Spirulina platensis growth curve.

The biomass concentration achieved in the 9th day was the highest value 111.70 $\pm$ 1.13 mg dry wt./100 ml. It was clear from the data presented in table (1) and figure (1). that, biomass production gradually increased from the 1st day until the 9th day then it decrease to the minimum biomass yield of 64.3 $\pm$ 0.78 mg dry wt./100 ml at the end of 13 days.

Effect of different nitrogen and phosphorus sources on biomass production of *S. platensis*.

Sodium nitrate (standard nitrogen source) concentration (2.5 g/L) gave the highest value of biomass 132.267±0.902 mg dry wt./100 ml as hllustrated in table (2). A significant decrease in biomass production was observed when sodium nitrate in the growth medium was replaced by urea 95.6 ± 1.1012 mg/ 100 ml, table (2). On the other hand K_2HPO_4 (standard phosphorus source) showed the maximum growth with dry weight 132.267 ± 0.902 mg/100 ml, table (2). However, no significant differences in biomass when culture media contained either Na_2HPO_4 or Na_3PO_4 (110.133 ± 0.4163 and 109.667 ±0.7571 mg dry wt./100ml, respectively).The lowest biomass concentration was observed when K_2HPO_4 was replaced by NaH_2PO_4 (96.067 ±0.5033 mg dry wt./100 ml).

Table (1): Growth curve of *Spirulina platensis* measured as mg dry wt./100 ml.

Day	Dry weight (mg/ 100 ml)
0	7.5 ±0.45
1	18.30 ±1.023
2	20.60 ±1.0
3	41.90 ±0.87
4	44.40 ±0.901
5	50.90 ±1.23
6	82.40 ±1.101
7	94.10 ±0.98
8	94.00 ±0.87
9	111.70 ±1.13
10	101.55 ±1.20
11	98.47 ±0.89
12	97.50 ±0.65
13	64.30 ±0.78

±SE: Standard error of three replicates

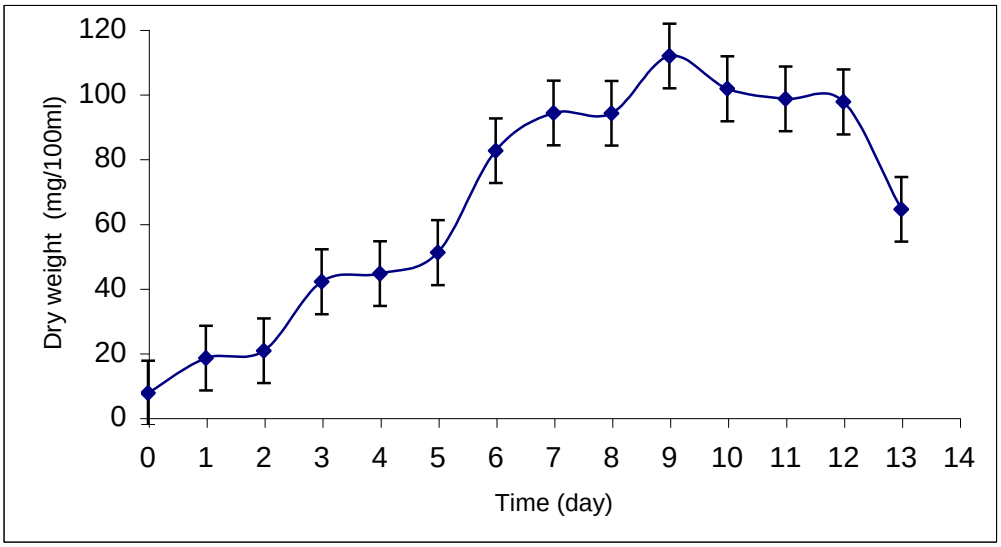


Fig. (1): Growth curve of *S. platensis* measured as mg dry wt./100 ml.

Table (2): Mean ±SE of dry weight of *Spirulina Platensis* grown in different media, using different nitrogen and phosphorus sources.

Medium	**Dry weight (mg/ 100 ml)**
Zarrouk's (**1966**)	137.73^{a} ± 0.611
Aiba & Ogawa (1977)	132.26^{b} ± 0.902
Nitrogen sources	
Urea	95.6^{b} ± 1.102
Sodium nitrate	132.267^{a} ± 0.902
Glycene	N.D
NH_4Cl	N.D
$(NH_4)_2SO_4$	N.D
Phosphorus sources	**Dry weight (mg/ 100 ml)**
K_2HPO_4	132.267^{a} ± 0.902
Na_2HPO_4	110.133^{b} ± 0.4163
Na_3PO_4	109.667^{b} ± 0.7571
KH_2PO_4	104.267^{c} ± 0.3055
NaH_2PO_4	96.067^{d} ± 0.5033

±SE: Standard error of three replicates
a,b,c,d…..etc different superscripts differ significantly ($P<0.05$)
ND = not detected

Screening of antimicrobial activity produced by *Spirulina platensis* using different solvents.

The data represented in Table 3 & 4 showed the biological activity of *S. platensis* extracts against different species of bacteria and fungi. It was found that the highest biological activity was recorded against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Aspergillus niger*. The results revealed that diethyl ether and ethyl acetate exhibited antimicrobial activity against Gram +ve and Gram -ve bacteria, while petroleum ether exhibited antimicrobial activity against Gram -ve only and n-hexane had no activity against all test organisms. On the other hand, among the water-miscible solvents (acetone, methanol, and ethanol) ethanol was the most effective solvent showed wide spectrum of antimicrobial activity against Gram +ve, Gram -ve bacteria and fungi as shown in figure 2 & 3.

Table (3): Antimicrobial activities of different water immiscible solvent extracts of *Spirulina platensis*.

Solvent	Mean diameter of inhibition zones (mm)*						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*B.th.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*S.c.*
Diethyl ether	0.00	14.00	0.00	15.00	21.00	0.00	16.00
Ethyl acetate	17.00	16.00	23.00	0.00	21.00	0.00	0.00
Petroleum ether	0.00	0.00	20.00	0.00	0.00	14.00	0.00
n-hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	19.00	0.00	0.00	21.00	0.00	0.00	0.00

*Mean of three replicates
B.s.:Bacillus subtilis NCTC 3610 *B.th: B. thuringiensis* ATCC 25597
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
S.c.: Saccharomyces cerevisiae ATCC2601

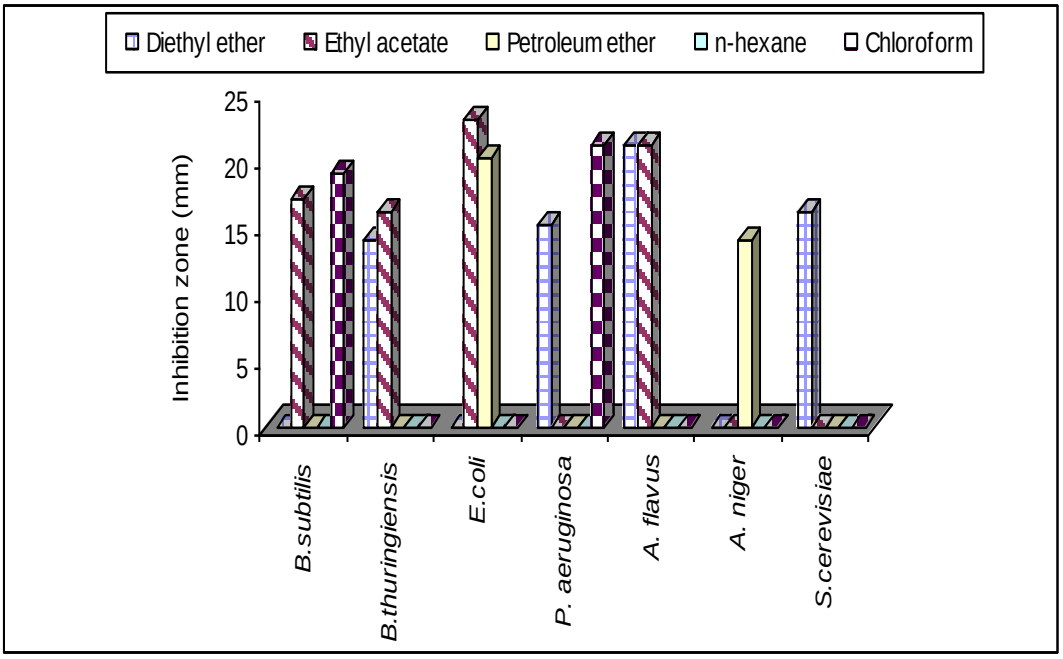


Fig. (2): antimicrobial activities of different water immiscible solvent extracts of *S. platensis*.

Table (4): Antimicrobial activities of different water miscible solvent extracts of *Spirulina platensis*.

Solvent	Mean diameter of inhibition zone (mm)*						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*B.th.*	E.c.	P.a.	A.f.	A.n.	*S.c.*
Acetone	0.00	0.00	16.00	15.00	18.00	16.00	0.00
Ethanol	15.00	0.00	21.00	0.00	0.00	14.00	0.00
Methanol	0.00	0.00	0.00	15.00	18.00	0.00	18.00

*Mean of three replicates
B.s.:Bacillus subtilis NCTC 3610 *B.th: Bacillus thuringiensis* ATCC 25597
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergilluss niger*
S.c.: Saccharomyces cerevisiae ATCC2601

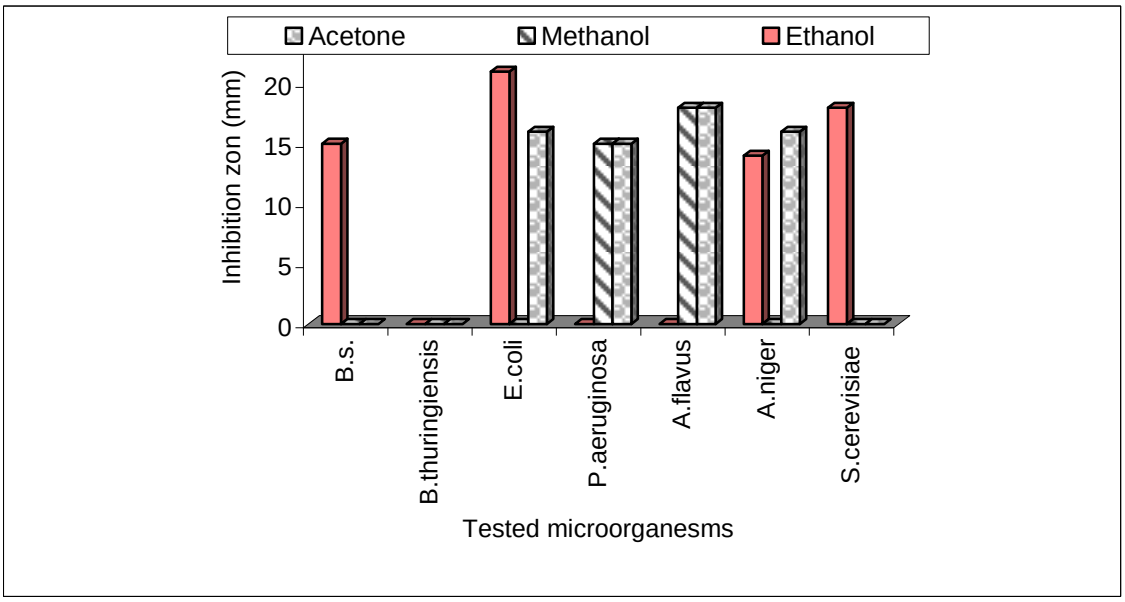


Fig. (3): antimicrobial activities of different water miscible solvent extracts of *Spirulina platensis*.

Effect of some culture conditions on biomass production (mg dry wt./100 ml) of *Spirulina platensis* and its antimicrobial activity.

This experiments aimed to study the effect of certain growth conditions as temperature, pH, light intensity, aeration and light duration on growth and antimicrobial activities of *S. platensis* .

Effect of different incubation temperature on biomass production.

Biomass of *S. platensis* was gradually increased significantly by increasing temperature from 20 ± 2 °C to 30 ± 2 °C where the maximum biomass concentration was 132.27 ± 0.9018 mg dry wt./100 ml. When temperature increased up to 40 ± 2 °C a significant decrease in algal biomass production was observed; 32.2 ± 2 mg dry wt./100 ml as illustrated at Table 5 and Fig.4.

Effect of different cultivation temperatures on antimicrobial activities of whole culture (cells and exometabolites).

The data presented in Table (6) showed that, no inhibitory activities were recorded with temperature degree 20 ± 2 °C and 40 ± 2 °C against all the tested microorganisms. The maximum inhibition zones were recorded at 30 ± 2 °C (32.67 ± 0.5773, 35.33 ± 0.5773, 31.0 ± 1.0, 31.0 ± 0.0, 15.67 ± 0.5773, 17.33 ± 2.0816 and 34.67 ± 0.577 mm with *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus*, *A. niger* and *Condida albicans* respectively) except that for *Pseudomonas aeruginosa*, where the maximum inhibition zone observed with whole culture (cells and exometabolites) grown at 25 ± 2 °C was 35.33 ±0.5773 mm.

Table (5): Effect of different cultivation temperatures on biomass production of *Siprulina platensis* measured as (mg dry wt. /100ml).

Temperature (±2°C)	Dry weight (mg/100 ml)
20	47.93^{d} ±0.3055
25	56.13^{c} ±0.3055
30	132.27^{a} ±0.9018
35	117.8^{b} ±0.5291
40	32.2^{a} ±0.20

±SE: standard error of three replicates
a,b,c etc……different superscripts differ significantly (P<0.05)

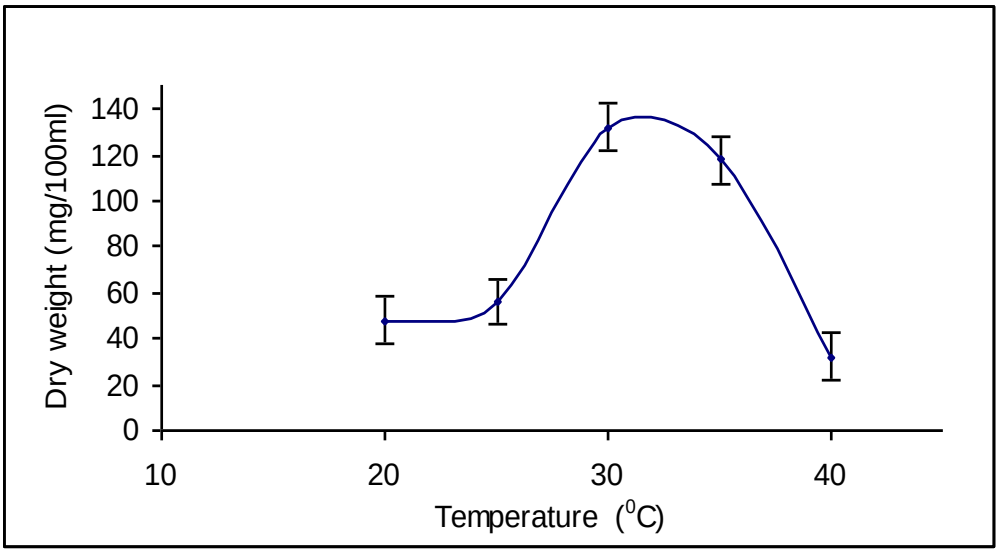


Fig.(4):Effect of different cultivation temperatures on biomass production of *S. platensis* measured as (mean ±SE mg dry wt. /100ml).

Table (6): Effect of different cultivtion temperatures on antimicrobial activities of *Spirulina platensis*.

