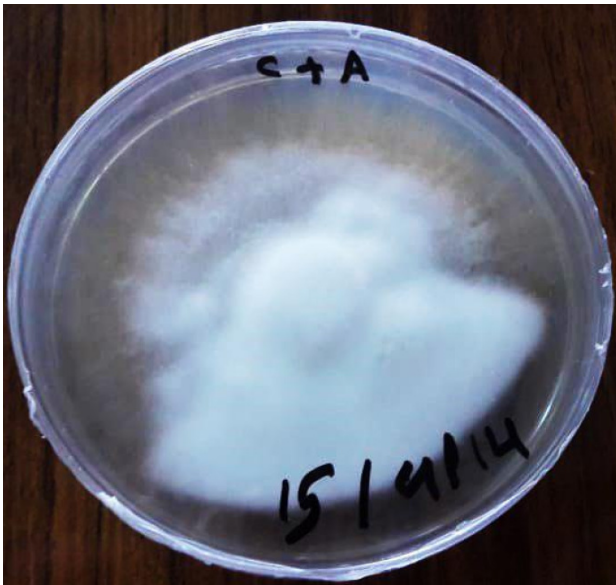
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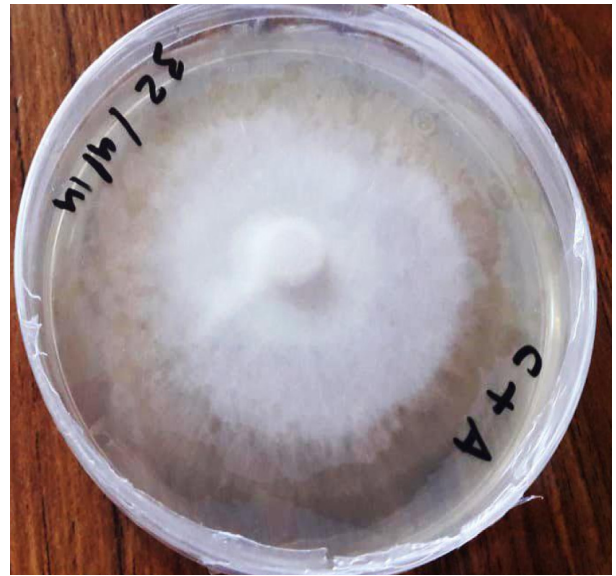
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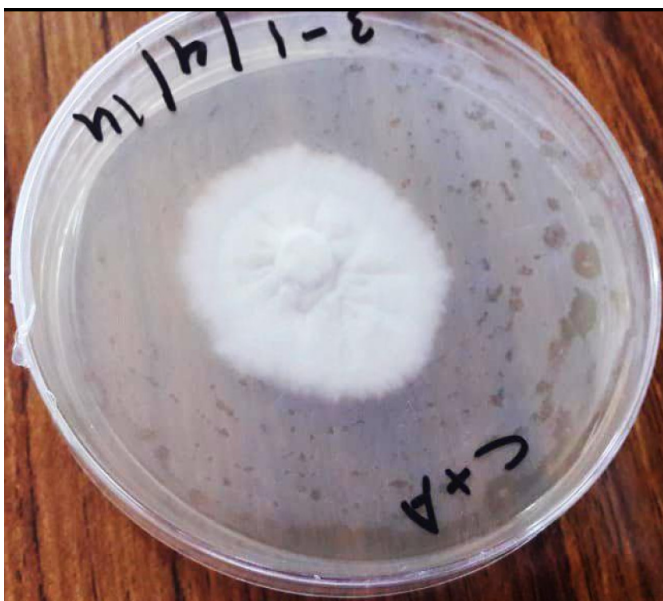
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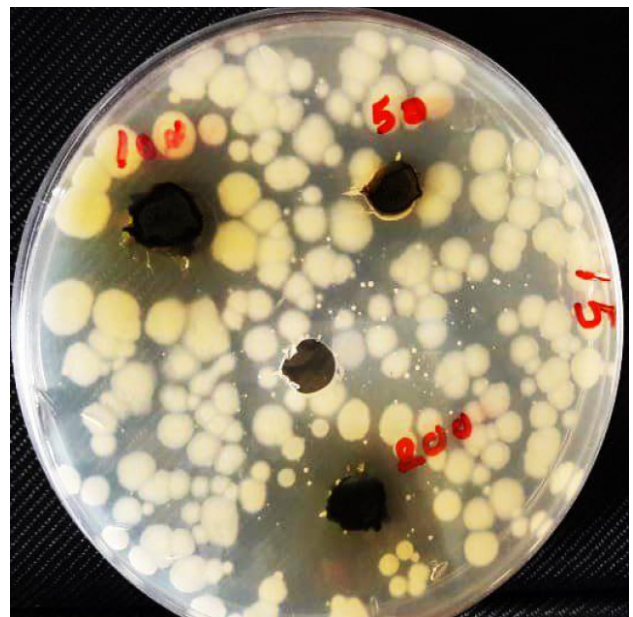
Appendix(1): *T. rubrum* on the Potato Dextrose Agar



