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BETA-CAROTENE EXTRACTION FROM SOME MICROALGAE, CYANOBACTERIA AND CHLOROPHYTA WITH ITS ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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Abstract

Beta-carotene pigment was extracted from 3 isolates of some microalgae (cyanobacteria and green algae) which were isolated from the local environment of Mosul city. The maximum concentration of the pigment in the acetone extract of *Chlorella* alga (RC) was (1179.1 ppm), whereas the minimum was in the lower layer after separation of cyanobacteria *Gloeocapsa* (F2G) and was (271.2 ppm). The efficacy of Beta- carotene pigment and the acetone extract against bacteria and pathogenic fungi was studied and the results showed that all the samples of the used genera (cyanobacteria and green algae) had showed inhibitive efficacy against bacteria and fungi. The acetone extract of *Chlorella* surpassed the other extracts of raw cyanobacteria (acetone) and this applies to the beta- carotene pigment F1 extracted from *Chlorella* and its efficacy against bacteria and fungi, whereas the lower layer F2 showed the least effect against bacteria and fungi.

Key word: b-carotene, Micro algae, Antimicrobial.

Introduction

Algae are simple autotrophic organisms, i.e. they have the ability to produce organic carbon through photosynthesis, their individuals vary in size, cellular structure and biological characteristic (Frost *et al.,* 2012). Microalgae are considered by some as being composed of bluish green algae (cyanobacteria) with primitive nuclei, some small real nuclei *chlorophyta* (Miazeketal., 2015) and *Gloeocapsa* which belongs to the family *Chroococcaceae* with oval cells and rounded ends, spherical or semi- spherical and each cells is surrounded by a distinguished gelatine sheath (Athbi, 2014). As for *Fischrella* algae, the thallus is diverse and of different shape, either single stranded or multi- stranded green coloured and usually trailing. A few of them consist of upright branches which consist of oval cells (Wehr and Sheat, 2003). The green alga *Chlorella* belongs to the family Chlorellaceae; it lives in sweet water and is of single cell, each of which (μ8-L) thick and is non- flagellate (Shim *et al.,* 2008). Algae are considered one of the richest biological sources with biologically effective compounds resulting from secondary metabolism, and they are sources for many of them such as Carotenoids, Terpens, Chlorophyll, fatty acids, alkaloids, phenols and other compounds used in manufacture and development of medicines to treat diseases such as cancer, aids, rheumatism, inflammations and viral, microbic or fungal infections (Almeida *et al.,* 2011; Harmsen, 2011).

Carotene pigments are natural pigments produced by different groups of living beings like plants, animals and microorganisms, and they take different colours like yellow, orange, red and purple (Li *et al.,* 2012). Carotene pigments are characterized by ease of extraction and purification, their low cost, and also they have no negative side effects (Frengova and Behkova, 2002; Wang *et al.,* 2012). Beta- carotene pigment is of a ring- shape composition made of a long chain of double bonds. Temperature consolidates the attachment of the double bonds resulting in their light colour (Fratianni *et al.,* 2010), it also does many functions in the human body, namely limiting the hazard of developing cancer, and heart and cardiovascular diseases due to their anti- oxidation characteristics (Wang *et al.,* 2008). Nowadays attention increased to the beta- carotene pigment which is one of the biologically active compounds that works with high efficacy (Ambati *et al.,* 2014).

To analyse the beta- carotene pigment, many methods were used including chromatography which is characterized by being on a high degree of sensitivity

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and particularity in establishing results. It includes thin layer chromatography (Sathya, 2017; Bhagarathy *et al.*,, 2011) and high performance liquid chromatography (HPLC) (Madhurima and Pooja, 2013). The study aims to extract the beta- carotene pigment from some microalgae and to study their effect on bacteria and pathogenic fungi.

Materials and Methods

Samples of the study were taken from higher education laboratories of the college of education/ department of biology, and they were diagnosed as:

Cyanobacteria, *Gloeocapsa*, *Fischrella* and *Chlorella Vulgaris.*

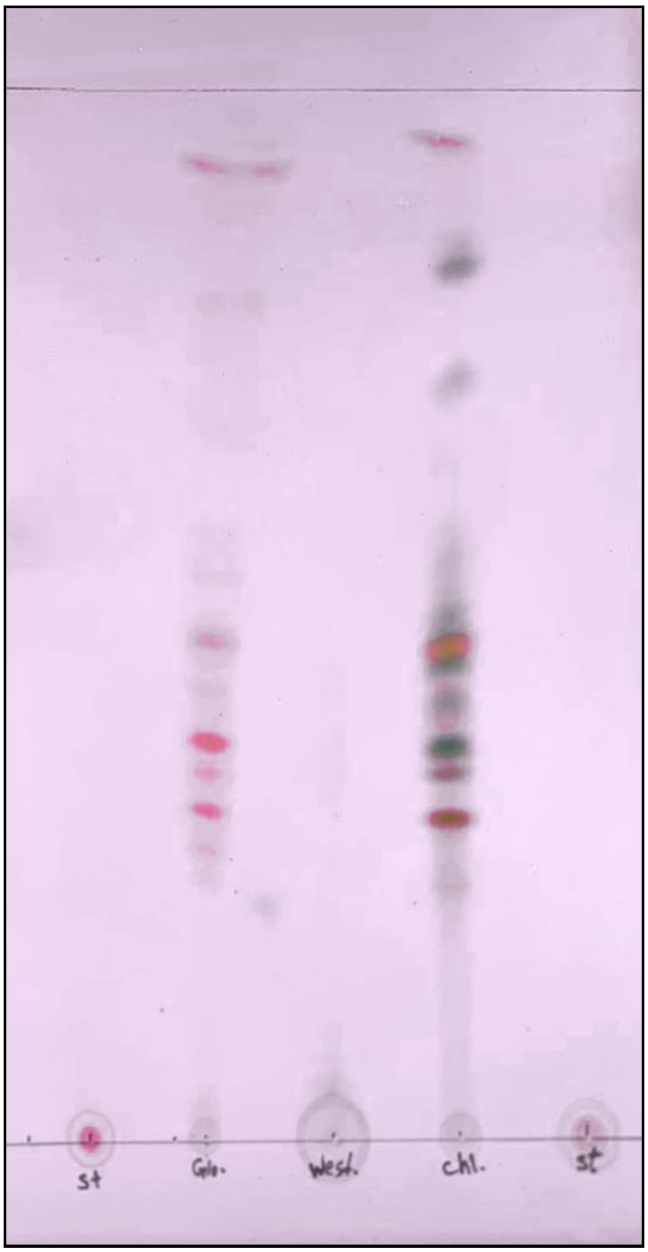


Fig. 1: Mobile Phase Petroleum Ether : Acetone.

Extraction of beta- carotene pigment

Beta- carotene pigment was extracted from some microalgae (cyanobacteria and green algae) after drying the wet mass taken from a farm of two weeks old using the method of (Harborne, 1984), a weight of 2g was taken and added to 50 ml of acetone then enwrapped with aluminium foil with stirring via magnetic stirrer for 24 hours, then put into the separating funnel and added to it a mixture of ether and methanol with a proportion of 37.5:5 ml respectively, then a polar an a non- polar layers were formed, and then the sample size was reduced via Rotary Vacuum Evaporator at a temperature of 40- 60 c˚ and then put in dark flasks.

Purification and diagnosis of beta- carotene pigment

Some steps and methods were followed to purify the

Fig. 2: Mobile Phase Hexane : Acetone.

Table 1: Values of R_f for Cyanobacteria (Gloeocapsa).

S. No.	Pigment	R_f values	Moving phase	Source
1-	Tycopene	0.07	Hexane: Acetone	Bhagavathy *et al.,*
2-	Myxoxanthin	0.15	Hexane: Acetone	2011
3-	Violaxanthin	0.22	Petroleum ether:	Bhagavathy *et al.,*
4-	Lutein	0.43	acetone	2011
5-	Chlorophyll b	0.54	Petroleum ether:	Alma *et al.,* (2018)
6-	Chlorophyll a	0.65	acetone	Alma *et al.,* (2018)
7-	B- carotene	0.94	Petroleum ether:	Sathya, (2017)
			acetone	Sathya, (2017)
			Petroleum ether:	Alma *et al.,* (2018)
			acetone	
			Petroleum ether:	
			acetone	

Table 2: Values of R_f for Cyanobacteria (*Fischrella)*

S. No.	Pigment	R_f values	Mobile phase	Source
1-	Neoxanthin	0.081	Petroleum ether:	Alma *et al.*, (2018)
2-	Myxoxanthin	0.15	Acetone	Bhagavathy *et al.*,
3-	Violaxanthin	0.2	Hexane: Acetone	(2011)
4-	Chlorophyll a	0.31	Hexane: Acetone	Bhagavathy *et al.*,
5-	B- carotene	0.94	Hexane: Acetone	Bhagavathy *et al.*,
			Petroleum ether:	(2011)
			Acetone	Alma *et al.*, (2018)

beta- carotene pigment among which were thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

1- Thin layer chromatography: Silica gel plates were used of dimensions (20×50 cm) where a spot of isolated beta- carotene samples was put on, and the plate loaded with the samples was put into a developing glass tank that contains solvents system; the first: petroleum ether: acetone (70:30ml) (Sathyu, 2017) and the second: Hexane: acetone (75:25ml) (Bhagavathy *et al.,* 2011), then standard samples were prepared of the beta- carotene pigment and after the separation was finished, separated