Temperature	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
20	0.00^{d} ±0.00	0.00^{d} ±0.00	0.00^{d} ±0.00	0.00^{d} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{d} ±0.00
25	30.33^{b} ±1.5275	31.00^{b} ±0.00	29.67^{b} ±0.5773	35.33^{a} ±0.5773	0.00^{b} ±0.00	0.00^{b} ±0.00	30.00^{b} ±0.00
30	32.67^{a} ±0.5773	35.33^{a} ±0.5773	31.00^{a} ±1.00	31.00^{b} ±0.00	15.67^{a} ±0.5773	17.33^{a} ±2.0816	34.67^{a} ±0.5773
35	19.67^{c} ±0.5773	27.00^{c} ±1.00	16.00^{c} ±1.00	28.33^{c} ±1.5275	0.00^{b} ±0.00	0.00^{b} ±0.00	25.00^{c} ±1.00
40	0.00^{d} ±0.00	0.00^{d} ±0.00	0.00^{d} ±0.00	0.00^{d} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{d} ±0.00

±SE: standard error of three replicates ,a,b,c,d…. etc different superscripts differ significantly ($P<0.05$) ,

B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565

E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145

A.f.: Aspergillus flavus *A.n.: Aspergillus niger*

C.a.: Candida albicans ATCC 10231

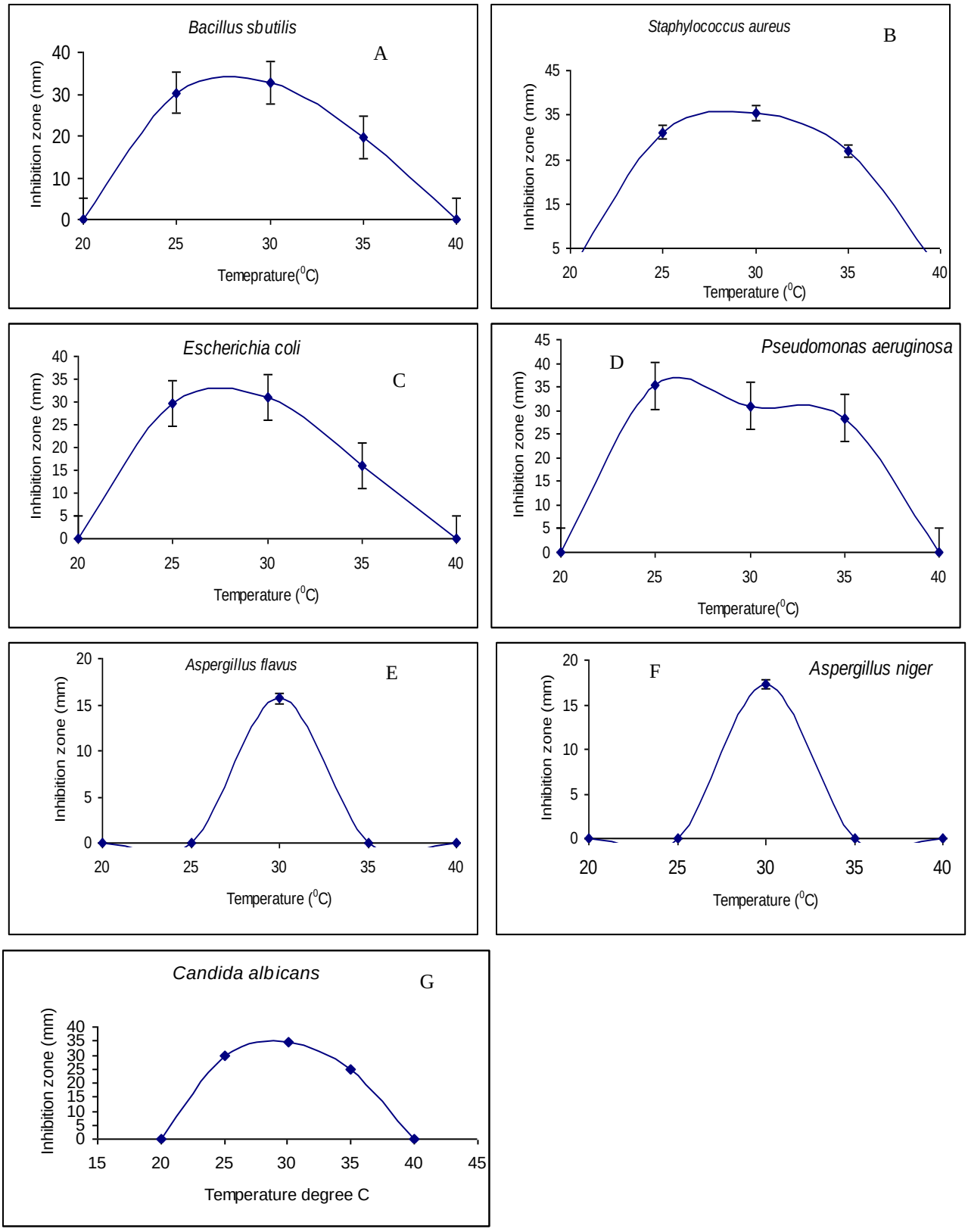


Fig. (5): Effect of different cultivation temperatures on antimicrobial activities of *Spirulina platensis* against some microorganisms.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* , **D**: *Pseudomonas aeruginosa*, **E**: *Aspergillus flavus* , **F**: *A. niger* and **G**: *Candida albicans*

Effect of different cultivation temperatures on antimicrobial activities of *Spirulina platensis* culture filtrate.

Data in Table (7) and figure (6) recorded the antimicrobial activities produced from *S. platensis* in culture filtrate. It was observed that maximum inhibition zones were achieved when the organisms was incubated at 30 ±2°C. *C. albicans* was the most sensitive organism, where the recorded inhibition zone was 35.67 ± 0.577 mm. Above or below 30 °C, the antimicrobial activities of the filtrate were significantly reduced except for those of *B. subtilis* and *E.coli*, where no significant differences were observed at 25 and 30 ±2°C as mentioned in whole culture (cells and exometabolites). There were no inhibitory activities at 20 and 40 ±2°C.

Effect of different incubation temperatures on antimicrobial activities of *S. platensis* extracted cells.

Results described in Table (8) and figure (7) showed that, ethanolic extract of *S. platensis* cells grown at 40 ±2°C did not show any antimicrobial activities against all the tested microorganisms. The extract of cells grown at 20 ±2°C inhibit only *S. aureus* (15.0 ± 1.0 mm) and *P. aeruginosa* (11.67 ± 0.577mm). Inhibition zones were significantly differed along with incubation temperature. The maximum antimicrobial activities (inhibition zones mm) with *B. subtilis* and *S. aureus* were observed from cells grown at 35 °C where inhibition zones were 27.0 ± 0.0 and 26.67 ± 1.155 mm, respectively. There was no significant difference between inhibition zones resulted from extracted cells grown at 30 and 35 ± 2°C against *E. coli* and *C. albicans*. However, the maximum inhibition zone of *P. aeruginosa* was recorded from extract of cells grown at 35 ± 2 °C (30.0 $\pm$ 1.0 mm). Extract did not show any antimicrobial activity against *A.flavus* while it showed moderate activity with *A.niger* (16.33 mm) only at 30 °C.

Table (7): Effect of different cultivation temperatures on antimicrobial activities of *Spirulina platensis* culture filtrate.

Temperature	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
20	0.00^{c} ±0.00	0.00^{d} ±0.00	0.00^{c} ±0.00	0.00^{d} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{d} ±0.00
25	32.67^{a} ±0.5773	30.67^{b} ±0.5773	31.33^{a} ±0.5773	34.67^{a} ±0.5773	0.00^{b} ±0.00	0.00^{b} ±0.00	32.00^{b} ±0.00
30	32.67^{a} ±0.5773	34.67^{a} ±0.5773	31.33^{a} ±0.5773	33.00^{b} ±1.00	12.33^{a} ±0.5773	16.33^{a} ±1.5275	35.67^{a} ±0.5773
35	19.67^{b} ±0.5773	27.00^{c} ±1.00	28.33^{b} ±1.5275	28.33^{c} ±1.5275	0.00^{b} ±0.00	0.00^{b} ±0.00	25.00^{c} ±1.00
40	0.00^{c} ±0.00	0.00^{d} ±0.00	0.00^{c} ±0.00	0.00^{d} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{d} ±0.00

±SE: standard error of three replicates.

a,b,c,d…… etc different superscripts differ significantly ($P<0.05$).

B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565

E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145

A.f.: Aspergillus flavus *A.n.: Aspergillus niger*

C.a.: Candida albicans ATCC 10231

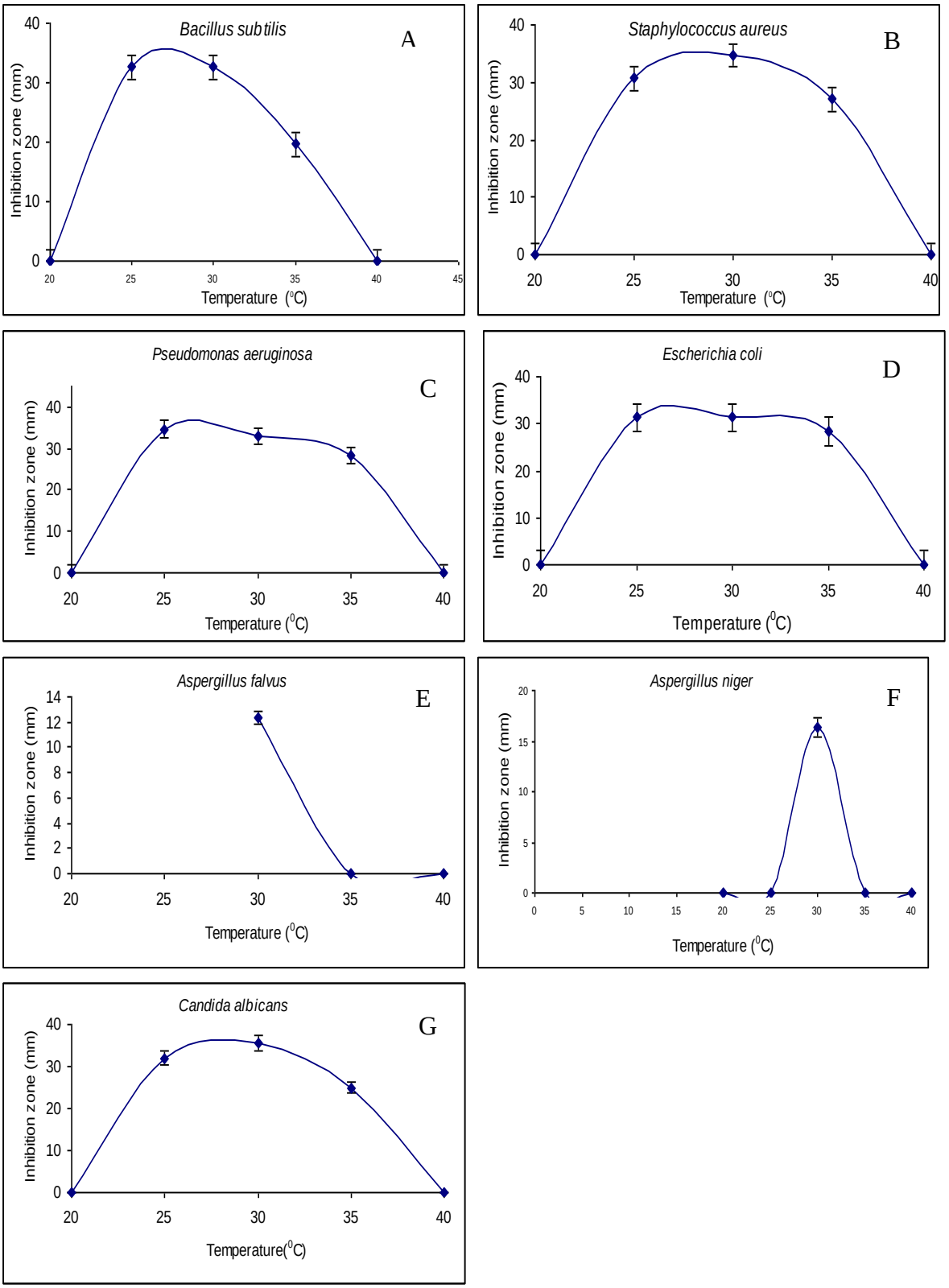


Fig.(6): Effect of different cultivation temperatures on antimicrobial activities of *Spirulina platensis* culture filtrate.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* , **D**: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Table (8): Effect of different cultivation temperatures on antimicrobial activities of ethanolic extracted cells of *Spirulina platensis* .

Temperature	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
20	0.00^{d} ±0.00	15.00^{d} ±1.00	0.00^{c} ±0.00	11.67^{d} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{c} ±0.00
25	18.33^{c} ±0.5773	17.33^{c} ±0.5773	20.67^{b} ±0.5773	17.33^{c} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	20.33^{b} ±0.5773
30	21.67^{b} ±0.5773	24.33^{b} ±0.5773	26.00^{a} ±0.00	27.33^{b} ±0.5773	0.00^{a} ±0.00	11.67^{a} ±0.5773	29.67^{a} ±0.5773
35	27.00^{a} ±0.00	26.67^{a} ±1.1547	25.67^{a} ±0.5773	30.00^{a} ±1.00	0.00^{a} ±0.00	0.00^{b} ±0.00	29.33^{a} ±0.5773
40	0.00^{d} ±0.00	0.00^{e} ±0.00	0.00^{c} ±0.00	0.00^{e} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{c} ±0.00

±SE: standard error of three replicates.
a,b,c,d…… etc different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

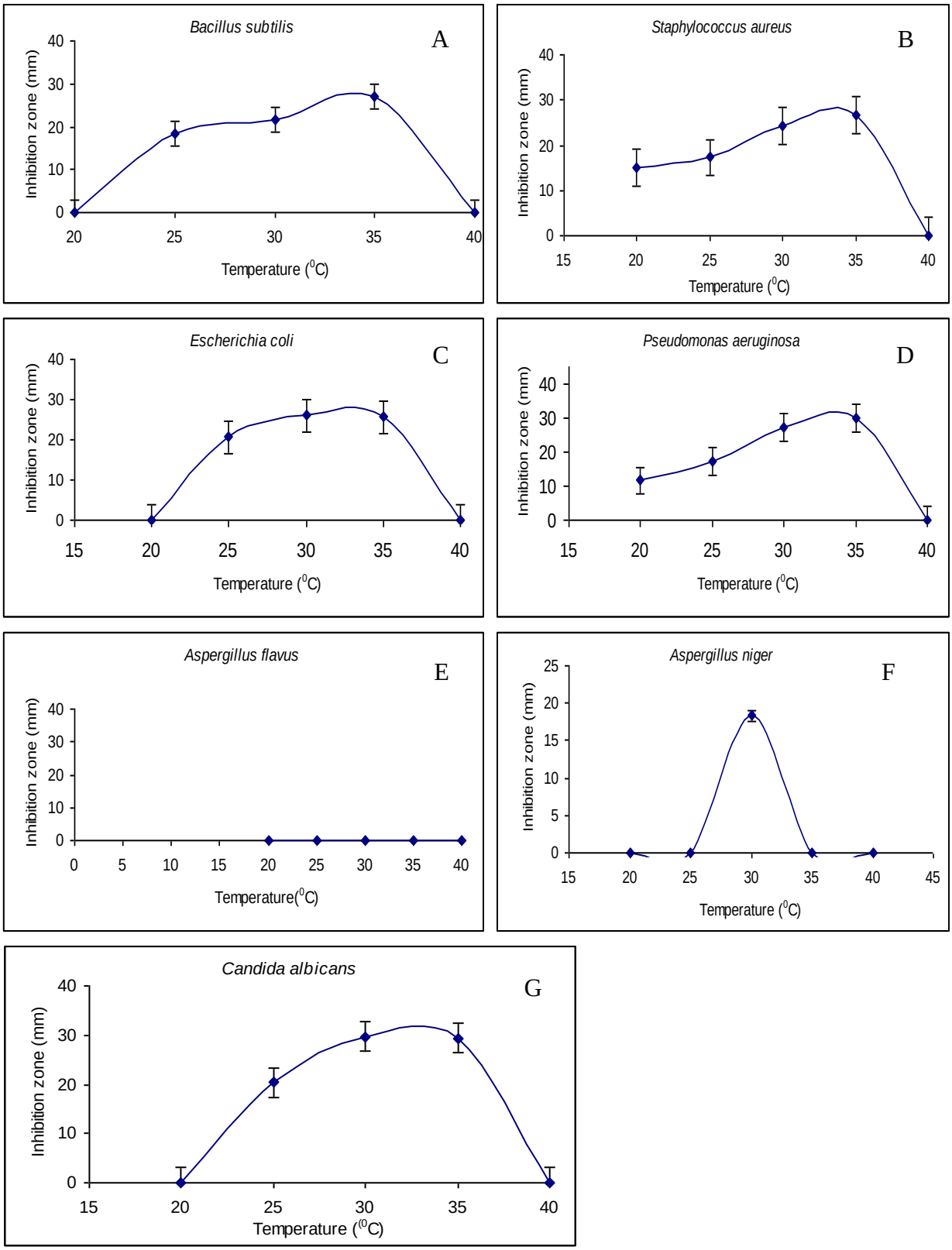


Fig.(7): Effect of different cultivation temperatures on antimicrobial activities of ethanolic extracted cells of *Spirulina platensis*.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Effect of different hydrogen ion concentrations (pH) on biomass production of *Spirulina platensis*.

Table (9) and figure (8) demonstrated that biomass concentration of *S. platenis* at different pH values ranged from 27.27 ± 0.1154 mg dry wt./100 ml to 92.13 ± 0.1151 mg dry wt./100 ml. The optimum pH for *S. platensis* growth was pH 9.0, which resulted in the highest biomass production (92.13 ± 0.1154 mg dry wt./100 mg).

Table (9): Effect of different hydrogen ion concentrations (pH) on biomass production of *Spirulina platensis* (mg dry wt./100 ml)

pH	Dry weight mg/100 ml
5.0	33.2^{f} ±0.40
6.0	52.60^{d} ±0.40
7.0	54.33^{c} ±0.2309
8.0	86.80^{b} ±0.3464
9.0	92.13^{a} ±0.1154
10.0	33.73^{e} ±0.1154
11.0	27.27^{g} ±0.1154

±SE: standard error of three replicates.
a,b,c,d…… etc different superscripts differ significantly (P<0.05).