Appendix(3): *T. interdigitale* on the Potato Dextrose Agar



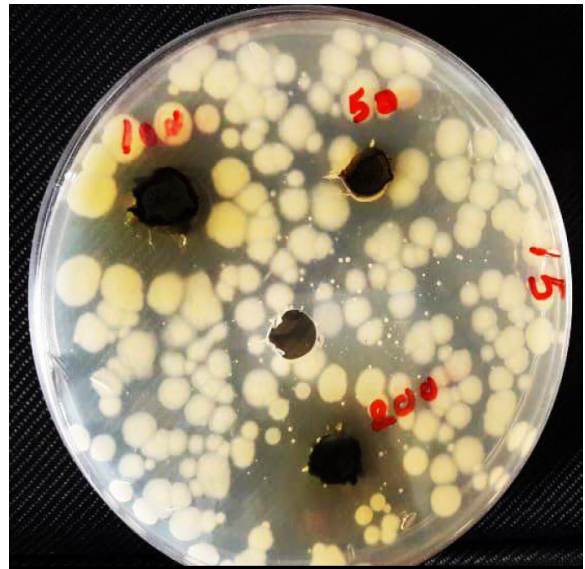
Appendix(2): *T. concentricum* on the Potato Dextrose Agar



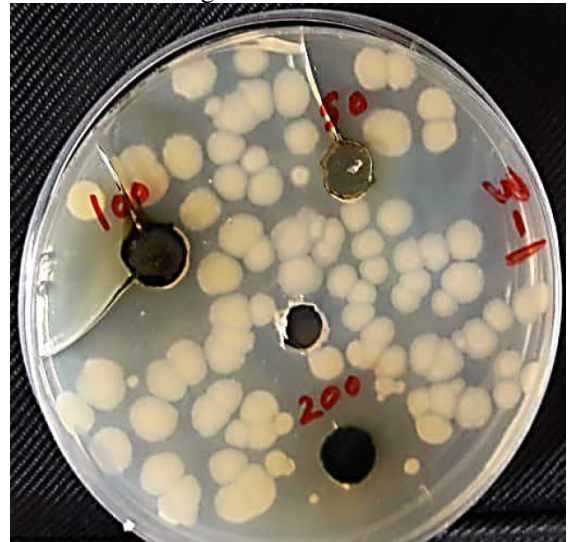
Appendix (4): Effect of alkaline extract of *S. platensis* against *T. rubrum*



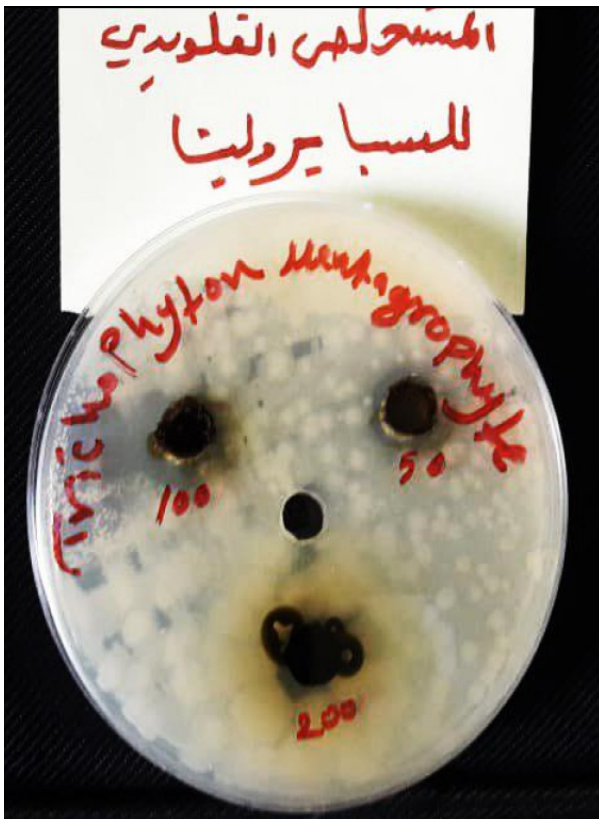
Appendix (5): Effect of alkaline extract of *S. platensis* against *T. concentricum*



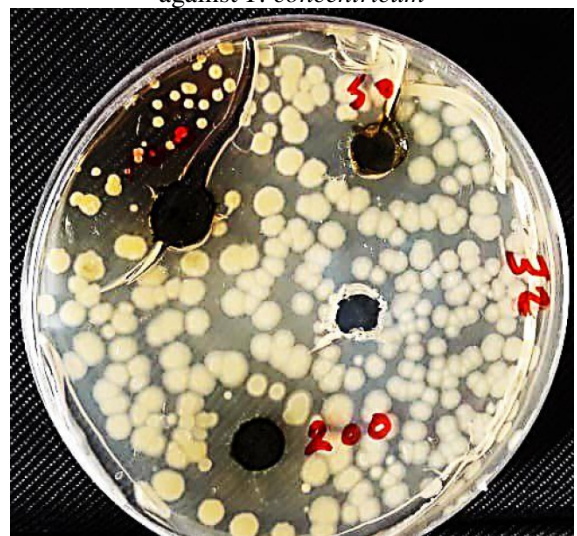
Appendix (7): Effect of alcoholic extract of *S. platensis* against *T. rubrum*



Appendix (8): Effect of alcoholic extract of *S. platensis* against *T. concentricum*



Appendix (6): Effect of alkaline extract of *S. platensis* against *T. interdigitale*



Appendix (9): Effect of alcoholic extract of *S. platensis* against *T. interdigitale*