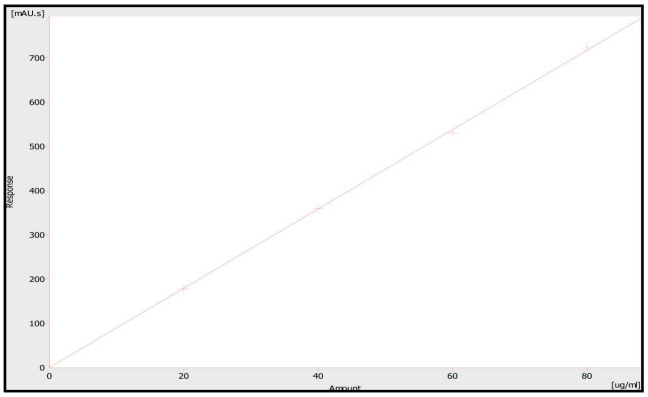


Fig. 3: Standard curve of beta-carotene pigment.

Table 3: Values of R_f for Cyanobacteria (*Chlorella*).

S. No.	Pigment	R_f values	Moving phase	Source
1-	Myxoxanthin	0.15	Hexane: Acetone	Bhagavathy *et al.,*
2-	Violaxanthin	0.22	Hexane: Acetone	(2011)
3-	Lutein	0.43	Petroleum ether:	Bhagavathy *et al.,*
4-	Fucoxanthin	0.5	acetone	(2011)
5-	Chlorophyll b	0.54	Petroleum ether:	Alma *et al.,* (2018)
6-	Chlorophyll a	0.69	acetone	Alma *et al.,* (2018)
7-	α- carotene	0.61	Petroleum ether:	Sathya, (2017)
8-	B- carotene	0.94	acetone	Alma *et al.,* (2018)
			Petroleum ether:	Bhagavathy *et al.,*
			acetone	(2011)
			Hexane: Acetone	Alma *et al.,* (2018)
			Petroleum ether:	
			acetone	

spots were noticed via ultra- violate ray source to calculate the rate of flow (RF).

Rate of flow (R_f) =

$$\frac{dis\tan ce\,\mathrm{cov}ered\ by\ the\ solved\ subs\tan ce}{dis\tan ce\,\mathrm{cov}ered\ by\ the\ solvent}$$

2- Beta- carotene pigment estimation using the high performance chromatography separation technique (HPLC)

Beta-carotene pigment was estimated in cyanobacteria and algae isolates according to (Madhurima and pooja, 2013) using the high performance liquid chromatography (HPLC) device where the carrier phase of acetonitrile: distilled water (80:20ml) was used as well as a separation column (C18- ODS) (25cm*4.6 cm), and the ultra- violate ray detector (uv- vis) was used with a wavelength of (450 nm) and a rate of flow (1 ml/minute). From the separation process resulted the drawing of peak curve for each sample along with its time of retention and the area of the curve for the standard beta- carotene pigment.

Anti microbial activity of beta carotene from micro algae

The method of well diffusion (Magaldie *et al.,* 2004) was followed, to test the inhibitive activity of B- carotene against Gram positive isolates *Staphylococcus aureus* and Gram negative isolates *Psuedomonas aeruginosa, Escherichia coli, Proteus and Klebsiella,* by growing them on petri dishes with Nutrient agar medium and

Table 4: Quantum estimation of B- carotene pigment extracted from cyanobacteria and green algae via the high performance liquid chromatography (HPLC).

Isolates	Extracts	Retention time	Concentration ppm
Gloeocapsa	Raw acetone extract (RG)	7.7037.7037.703	924.2638.6271.2
	Upper layer ether (F1G)		
	Lower layer methanol (F2G)		
Fischrella	Raw acetone extract (RF)	7.7037.7037.703	1092.2891.2354.8
	Upper layer ether (F1F)		
	Lower layer methanol (F2F)		
Chlorella	Raw acetone extract (RC)	7.7037.7037.703	1179.1780.3410.3
	Upper layer ether (F1C)		
	Lower layer methanol (F2C)		
Standard beta- carotene pigment		7.767	20

inoculum by the spreading method in Broth agar medium. The dishes were left for 5 minutes, then 5 holes of 6mm were made in each dish, 50 µl of acetone extract (R) were added, and B- carotene pigment (F1) and the lower layer after separating the pigment (F2) and acetone of 100% concentration as a control element (C1) and a mixture of ether and methyl with a proportion of (15:2ml), respectively as a second control element (C2).