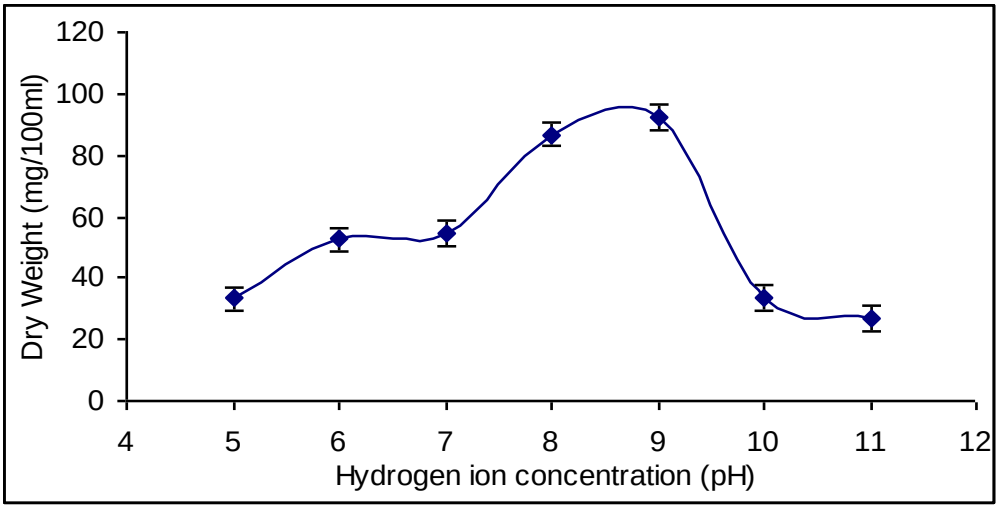


Fig.(8):Effect of different hydrogen ion concentrations (pH) on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites).

The data in Table (10) and figure (9) illustrate the influence of hydrogen ion concentration (pH) on *S. platensis* antimicrobial activities of the whole culture (cells and exometabolites). There was no inhibitory effect occurred against any test microorganisms at pH 5.0 and 11.0. While *Bacillus subtilis* (Gram +ve bacteria) was the most sensitive bacterial isolate represented 45.0±1.0 mm, zone of inhibition followed by *Escherichia coli*, 44.0 ± 0.0 mm, *Staphylococcus aureus*, 40.67 ± 0.5773 and *Pseudomonas aeruginosa*, 39.0 ± 0.5773 mm.

It was obvious from the results that *Candida albicans, B. subtilis* and *E. coli* followed by *S. aureus* and *P. aerugenosa* were the most susceptible microorganisms affected by *S. platensis* whole culture (cells and exometabolites), while the most resistance isolates were *Aspergillus flavus* and *A. niger* showed inhibition zone 15.67 ± 0.5773 and 17.33 ± 2.082 mm, respectively. A weak inhibition was also observed for all tested fungal isolates.

The antifungal activity of *S. platensis* whole culture, represented in Table (10) showed that mould *A. flavus* and *A. niger* were more resistant than yeast, *C. albicans*, where inhibition zone occurred with mould ranged from 11.00 ± 0.0 mm to 17.33 ± 2.082 mm and 28.33 ± 2.082 mm to 48.0 ± 0.0 mm for yeast. It is clear from the data that, pH 7.0 and pH 8.0 showed moderate inhibition activities against Gram -ve bacteria and *A. niger* without significant differences.

Table (10): Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites).

pH	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
5.0	0.00^{f} ±0.00	0.00^{f} ±0.00	0.00^{e} ±0.00	0.00^{d} ±0.00	0.00^{a} ±0.00	0.00^{c} ±0.00	0.00^{e} ±0.00
6.0	24.00^{e} ±1.00	29.33^{e} ±0.5773	29.67^{d} ±0.5773	29.33^{c} ±0.5773	0.00^{a} ±0.00	0.00^{c} ±0.00	28.33^{d} ±2.082
7.0	38.00^{d} ±0.00	39.33^{b} ±0.5773	43.00^{b} ±0.00	33.67^{b} ±1.1547	0.00^{a} ±0.00	11.00^{b} ±0.00	46.67^{ab} ±1.5275
8.0	40.00^{b} ±0.00	37.00^{c} ±1.00	43.00^{b} ±0.00	34.33^{b} ±0.5773	0.00^{a} ±0.00	11.00^{b} ±0.00	44.00^{c} ±0.00
9.0	45.00^{a} ±1.00	40.67^{a} ±0.5773	44.00^{a} ±0.00	39.00^{a} ±0.5773	15.67^{a} ±0.5773	17.33^{a} ±2.0816	48.00^{a} ±0.00
10.0	39.00^{c} ±0.00	34.00^{d} ±1.000	39.00^{c} ±0.00	34.67^{b} ±0.5773	11.67^{b} ±1.1547	12.33^{b} ±1.5275	45.00^{bc} ±0.00
11.0	0.00^{f} ±0.00	0.00^{f} ±0.00	0.00^{e} ±0.00	0.00^{d} ±0.00	0.00^{a} ±0.00	0.00^{c} ±0.00	0.00^{e} ±0.00

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

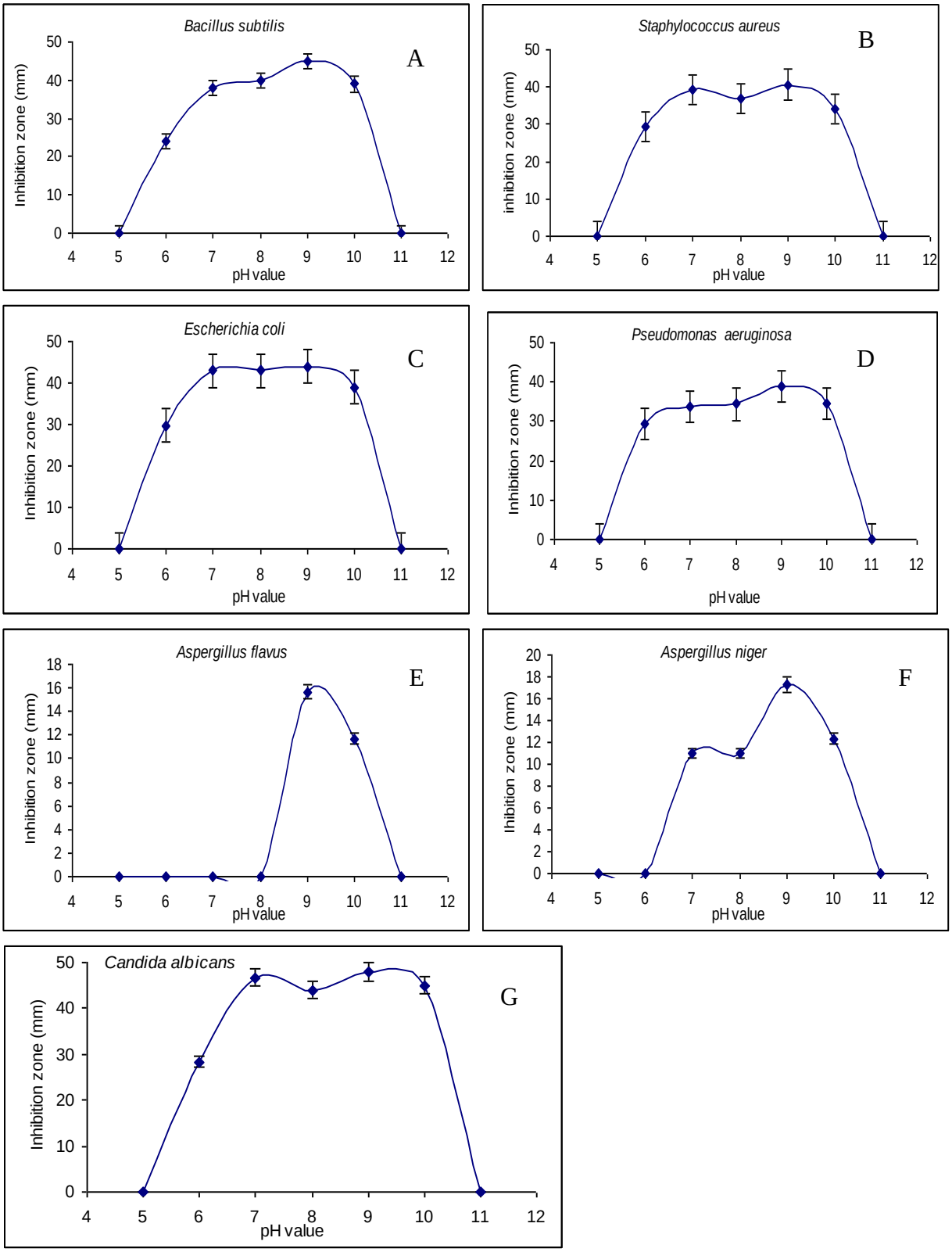


Fig.(9): Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites).

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* culture filtrate.

Results presented in Table (11) and figure (10), showed the maximum antimicrobial activities produced by *S. platensis* culture filtrate, obtained at pH 9.0.

The antimicrobial activities of *S. platensis* were pH dependent, where culture filtrate at pH 5.0 and 11.0 did not exhibit any antimicrobial activities. By increasing pH from 6.0 to 8.0 an inhibition zones were observed with the tested Gram +ve, Gram -ve and unicellular fungi. No significant differences were recorded in activity at pH 7.0, 8.0 and 10.0 against *Pseudomonas aeruginosa* and *Candida albicans*. There were no inhibitory effect occurred at pH values 5.0, 6.0, 7.0, 8.0 and 11.0 against multi-cellular fungi (*Aspergillus flaves* and *A. niger*). The maximum antimicrobial activities of culture filtrate reached 39.67 ± 1.1547, 16.33 ± 1.1547 and 35.67 ±0.5773 mm for *P. aeruginosa, A. niger* and *C. albicans*, respectively.

Table (11): Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* culture filtrate.

pH	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
5.0	0.00^{d} ±0.00	$0.00.00^{f}$ ±0.00	0.00^{e} ±0.00	0.00^{d} ±0.00	0.00^{c} ±0.00	0.00^{c} ±0.00	0.00^{c} ±0.00
6.0	25.00^{c} ±0.00	25.00^{e} ±1.00	28.00^{d} ±0.00	29.33^{c} ±0.5773	0.00^{c} ±0.00	0.00^{c} ±0.00	32.67^{b} ±0.5773
7.0	32.33^{b} ±0.5773	32.67^{d} ±0.5773	30.33^{c} ±0.5773	33.67^{b} ±1.1547	0.00^{c} ±0.00	0.00^{c} ±0.00	33.00^{b} ±1.00
8.0	32.67^{b} ±0.5773	35.67^{b} ±0.5773	32.33^{b} ±0.5773	34.33^{b} ±0.5773	0.00^{c} ±0.00	0.00^{c} ±0.00	33.33^{b} ±0.5773
9.0	34.67^{a} ±0.5773	37.00^{a} 0.00	34.33^{a} ±0.5773	39.67^{a} ±1.1547	12.33^{a} ±0.5773	16.33^{a} 1.1547	35.67^{a} ±0.5773
10.0	34.67^{a} ±0.5773	34.00^{c} ±1.00	31.00^{c} ±1.00	34.67^{b} ±0.5773	11.33^{b} ±0.5773	14.33^{b} ±0.5773	33.00^{b} ±1.0
11.0	0.00^{D} ±0.00	0.00^{F} ±0.00	0.00^{E} ±0.00	0.00^{D} ±0.00	0.00^{C} ±0.00	0.00^{C} ±0.00	0.00^{C} ±0.00

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly ($P<0.05$).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

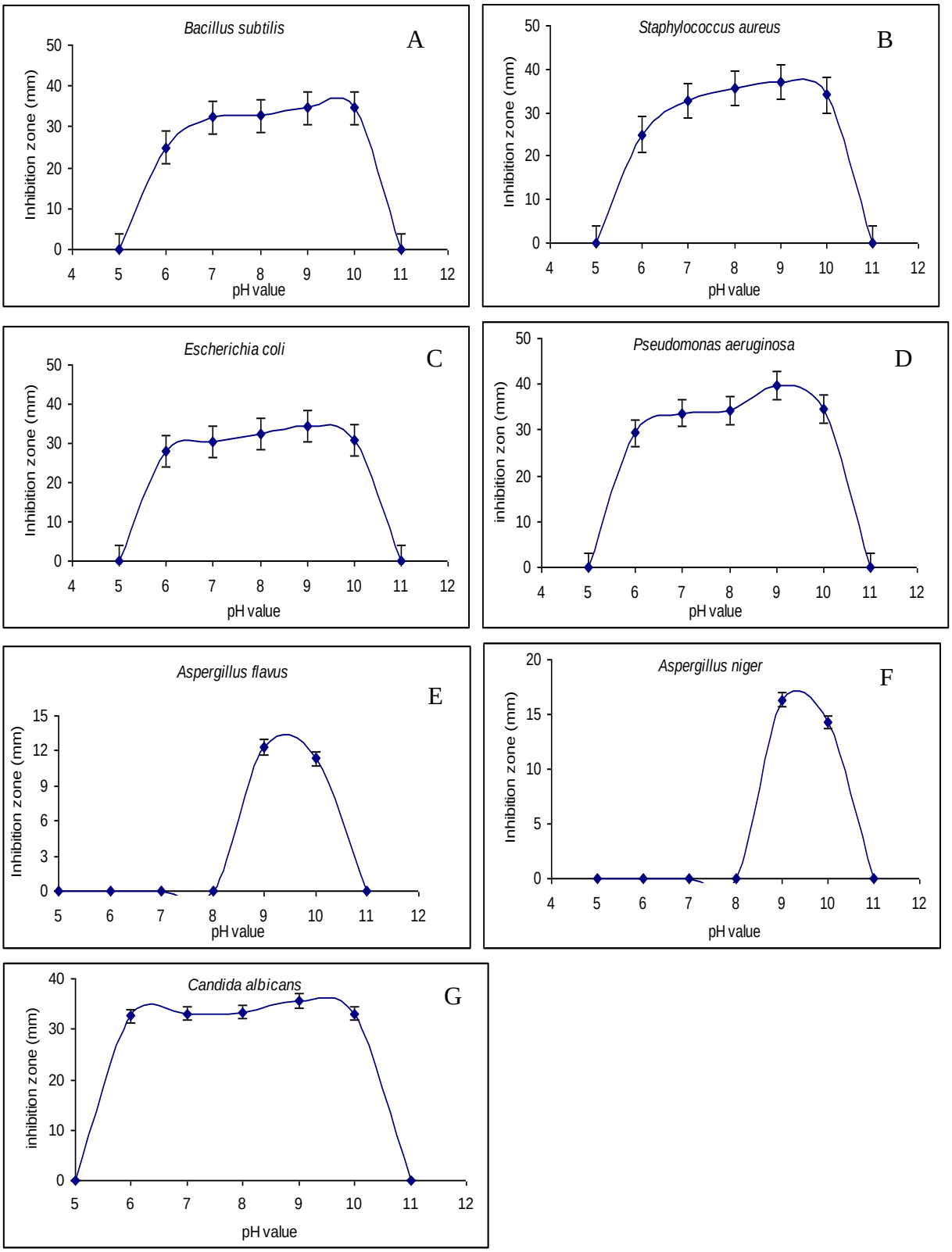


Fig.(10):Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of culture filtrate of *Spirulina platensis*.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E**: *Aspergillus flavus* , **F**: *A. niger* and **G**: *Candida albicans*

Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* extracted cells.

The antimicrobial activity of *S. platensis* cells extracts was reported in Table (12) and figure (11) showed the inhibition of all tested microorganisms except *A. flavus*. The extracts of cells which were grown at pH 5.0 and 11.0 did not exhibited antimicrobial activities.

A weak to moderate inhibition zones were also observed with extracts of cells that grown at pH 6.0 and 10.0. Results in table (12), showed that *S. platensis* extracted cells that grown at pH 8.0 and 9.0 showed the highest antimicrobial activities. However, *A. flavus* was the most resistance organism, while *P. aeruginosa* and *E. coli* (Gram -ve bacteria) were the most sensitive species where inhibition zone were 29.67 ± 0.5773 mm and 29.0 ± 1.732 mm, respectively.

There was no significant difference between inhibition zones from extracted cells that were grown at pH 8.0 and 9.0 against *B. subtilis*, *S. auerus* and *P. aerugonisa*.

Table (12): Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* extracted cells.

pH	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
5.0	0.00^{d} ±0.00	0.00^{e} ±0.00	0.00^{e} ±0.00	0.00^{d} ±0.00	0.00^{a} ±0.00	0.00^{c} ±0.00	0.00^{f} ±0.00
6.0	18.33^{c} ±0.5773	20.33^{b} ±0.5773	21.67^{c} ±0.5773	20.67^{c} ±0.5773	0.00^{a} ±0.00	0.00^{c} ±0.00	27.00^{c} ±0.00
7.0	23.00^{b} ±1.00	19.00^{c} ±1.00	22.33^{c} ±1.1547	22.67^{b} ±0.5773	0.00^{a} ±0.00	11.33^{a} ±0.5773	22.33^{d} ±0.5773
8.0	26.00^{a} ±1.00	25.67^{a} ±0.5773	27.00^{b} ±0.00	29.33^{a} ±0.5773	0.00^{a} ±0.00	11.33^{a} ±0.5773	28.67^{b} ±0.5773
9.0	26.67^{a} ±0.5773	26.67^{a} ±0.5773	29.00^{a} ±1.7320	29.67^{a} ±0.5773	0.00^{a} ±0.00	11.67^{a} ±0.5773	29.67^{a} ±0.5773
10.0	18.00^{c} ±0.00	16.00^{d} ±1.00	12.67^{d} ±0.5773	21.33^{c} ±0.5773	0.00^{a} ±0.00	10.00^{b} ±0.00	15.33^{e} ±0.5773
11.0	0.00^{d} ±0.00	0.00^{e} ±0.00	0.00^{e} ±0.00	0.00^{d} ±0.00	0.00^{a} ±0.00	0.00^{c} ±0.00	0.00^{f} ±0.00

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

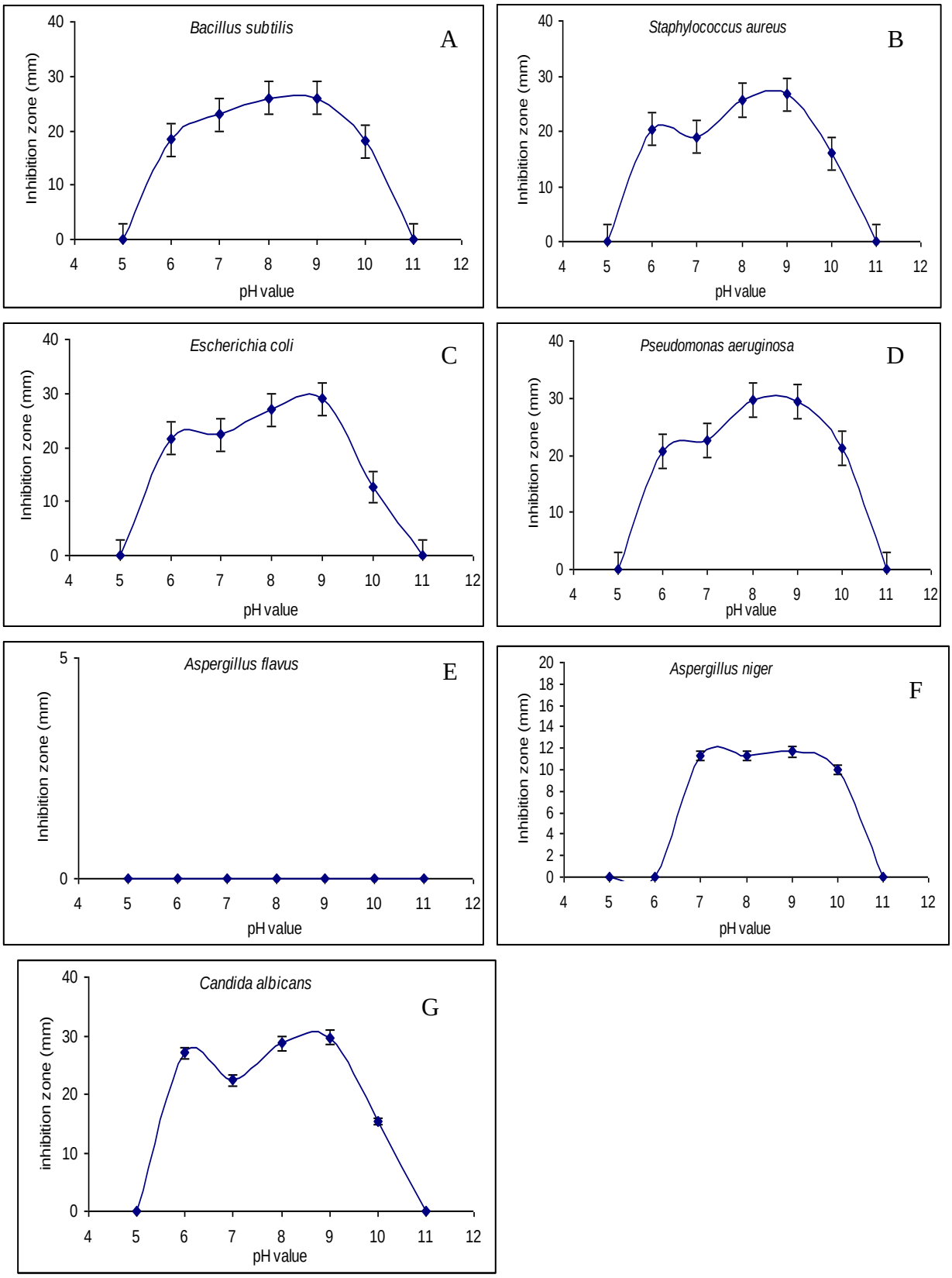


Fig. (11): Effect of different hydrogen ion concentrations (pH) on antimicrobial activities of *Spirulina platensis* extracted cells.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Effect of different light intensities (Klux) on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

Data recorded in the Table (13) and figure (12) revealed that, *S. platensis* biomass production (mg dry wt./100 ml) recorded the highest value with 2.25 klux and the lowest one was observed in case of 4.0 klux (136.27 ± 0.808 and 87.67 ± 0.231 mg dry wt./100 ml, respectively).

Effect of different light intensities (Klux) on antimicrobial activities produced by *S. platensis* whole culture (cells and exometabolites).

The data in Table (14) and figure (13) showed the influence of light intensities on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites) grown at different light intensities. There were no significant differences in antimicrobial activities produced by *S. platesis* whole culture grown at light intensity 2.0 Klux and 2.25 Klux against *B. subtilis, E. coli* and *C.albicans*.

The inhibition zones obtained varied according to light intensity, where *S. aureus* has the highest sensitivity to the whole culture (cells and exometabolites) grown at light intensity 2.5 and 2.75 Klux with inhibition zone 35.00± 1.0 mm and 2.5 Klux 34.67 ± 0.577 without significant differences between them.Whole cultures (cells and exometabolites) grown at light intensity 3.75 and 4.0 Klux exhibited the lowest inhibition zones with *B.subtilis, E. coli, A.niger* and *C. alibicans*, without significant difference while the lowest inhibition zone of *S. aureus* was observed at 3.5 Klux. Generally, 2.5 klux was the best light intensity for antimicrobial activities produced from *S. platensis* whole culture (cells and exometabolites).

Table (13): Effect of different light intensities (Klux) on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

Light intensity (Klux)	Dry weight (mg/100 ml)
1.50	103.67^{e} ±0.462
1.75	120.53^{c} ±0.611
2.00	137.00^{a} ±0.20
2.25	136.27^{a} ±0.808
2.50	132.27^{b} ±0.902
2.75	120.73^{c} ±0.306
3.00	114.40^{d} ±0.60
3.25	104.47^{e} ±1.102
3.50	97.80^{f} ±0.40
3.75	98.73^{f} ±0.306
4.00	87.67^{g} ±0.231

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).

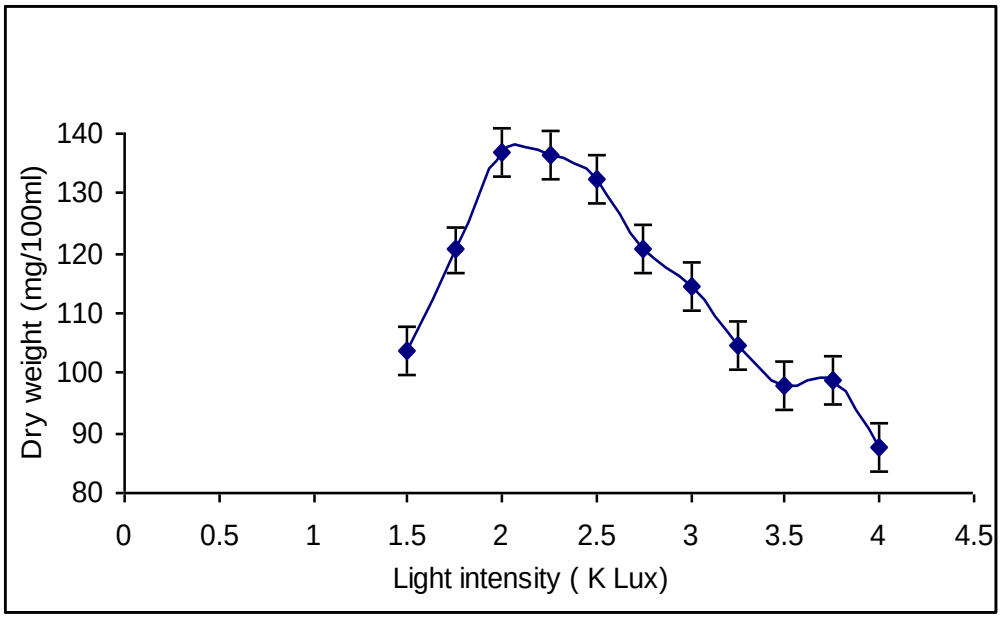


Fig. (12): Effect of different light intensities (Klux) on biomass production of *Spirulina platensis* (mg dry wt./100ml).

Table (14): Effect of different light intensities (Klux) on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites).

Light intensity (Klux)	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
1.50	29.00^{ab} ±0.00	33.00^{b} ±0.00	28.00^{c} ±0.00	31.00^{c} ±0.00	0.00^{b} ±0.00	15.33^{c} ±0.5773	31.00^{c} ±0.00
1.75	24.67^{c} ±0.5773	31.00^{c} ±0.00	25.00^{d} ±0.00	26.67^{d} ±0.5773	0.00^{b} ±0.00	15.67^{c} ±0.5773	33.67^{b} ±0.5773
2.00	28.00^{b} ±1.00	25.00^{d} ±0.00	30.00^{ab} ±1.00	30.67^{c} ±0.5773	0.00^{b} ±0.00	16.67^{b} ±0.5773	31.00^{c} ±0.00
2.25	28.00^{b} ±1.00	30.67^{c} ±0.5773	31.00^{a} ±1.00	34.33^{a} ±0.5773	0.00^{b} ±0.00	17.67^{a} ±0.5773	31.00^{c} ±0.00
2.50	30.00^{a} ±1.00	34.67^{a} ±0.5773	31.00^{a} ±1.00	31.00^{c} ±0.00	15.67^{a} ±0.5773	18.67^{a} ±0.5773	34.67^{a} ±0.5773
2.75	29.67^{a} ±0.5773	35.00^{a} ±1.00	29.00^{bc} ±0.00	30.33^{c} ±0.5773	0.00^{b} ±0.00	15.67^{b} ±0.5773	34.33^{a} ±0.5773
3.00	28.33^{b} ±0.5773	25.00^{d} ±0.00	29.67^{abc} ±0.5773	33.33^{ab} ±1.1547	0.00^{b} ±0.00	16.33^{b} ±0.5773	34.00^{a} ±1.00
3.25	30.00^{a} ±0.00	30.67^{c} ±0.5773	29.33^{abc} ±0.5773	32.67^{b} ±0.5773	0.00^{b} ±0.00	15.67^{c} ±0.5773	30.00^{c} ±0.5773
3.50	25.67^{c} ±0.5773	15.67^{g} ±0.5773	21.33^{e} ±0.5773	22.67^{e} ±0.5773	0.00^{b} ±0.00	15.0^{d} ±0.00	20.67^{d} ±0.5773
3.75	15.67^{d} ±0.5773	23.67^{e} ±0.5773	20.67^{e} ±0.5773	21.33^{f} ±0.5773	0.00^{b} ±0.00	15.0 ±0.00	20.33^{d} ±0.5773
4.00	16.67^{d} ±0.5773	20.67^{f} ±0.5773	21.33^{e} ±2.5166	17.67^{g} ±0.5773	0.00^{b} ±0.00	15.0^{d} ±0.00	17.00^{e} ±1.00

±SE: standard error of three replicates.

a,b,c,d…… etc, different superscripts differ significantly (P<0.05).

B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565

E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145

A.f.: Aspergillus flavus *A.n.: Aspergillus niger*

C.a.: Candida albicans ATCC 10231

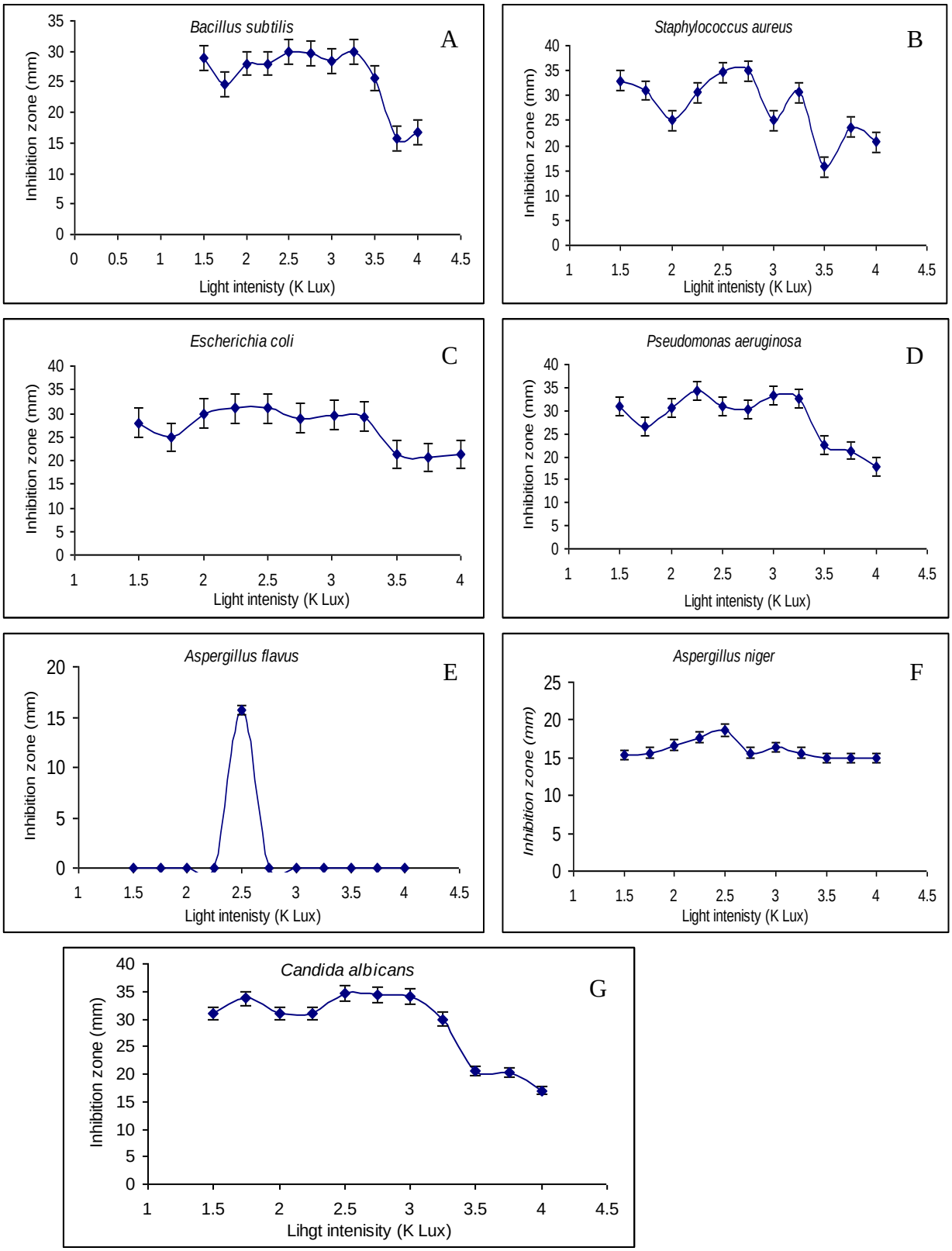


Fig. (13): Effect of different light intensities (Klux) on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites)

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Effect of different light intensities (Klux) on antimicrobial activities of *Spirulina platensis* culture filtrate.

Data presented in Table (15) and figure (14) showed the effect of light intensities on *S. platensis* culture mfiltrate (exometabolites) as antimicrobial activities.

The data revealed that culture filtrate of *S. platensis* inhibited the growth of the tested microorganisms, the highest light intensities of 3.75 Klux and 4.0 Klux showed weak inhibition zones. A moderate antimicrobial activities without significant difference were recorded of culture filtrate of cells grown at 1.5 Klux and 1.75 Klux against *B.subtilis*, *S.aureus*, *P. aeruginosa* and *A. niger*.

Culture filtrate obtained from *S. platensis* culture grown at 2.5 and 2.75 Klux exhibited the highest inhibition zones against all the tested microorganisms without significant difference in case of *B.subtilis*, *E.coli* and *A.niger*. On the other hand light intensity of 2.5 Klux exhibited the largest inhibition zone of *S.aureus* (34.67 ± 0.5773 mm) and 2.75 Klux for *P. aeruginosa* and *C.albicans* with inhibition zones 34.67 ± 0.5773 mm and 40.0 ± 1.0 mm, respectively.

A. flavus was affected only by culture filtrate of cells grown at 2.5 Klux while *A. niger* was moderately inhibited by all culture filtrates as inhibition zones were fluctuated between 14.33 ± 0.5773 and 18.33 ± 1.00 mm.

Table (15): Effect of different light intensities (Klux) on antimicrobial activities of culture filtrate of *Spirulina platensis*.

Light intensity (Klux)	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
1.50	29.67^{bc} ±0.5773	28.00^{e} ±0.00	30.67^{a} ±0.5773	31.33^{cd} ±0.5773	0.00^{b} ±0.00	14.33^{d} ±0.5773	35.33^{b} ±0.5773
1.75	28.67^{cd} ±0.5773	28.33^{e} ±0.5773	24.67^{b} ±2.8867	30.67^{d} ±0.5773	0.00^{b} ±0.00	15.33^{bcd} ±0.5773	30.33^{de} ±0.5773
2.00	28.67^{cd} ±0.5773	28.00^{e} ±0.00	29.67^{a} ±0.5773	31.33^{cd} ±0.5773	0.00^{b} ±0.00	16.00^{b} ±1.00	30.33^{de} ±0.5773
2.25	30.00^{b} ±1.1547	29.67^{d} ±0.5773	30.00^{a} ±1.00	31.00^{d} ±0.00	0.00^{b} ±0.00	18.33^{a} ±1.00	29.67^{e} ±0.5773
2.50	32.67^{a} ±0.5773	34.67^{a} ±0.5773	31.33^{a} ±0.5773	33.00^{b} ±1.00	12.33^{a} ±0.5773	17.67^{a} ±0.5773	35.67^{b} ±0.5773
2.75	33.33^{a} ±0.5773	31.67^{b} ±0.5773	31.00^{a} ±0.00	34.67^{a} ±0.5773	0.00^{b} ±0.00	15.67^{bc} ±0.5773	40.00^{a} ±1.00
3.00	28.33^{d} ±0.5773	30.67^{c} ±0.5773	29.67^{a} ±0.5773	33.00^{b} ±1.00	0.00^{b} ±0.00	15.00^{bcd} ±0.00	32.00^{c} ±1.00
3.25	30.00^{b} ±0.00	30.67^{c} ±0.5773	29.33^{a} ±0.5773	32.33^{bc} ±0.5773	0.00^{b} ±0.00	15.33^{bcd} ±0.5773	30.67^{de} ±0.5773
3.50	25.67^{e} ±0.5773	12.00^{f} ±0.00	17.00^{c} ±0.00	2267^{e} ±0.5773	0.00^{b} ±0.00	15.00^{bcd} ±0.00	31.00^{cd} ±1.00
3.75	15.67^{f} ±0.5773	0.00^{g} ±0.5773	17.00^{c} ±0.00	0.00^{f} ±0.00	0.00^{b} ±0.00	14.67^{d} ±0.5773	26.67^{f} ±0.5773
4.00	0.00^{g} ±0.00	0.00^{g} ±0.00	0.00^{d} ±0.00	0.00^{f} ±0.00	0.00^{b} ±0.00	14.67^{d} ±0.5773	29.67^{e} ±0.5773

±SE: standard error of three replicates.

a,b,c,d…… etc, different superscripts differ significantly (P<0.05).

B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565

E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145

A.f.: Aspergillus flavus *A.n.: Aspergillus niger*

C.a.: Candida albicans ATCC 10231

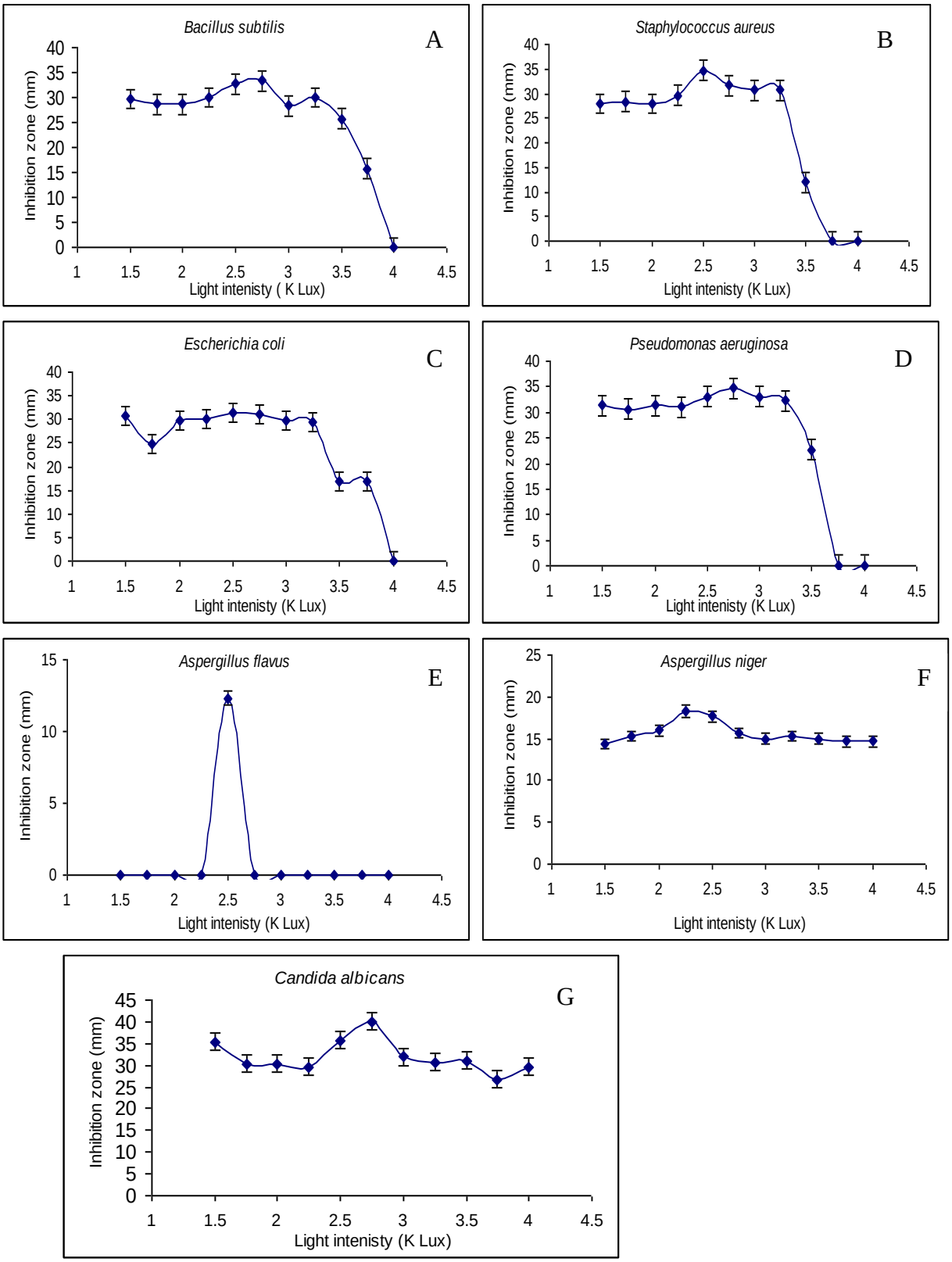


Fig. (14): Effect of different light intensities (Klux) on antimicrobial activities of culture filtrate of *Spirulina platensis*.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Effect of different light intensities on antimicrobial activities of *Spirulina platensis* cells extract.

The data in Table (16) and graphically presented in figure (15) showed the influence of light intensities on biomass production and antimicrobial activities of *S. platensis* cells extract. A significant difference ($P<0.05$) was found in inhibition zones of the tested microorganisms treated with cells extract. The extraction of cells grown at light intensity 1.5, 1.75 and 2.0 Klux showed nonsignificantly differences of antimicrobial activities against Gram +ve bacteria

The extract of *S. platensis* cells exhibited the highest antimicrobial activity at light hntensity of 2.5 Klux, where the inhibition zones were between 11.67 ± 0.5773 mm to 30.0 ± 1.0 mm, according to the test organisms.

However, no significant difference was detected in extract produced by cells grown at light intensity of 2.25 and 2.5 Klux against *Bacillus subtilis*, 21.67 ± 0.5773 mm. The extraction of cells grown at light intensity above 3.0 Klux did not exhibit any antimicrobial activities against all the tested microorganisms.

No antimicrobial activities were deteced with *A. flavus* and *A.niger* at all light intensities except a weak activity at light intensity of 2.5 Klux in case of *A. niger*.

Table (16): Effect of different light intensities (Klux) on antimicrobial activities of *Spirulina platensis* cells extract

Light intensity (Klux)	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
1.50	18.67^{b} ±1.00	20.33^{cd} ±0.5773	18.00^{d} ±0.00	21.33^{c} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	24.33^{c} ±0.5773
1.75	19.00^{b} ±1.00	21.33^{bcd} ±0.5773	20.00^{c} ±1.00	20.00^{d} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	24.33^{c} ±0.5773
2.00	19.33^{b} ±0.5773	20.00^{d} ±1.00	22.00^{b} ±1.732	18.67^{e} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	22.67^{d} ±0.5773
2.25	21.67^{a} ±0.5773	22.33^{b} ±0.5773	21.67^{b} ±0.5773	22.00^{c} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	25.00^{bc} ±0.00
2.50	21.67^{a} ±0.5773	24.67^{a} ±0.5773	26.00^{a} ±0.00	30.00^{a} ±1.00	0.00^{a} ±0.00	11.67^{a} ±0.5773	29.67^{a} ±0.5773
2.75	19.33^{b} ±0.5773	21.67^{bc} ±0.5773	18.00^{d} ±0.00	25.33^{b} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	25.67^{b} ±0.5773
3.00	15.33^{c} ±0.5773	13.67^{e} ±2.3090	0.00^{e} ±0.00	17.67^{f} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	10.33^{e} ±0.5773
3.25	0.00^{d} ±0.00	0.00^{f} ±0.00	0.00^{e} ±0.00	0.00^{g} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{f} ±0.00
3.50	0.00^{d} ±0.00	0.00^{f} ±0.00	0.00^{e} ±0.00	0.00^{g} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{f} ±0.00
3.75	0.00^{d} ±0.00	0.00^{f} ±0.00	0.00^{e} ±0.00	0.00^{g} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{f} ±0.00
4.00	0.00^{d} ±0.00	0.00^{f} ±0.00	0.00^{e} ±0.00	0.00^{g} ±0.00	0.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{f} ±0.00

±SE: standard error of three replicates.

a,b,c,d…… etc, different superscripts differ significantly (P<0.05).

B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565

E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145

A.f.: Aspergillus flavus *A.n.: Aspergillus niger*

C.a.: Candida albicans ATCC 10231

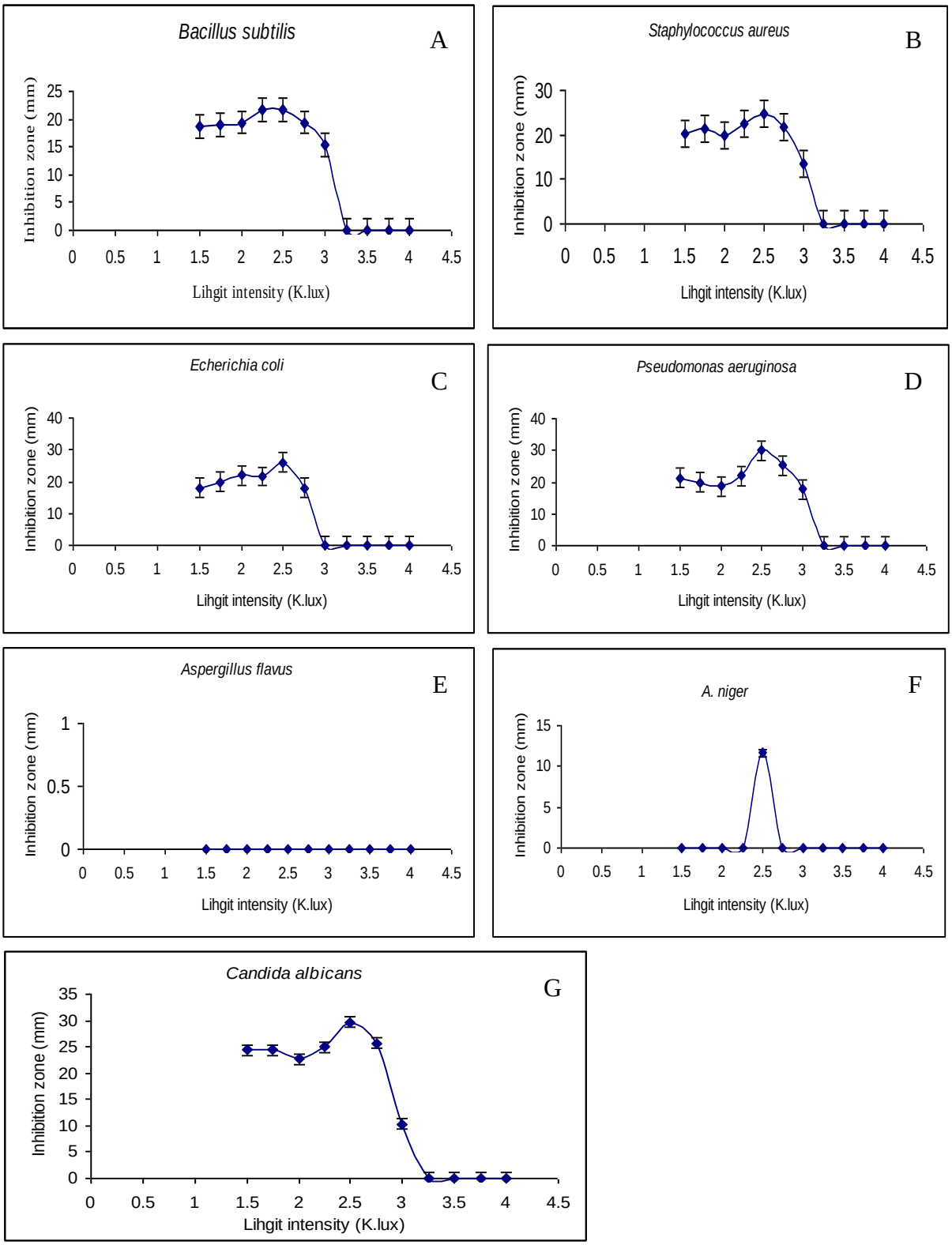


Fig.(15): Effect of different light intensities (Klux) on antimicrobial activities of *Spirulina platensis* cells extract.

A: *Bacillus subtilis* , **B**: *Staphylococcus aureus* , **C**: *Echerichia coli* ,
D: *Pseudomonas aeruginosa*, **E***: Aspergillus flavus* , **F***: A. niger* and **G**: *Candida albicans*

Influence of aeration on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

At the end of incubation period (9 days), biomass production in the aerated culture was significantly increased by 55.98 % over that of the non-aerated culture as illustrated in table (17) and figure (16).

Influence of aeration on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites) .

The data in the table (18) and figure (17) represent the antimicrobial activities of *S. platensis* whole culture (cells and exometabolites) of aerated and non-aerated conditions. The highest inhibition zones were recorded in aerated culture against all the tested microorganisms comparing with that of non-aerated culture. *Candida albicans* was the most sensitive organism to the whole culture extract (cells and exometabolites), with inhibition zone 48.67 ± 0.5773 mm, followed by *B. subtilis*, 47.0 ± 0.0 mm, and *E. coli*, 46.0 ±0.0 mm. The fungal species were the most resistant isolates, where *A. flavus* and *A. nigher* inhibition zones were 18.33 ± 0.577 mm and 13.0 ± 1.0 mm respectively.

Table (17): Influence of aeration on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

Aeration	Dry wteight mg/100 ml
Aerated culture	94.73^{a} ±0.1154
Non aerated culture	60.73^{b} ±0.1154

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).

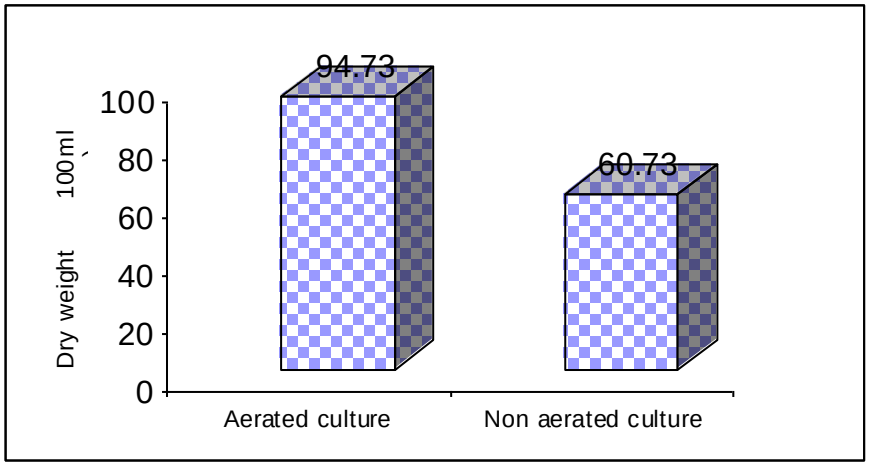


Fig.(16): Influence of aeration on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

Table (18): Influence of aeration on antimicrobial activities of whole culture (cells and exometabolites) of *Spirulina platensis.*

Aeration	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
Aerated culture	47.00[a] ±0.00	41.33[a] ±0.5773	46.00[a] ±0.5773	44.33[a] ±0.5773	18.33[a] ±0.5773	13.00[a] ±1.00	48.67[a] ±0.5773
Non aerated culture	45.00[b] ±0.00	39.00[b] ±0.00	30.33[b] ±0.5773	31.00[b] ±0.00	11.00[b] ±0.00	11.00[b] ±0.00	35.33[b] ±0.5773

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

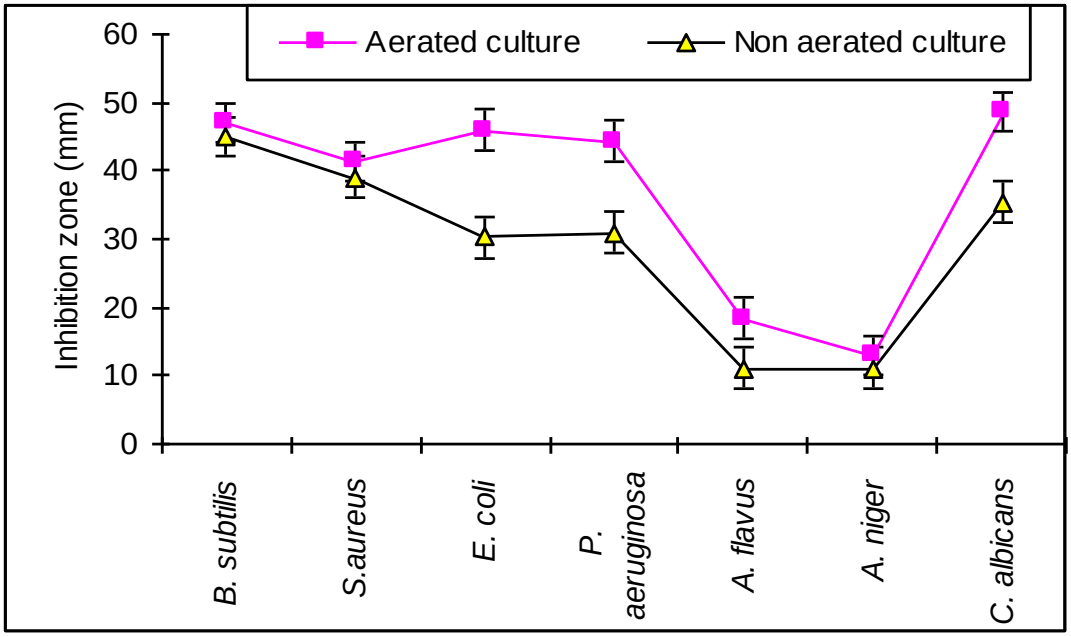


Fig. (17): Influence of aeration on antimicrobial activities of whole culture (cells and exometabolites) of *Spirulina platensis*.

Influence of aeration on antimicrobial activities of *Spirulina platensis* culture filtrate.

It was obvious from the data in the table (19) and figure (18) that *S. platensis* cultural filtrate of aerated and non aerated cultures showed antimicrobial activities against all the tested microorganisms. *Candida albicans* was the most sensitive species to the culture filtrate with inhibition zone 38.67 ± 0.577 mm, while the lowest inhibition zones were recorded with non-aerated culture filtrate for *A. flavus* and *A. nigher* with inhibition zone 11.0 ± 0.0 and 12.0 ± 0.0 mm, respectively.

Influence of aeration on antimicrobial activities of *S. platensis* cells extract.

The extraction of aerated culture cells of *S. platensis* exhibited antimicrobial activities against all the tested microorganisms.There were no significant differences of antimicrobial activities of *S. platensis* culture filtrate of aerated and non-aerated culture against both *S. aureus* and *A. niger*. The highest inhibition zone was recorded for *P. aeruginosa* 25.33 ± 1.5275 mm while the lowest inhibition zones were recorded with *A.niger*. as shown in table (20) and figure (19).

Table (19): Influence of aeration on antimicrobial activities of culture filtrate of *Spirulina platensis.*

Aeration	Mean diameter (±SE) of inhibition zone (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
Aerated culture	35.00[a] ±0.00	31.33[a] ±0.5773	32.67[a] ±0.5773	33.67[a] ±0.5773	15.67[a] ±0.5773	14.00[a] ±1.7230	38.67[a] ±0.5773
Non aerated culture	34.00[b] ±0.00	30.67[a] ±0.5773	30.33[b] ±0.5773	31.00[b] ±0.00	11.00[b] ±0.00	12.00[a] ±0.00	35.33[b] ±0.5773

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

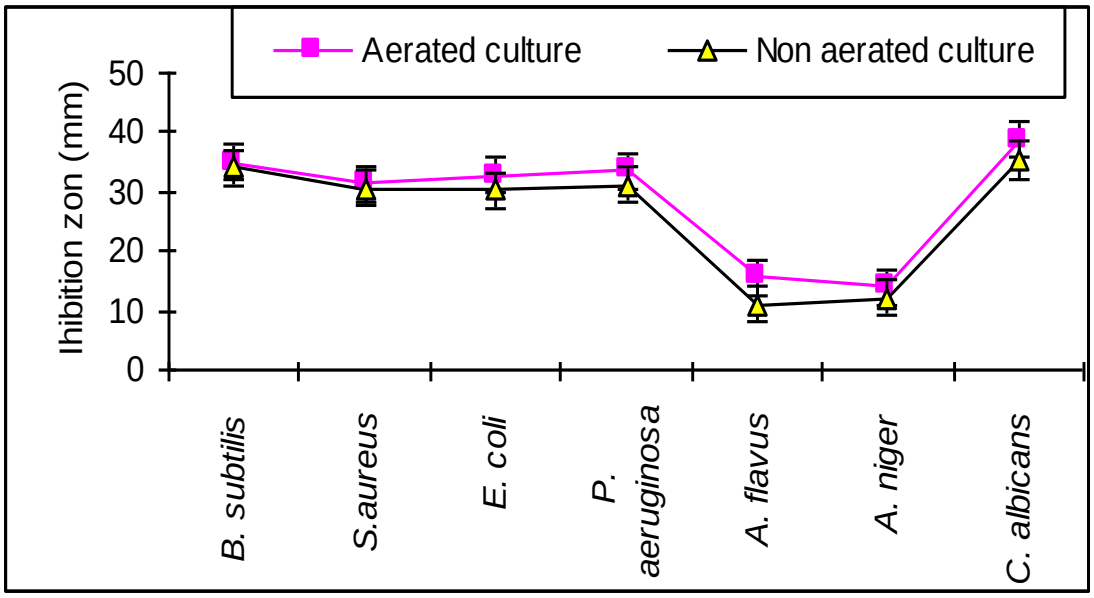


Fig. (18): Influence of aeration on antimicrobial activities of culture filtrate of *Spirulina platensis.*

Table (20): Influence of aeration on antimicrobial ativityies of cells extract of *Spirulina platensis.*

Aeration	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
Aerated culture	21.67^{a} ±0.5773	22.33^{a} ±0.5773	23.67^{a} ±0.5773	25.33^{a} ±1.5275	0.00^{a} ±0.00	11.67^{a} ±0.5773	22.00^{a} ±1.00
Non aerated culture	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.00^{a} ±0.00	11.33^{a} ±0.5773	11.67^{b} ±0.5773

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

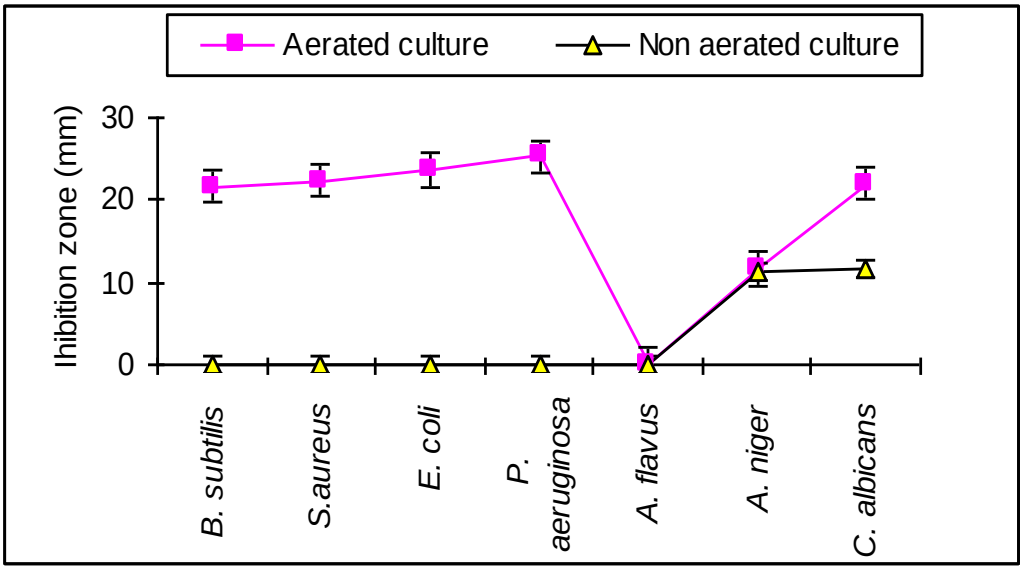


Fig. (19): Influence of aeration on antimicrobial activities of cells extract of *Spirulina platensis.*

The effect of light duration on biomass production of *Spirulina platensis* (mg dry wt./100 ml).

Table (21) and figure (20) demonstrated that *Spirulina platensis* had better biomass production in the 24h. photoperiod than 12 h. photoperiod. Twenty-four hour illumination biomass production was 107.14% greater than that obtained at 12 h. illumination.

Effect of light duration on antimicrobial activities of *S. platensis* whole culture (cells and exometabolites).

There was no significant difference between antimicrobial activities of *S. platensis* whole culture (cells and exometabolites) grown at 24 hour and 12/12 hour (dark/light) photoperiod against Gram-ve bacteria and *C. albicans*.Whole culture (cells and exometabolites) of *S. platensis* grown at 12hour Photoperiod had no inhibitory effect against *A.flavus* and *A.niger*. Table (22) and figure (21) showed the highest inhibition zone of *S. platensis* whole culture (cells and exometabolites) grown at 24 h. photoperiod, which was recorded against *E. coli* with an inhibition zone of 32.0 ±2.0 mm.

Table (21). Effect of light duration on biomass production of *Spirulina platensis* (mg dry wt./100ml).

Light duration (hour)	Dry wghiet mg/100 ml
12/12 (dark/light)	56. 87[b] ±0.4618
24 photoperiod	117.80[a] ±0.5291

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).

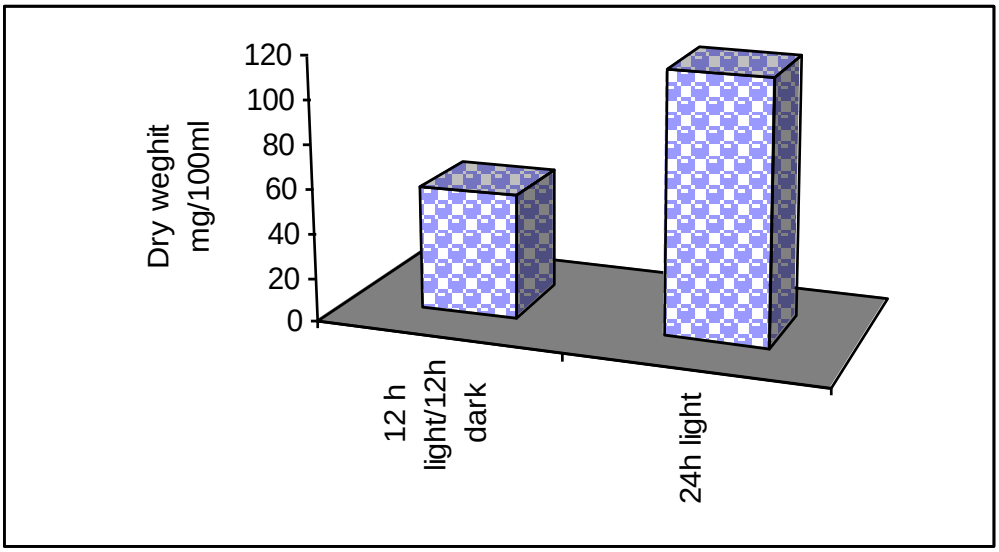


Fig. (20): Effect of light duration on biomass production of *Spirulina platensis* (mg dry wt./100ml).

Table (22) Effect of light duration on antimicrobial activities of *Spirulina platensis* whole culture (cells and exometabolites).

Light duration (hour)	Mean diameter (±SE) of inhibition zones (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
12/12 (dark/light)	23.33[b] ±1.1547	20.33[b] ±0.5773	23.33[b] ±1.1547	25.00[b] ±1.00	0.00[b] ±0.00	0.00[b] ±0.00	22.00[a] ±2.83
24 photoperiod	28.00[a] ±3.829	27.00[a] ±1.00	32.00[a] ±2.00	28.33[a] ±1.5275	15.67[a] ±0.5773	17.33[a] ±2.0816	23.00[a] ±1.00

±SE: standard error of three replicates.
,a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC3610 *S.a: Staphylococcus aureus* ATCC
E.c.: Echerichia coli NCTC9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavu , *A.n.: Aspergillus niger*
C.a.: Candida albicans

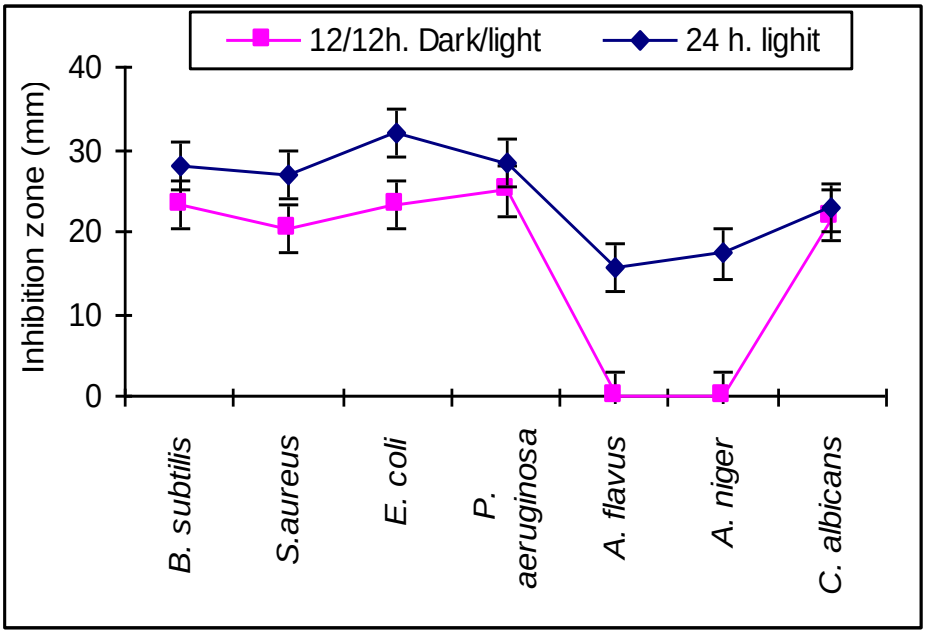


Fig. (21): effect of light duration on antimicrobial activities of *S. platensis* whole culture (cells and exometabolites).

Effect of light duration on antimicrobial activities of *S. platensis* culture filtrate.

The data represented in table (23) and figure (22) revealed the antimicrobial effect of *S. platensis* grown at 12/12h. (dark/light) and 24 h. photoperiod.

A significant difference between the antimicrobial effects of the two photoperiods was recorded as inhibition zones of *B. substilis, S. aureus, A. falvus* and *A. nigher*. Antimicrobial sensitivity of the tested microorganisms followed the order *B. subtilis* > *E. coli* ≥ *P. aeruginosa* > *S. aureus* > *C.albicans*, with inhibition zones of 29.67 ± 0.5773, 28.33 ± 1.527, 27.0 ± 1.0, and 25.0 ± 3.829 mm, respectively. While *A. flavus* was the most resistant species followed by *A. niger* with inhibition zones 12.33 ±0.5773 and 16.33 ±0.5773 mm, respectively.

Table (23): Effect of light duration on antimicrobial activities of *Spirulina platensis* culture filtrate.

Light duration (hour)	Mean diameter (±SE) of inhibition zone (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
12/12 (dark/light)	21.33^{b} ±0.5773	20.33^{b} ±0.5773	28.00^{a} ±0.00	28.00^{a} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	23.00^{a} ±1.00
24 photoperiod	29.67^{a} ±0.5773	27.00^{a} ±1.00	28.33^{a} ±1.5275	28.33^{a} ±1.5275	12.33^{a} ±0.5773	16.33^{a} ±0.5773	25.00^{a} ±3.829

±SE: standard error of three replicates.
,a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

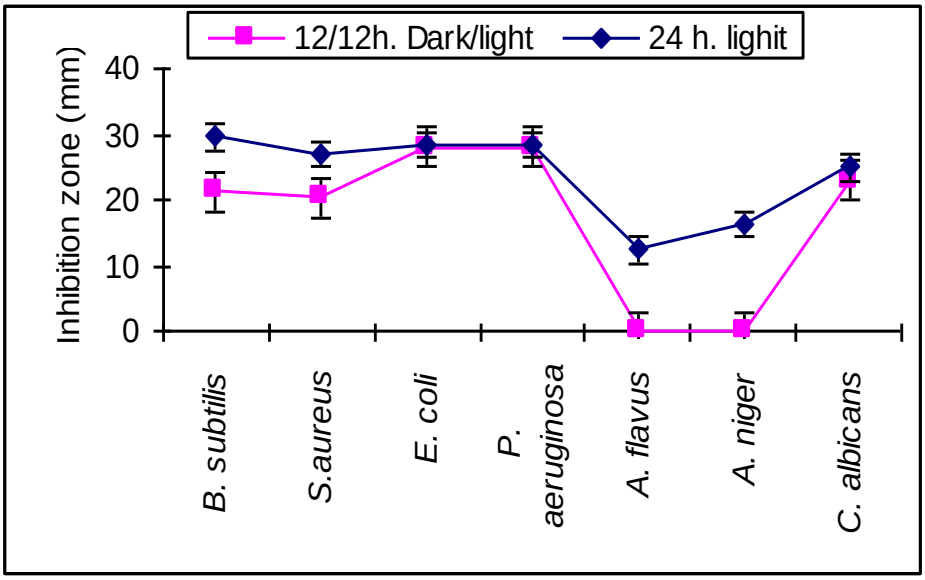


Fig. (22): Effect of light duration on antimicrobial activities of *S. platensis* culture filtrate.

Effect of light duration on antimicrobial activities of *Spirulina platensis* cells extract.

The extracted cells of *S. platensis* previously grown at 24 h. photoperiod showed significantly higher antimicrobial activities than that grown at 12 h. photoperiod. The highest inhibition zone was observed with *B. subtilis* and *P. aeruginosa,* 31.67 ± 1.528 nm and 31.33 ± 0.5773 mm respectively. On the other hand the lowest inhibition zone was observed with *A. niger* (11.67 ± 0.5773 mm) with *S. platensis* extracted cells that previously grown at 24 h. photoperiod while those of 12/12 photoperiod had not antimicrobial activities with either *A. flavus* or *A. niger*.

The test microorganisms differed in relation to their susceptibility to purified antibiotic produced by *S. platensis*. The Gram positive bacterium *Bacillus subtilis* was the most susceptibile bacterial species, while the Gram negative bacterium *P. aeruginosa* was the least susceptible, the purified antibiotic produced by *S. platensis* was observed to be more active against Gram positive, Gram negative bacteria and unicellular fungi,*C. albicans*. On the other hand most resistance species were the multicellular fungi as recorded in table (24) and figure (23)

Table (24) Effect of light duration on antimicrobial activities of *Spirulina platensis* cells extract.

light duration (hour)	Mean diameter (±SE) of inhibition zone (mm)						
	Bacteria				Fungi		
	G +ve		G -ve		Multicellular		Unicellular
	B.s.	*S.a.*	*E.c.*	*P.a.*	*A.f.*	*A.n.*	*C.a.*
12/12 (dark/light)	27.00^{b} ±0.00	26.67^{b} ±1.1547	25.67^{b} ±0.5773	27.67^{b} ±0.5773	0.00^{a} ±0.00	0.00^{b} ±0.00	29.67^{a} ±0.5773
24 photoperiod	31.67^{a} ±1.5275	29.67^{a} ±0.5773	28.00^{a} ±0.00	31.33^{a} ±0.5773	0.00^{a} ±0.00	11.67^{a} ±0.5773	29.33^{a} ±0.5773

±SE: standard error of three replicates.
a,b,c,d…… etc, different superscripts differ significantly (P<0.05).
B.s.:Bacillus subtilis NCTC 3610 *S.a: Staphylococcus aureus* ATCC 13565
E.c.: Echerichia coli NCTC 9132 *P.a.: Pseudomonas aeruginosa* ATCC10145
A.f.: Aspergillus flavus *A.n.: Aspergillus niger*
C.a.: Candida albicans ATCC 10231

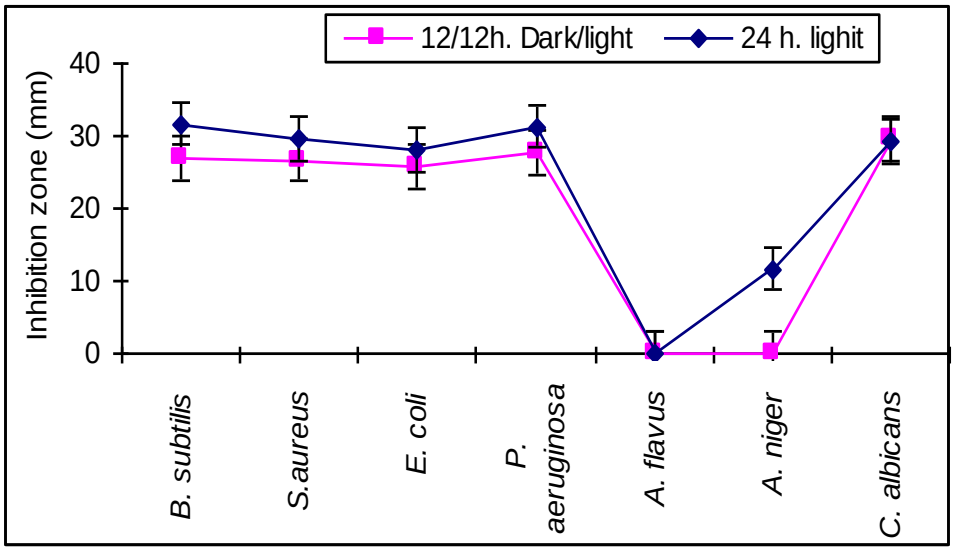


Fig.(23): Effect of light duration on antimicrobial activities of *S. platensis* cells extract.

The antimicrobial potentialities of the purified antibiotic produced by *Spirulina platensis.*

Data in table (25), showed that, the test microorganisms differed in their susceptibility to purified antibiotic produced by *S. platensis*.

C. albicans was the most susceptible test organism with minimal inhibition concentration of 30.0μg ml^{-1}, while the Gram –ve bacterium *P. aeruginosa* was the least susceptible one (MIC,85μg ml^{-}). The purified antibiotic produced by *S. platensis* was more active against Gram +ve, Gram -ve and *C. albicans*. The most resistance isolates were the multicellular fungi *A. flavus* and *A. niger*

Table (25): The antimicrobial activities of purified antimicrobial substance produced by *Spirulina platensis.*

Microorganism	MIC μg ml^{-1}	MCC μg ml^{-1}	Streptomycin	Polymyin
B. subtilis NCTC 3610	60.0	80.0	20.0	ND
S. auerus ATCC 13565	65.0	90.0	25.0	ND
E. coli NCTC 9132	80.0	110.0	30.0	ND
P. aeruginosa ATCC 10145	85.0	120.0	35.0	ND
C. albicans ATCC 10231	30.0	45.0	15.0	20
Aspergillus flavus	> 120	>200	ND	R
A. niger	> 120	>200	ND	R

ND: Not detected, R: Resistance
MIC: Minimum inhibition concentration.
MCC: Minimum cidal concentration.

Characterization of the antimicrobial product produced by *Spirulina platensis.*

-The physical properties.

The purified compound was found to be yellowish green with no characteristic odor, soluble in methanol, diethyl ether, chloroform and dimethyl sulfoxide, but sparingly soluble in water and acetone. Melting point was 37-40 °C .

The spectroscopic analysis of the purified anti-microbial product

The compound showed the following data:

I) IR spectrum showed bands at 1269 cm^{-1}, 1414 cm^{-1} (C–O–C), 1643 cm^{-1} (CO of amide),1563 cm^{-1} (C=C) and broad band 3441 cm^{-1} (of OH and NH).

II) ^{1}HNMR showed δ 0.8 (-CH3), δ 1.2 (-CH2), δ 4.2(-OH), δ 7.2(-NH), δ 7.4 and δ 7.7 (aromatic CH).

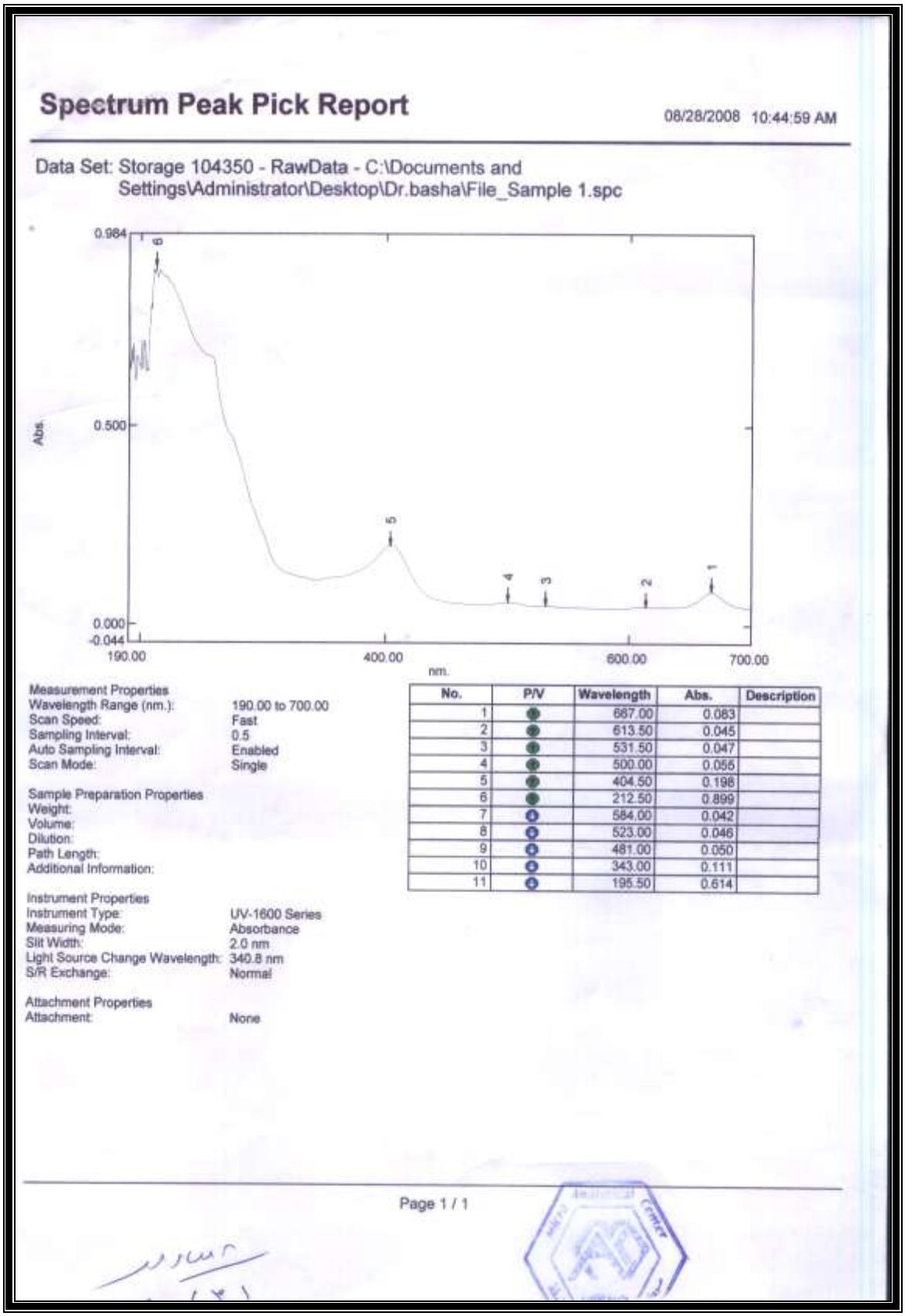
III) Mass spectrum showed molecular ion beak at m/z = 341(abundance (0.03%).

Also, the elemental analysis gave molecular formula:

$C_{15}H_{18}NO_8$.

Table (26): Biological activity of fractions obtained from column.

Fraction	**Inhibition zone (mm)**	
	Gram -Ve	**Gram +Ve**
	Echerichia coli	*Staphylococcus aureus*
1	25.0	26.0
2	18.0	17.0
3	-Ve	-Ve
4	-Ve	-Ve
5	-Ve	-Ve
6	-Ve	-Ve
7	-Ve	-Ve
8	-Ve	-Ve
9	-Ve	-Ve
10	-Ve	-Ve
11	-Ve	-Ve



Spectrum Peak Pick Report

08/28/2008 10:44:59 AM

Data Set: Storage 104350 - RawData - C:\Documents and Settings\Administrator\Desktop\Dr.basha\File_Sample 1.spc

Measurement Properties

Wavelength Range (nm.):	190.00 to 700.00
Scan Speed:	Fast
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

Instrument Properties

Instrument Type:	UV-1600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Light Source Change Wavelength:	340.8 nm
S/R Exchange:	Normal

Attachment Properties

Attachment:	None

No.	P/V	Wavelength	Abs.	Description
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2	▼	613.50	0.045	
3	▼	531.50	0.047	
4	▼	500.00	0.055	
5	▼	404.50	0.198	
6	▼	212.50	0.899	
7	▲	584.00	0.042	
8	▲	523.00	0.046	
9	▲	481.00	0.050	
10	▲	343.00	0.111	
11	▲	195.50	0.614	

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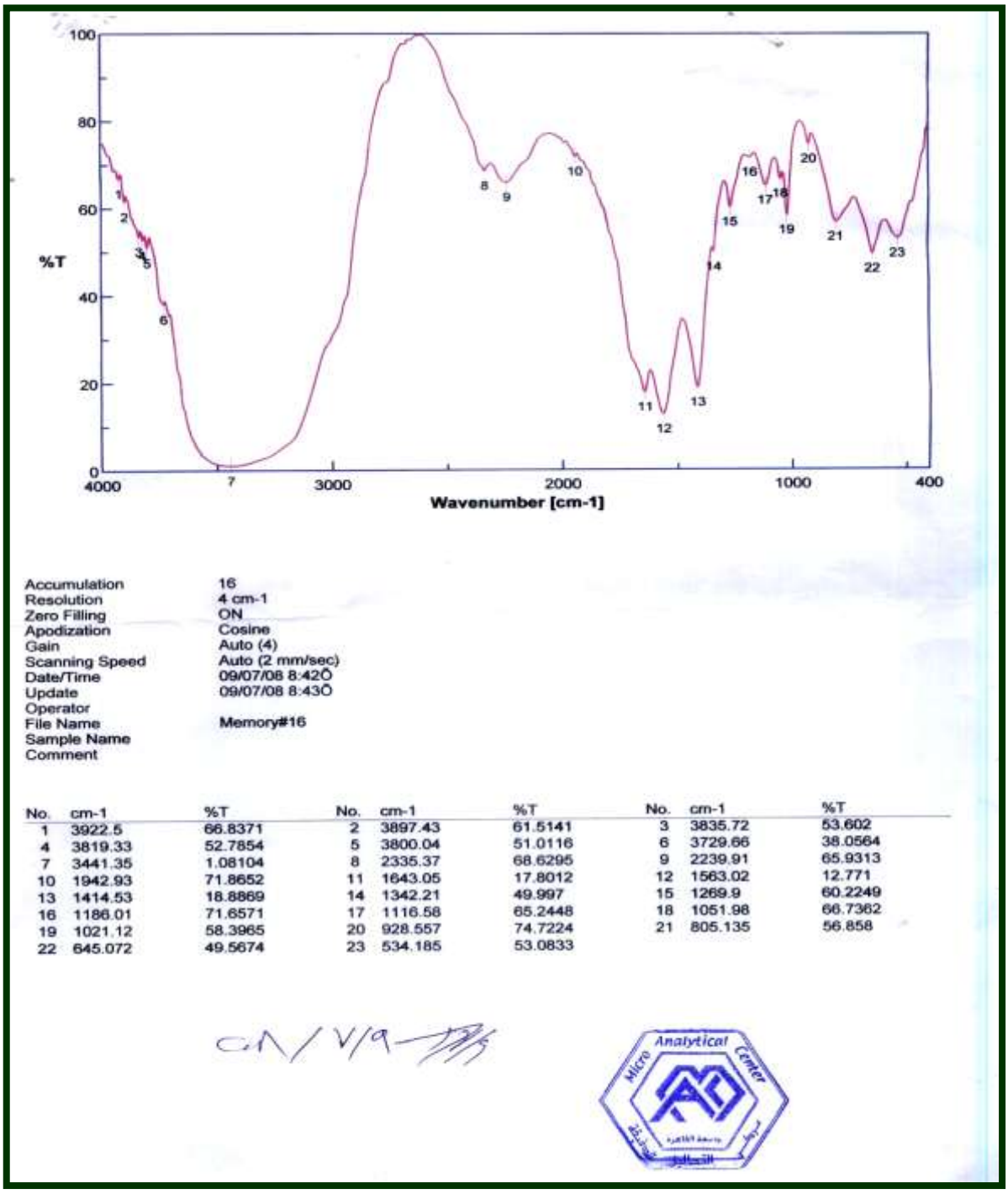
Fig. (24) : UV Spectrum of the antimicribially active compound produced by *Spirulina platensis.*



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Resolution	4 cm-1
Zero Filling	ON
Apodization	Cosine
Gain	Auto (4)
Scanning Speed	Auto (2 mm/sec)
Date/Time	09/07/08 8:42Ô
Update	09/07/08 8:43Ô
Operator	
File Name	Memory#16
Sample Name	
Comment	

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3922.5	66.8371	2	3897.43	61.5141	3	3835.72	53.602
4	3819.33	52.7854	5	3800.04	51.0116	6	3729.66	38.0564
7	3441.35	1.08104	8	2335.37	68.6295	9	2239.91	65.9313
10	1942.93	71.8652	11	1643.05	17.8012	12	1563.02	12.771
13	1414.53	18.8869	14	1342.21	49.997	15	1269.9	60.2249
16	1186.01	71.6571	17	1116.58	65.2448	18	1051.98	66.7362
19	1021.12	58.3965	20	928.557	74.7224	21	805.135	56.858
22	645.072	49.5674	23	534.185	53.0833			

Fig. (25): IR Spectrum of the antimicribially active compound produced by *Spirulina platensis.*

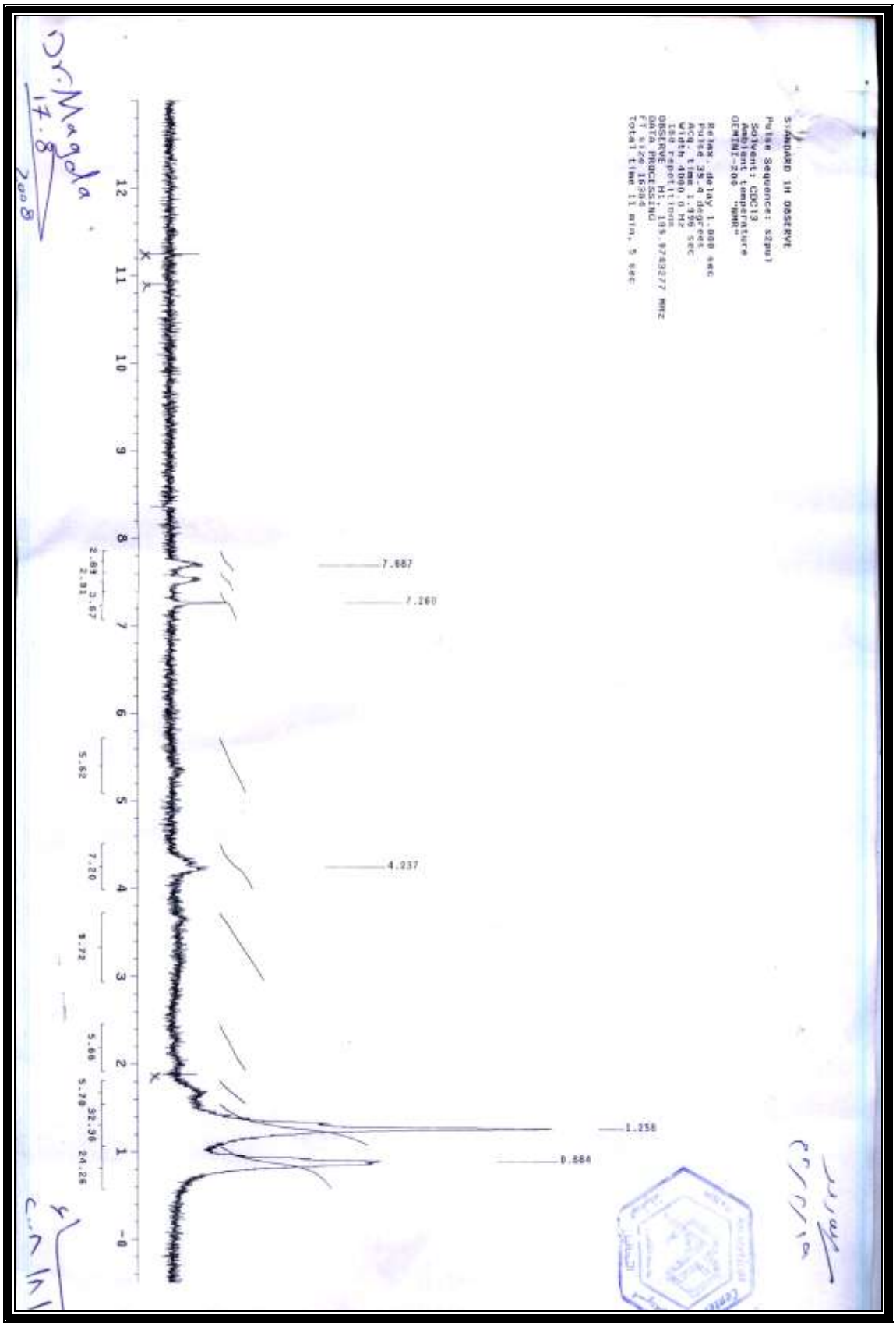

Fig. (26) : ^{1}HNMR Spectrum of the antimicribially active compound produced by *Spirulina platensis*.

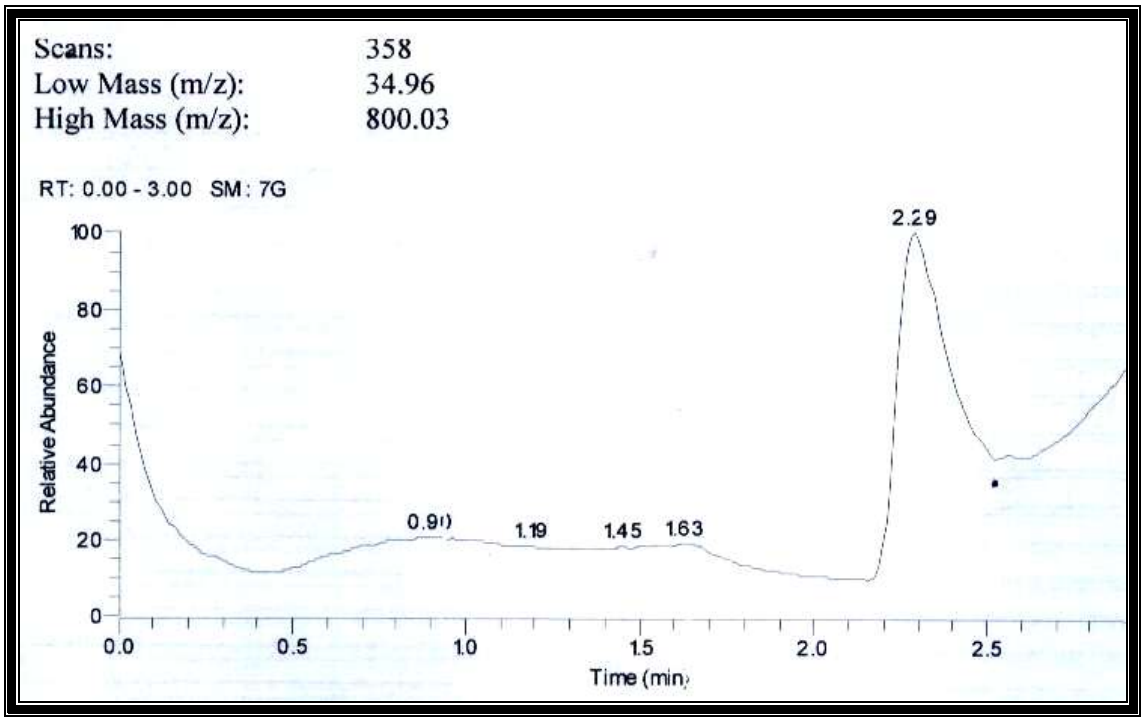

Fig. (27) : Mass Spectrum of the antimicribially active compound produced by *Spirulina platensis*

Table (27): Behaviour of the active compound towards differed chemical reactions

Test	Reaction	Result
1. Ninhydrine Test	-Ve	No proteins
2. Erlish's Reaction	-Ve	No group of tryptophan.
3. Nitroprosside Reaction	-Ve	No phenolic group.
4. Ferric chloride Reaction	-Ve	No diketons.
5. Fehling's Reaction	-Ve	No free aldehyde group.
6. Meyer's Reaction	-Ve	No nitro group
7. Tollen's Reaction	+Ve	An aromatic aldhyde, diketons or aromatic amines
8. Lead sulphide reaction	-Ve	No aminoacid contains sulfur.
9. Molish's reaction	-Ve	No carbohydrates
10. Sakaguchi's Reaction	-Ve	No guanidine group of arginine

Proximal composition of *Spirulina platensis* .

At pH 9.0, 30 °C and continous light of 2.50 Klux after 9 days of incubation the organism produced the highest biomass. Table (28) showed the proximal composition of *S. platensis* where protein amount was 52.9 ± 0.21 g/100g, crude fiber was 13.07 ± 2.67, while carbohydrate amount was 14.3 ± 1.113 g/100 g (dry wt). Total ash in the biomass was 10.77 ± 0.252 and lipids 5.94 ± 0.561 g/100g (dry wt.).

Table (28): Proximal composition of *Spirulina platensis* grown at 30 °C and pH 9 for 9 days (g/100 g dry weight)

Moisture	11.1 ± 0.557
Crude protein	52.9 ± 0.21
Crude fiber	13.07 ± 2.67
Carbohydrate	14.3 ± 1.113
Ash	10.77 ± 0.252
Lipids	5.94 ± 0.561

Plate(1) : *Spirulina platensis* under the light microscope.

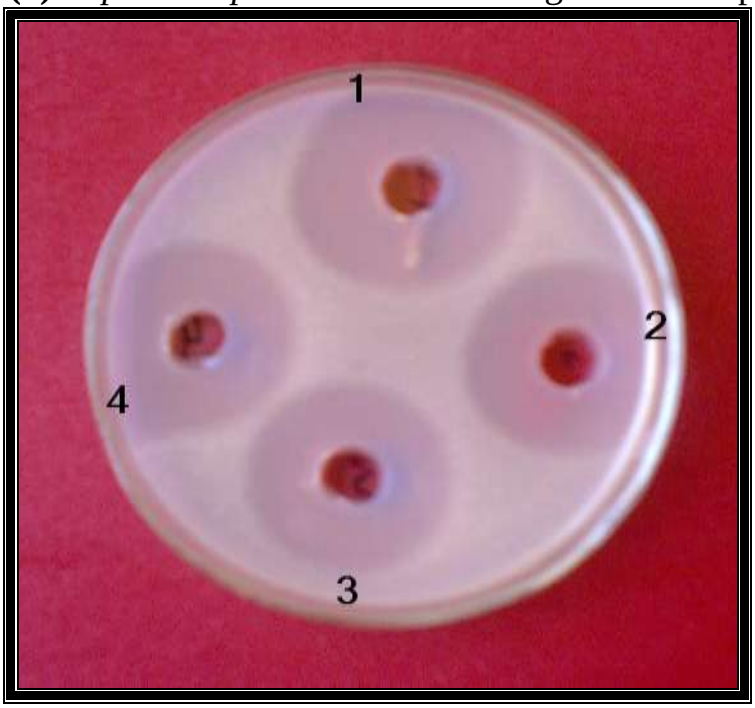

Plate (2): Antimicrobial activity of *Spirulina platensis* culture filtrate grown at different light intensities against *Candida albicans*.
1: 1.5 Klux 2: 1.75 Klux 3: 2.00 Klux 4: 2.25 Klux

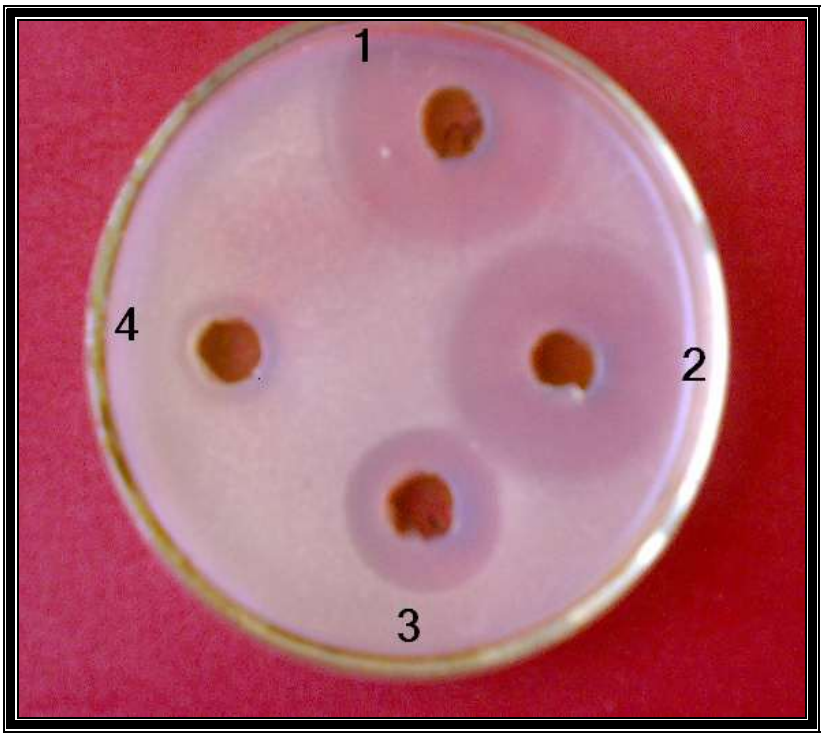

Plate (3) : Antimicrobial activity of cells extract of *Spirulina platensis* grown at different pH values against *Bacillus subtilis*.
1: pH 8 2: pH 9 3: pH 10 4: pH 11

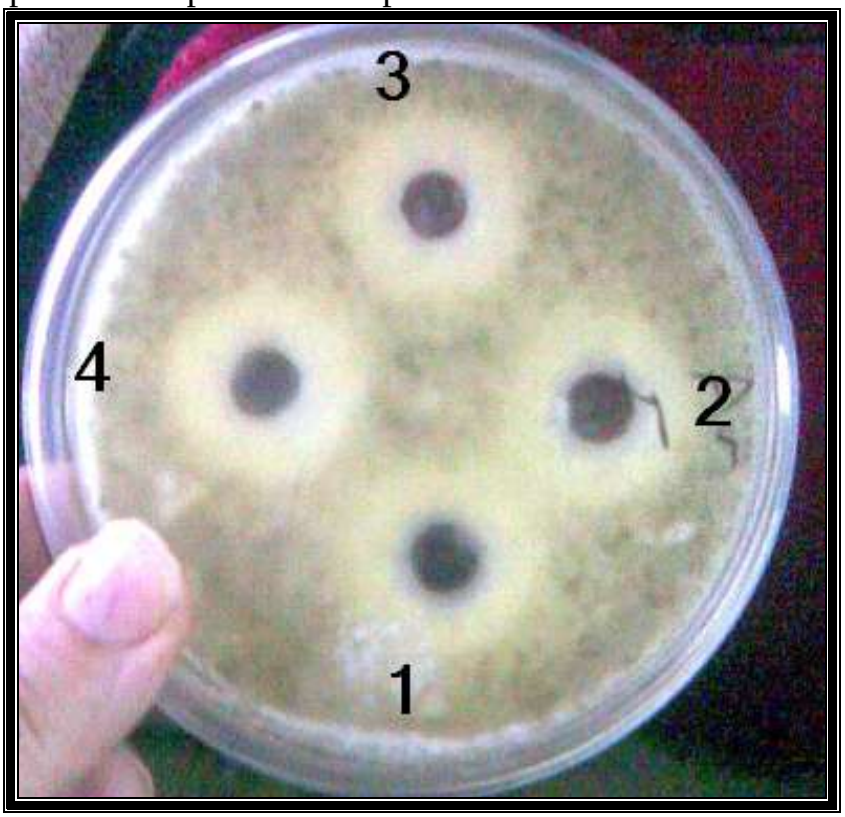

Plate (4) : Antimicrobial activity of *Spirulina platensis* culture filtrate grown at different light intensities against *Aspergillus niger* .
1: 1.5 Klux 2: 1.75 Klux 3: 2.00 Klux 4: 2.25 Klux

Plate (5): *Spirulina platensis* cells on the naylon mesh.

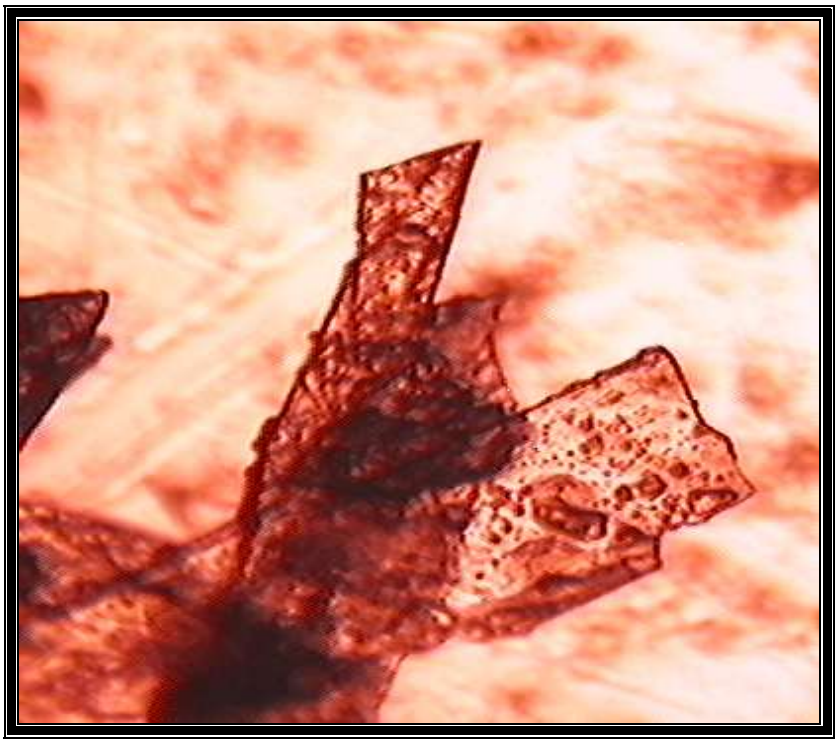

Plate (6) : The antagonistic compound under the light microscope.

Plate (7) : Thin layer chromatography of *Spirulina platensis* ethanolic cells extract under the long wave ultra violet lamp (360 nm).