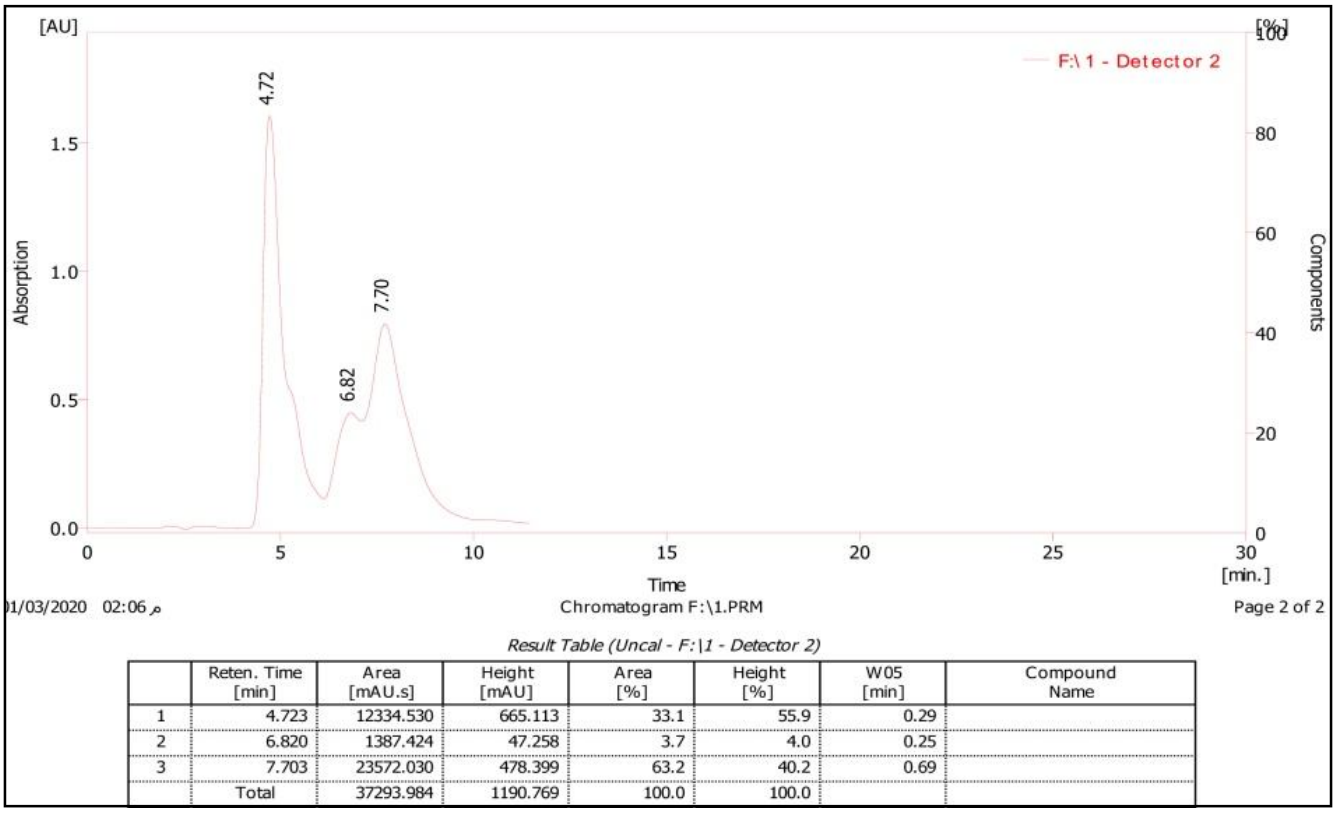
As for the fungi isolates used they are: *Candida albicans, Alternaria alternaria* and *Fosarium solani.* They were grown on PDA medium and pollinated by spreading from PDA slants and containing saline liquid (5ml). The dishes were left to dry and 5 holes were made in each dish in the same fashion of the method used earlier for bacteria.

Results and Discussion

Extraction of beta- carotene pigment

The extraction of beta- carotene pigment from the cyanobacteria and algae isolates was congruent with the study of Ruivo *et al.,* (2014), as it indicated that the efficacy of carotenes pigment extraction depends on the polarity of solvents and the pigments. Non- polar carotenes were extracted using acetone, whereas polar ones were extracted using methanol.

The study of (Al- Mandeel, 2010; Naghavi *et al.,* 2015) showed the use of solvents that contain ether polar solvent and that acetone is of the solvents preferred to be used as polar constituents in the solvent systems used in efficient separation of most of the common pigments including carotenoids. Mezzomo *et al.,* (2011) showed that the maximum total productivity of carotenoids pigment was by soaking in acetone, hence the Harborne method was used.



Result Table (Uncal - F:\1 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	12334.530	665.113	33.1	55.9	0.29	
2	6.820	1387.424	47.258	3.7	4.0	0.25	
3	7.703	23572.030	478.399	63.2	40.2	0.69	
	Total	37293.984	1190.769	100.0	100.0		

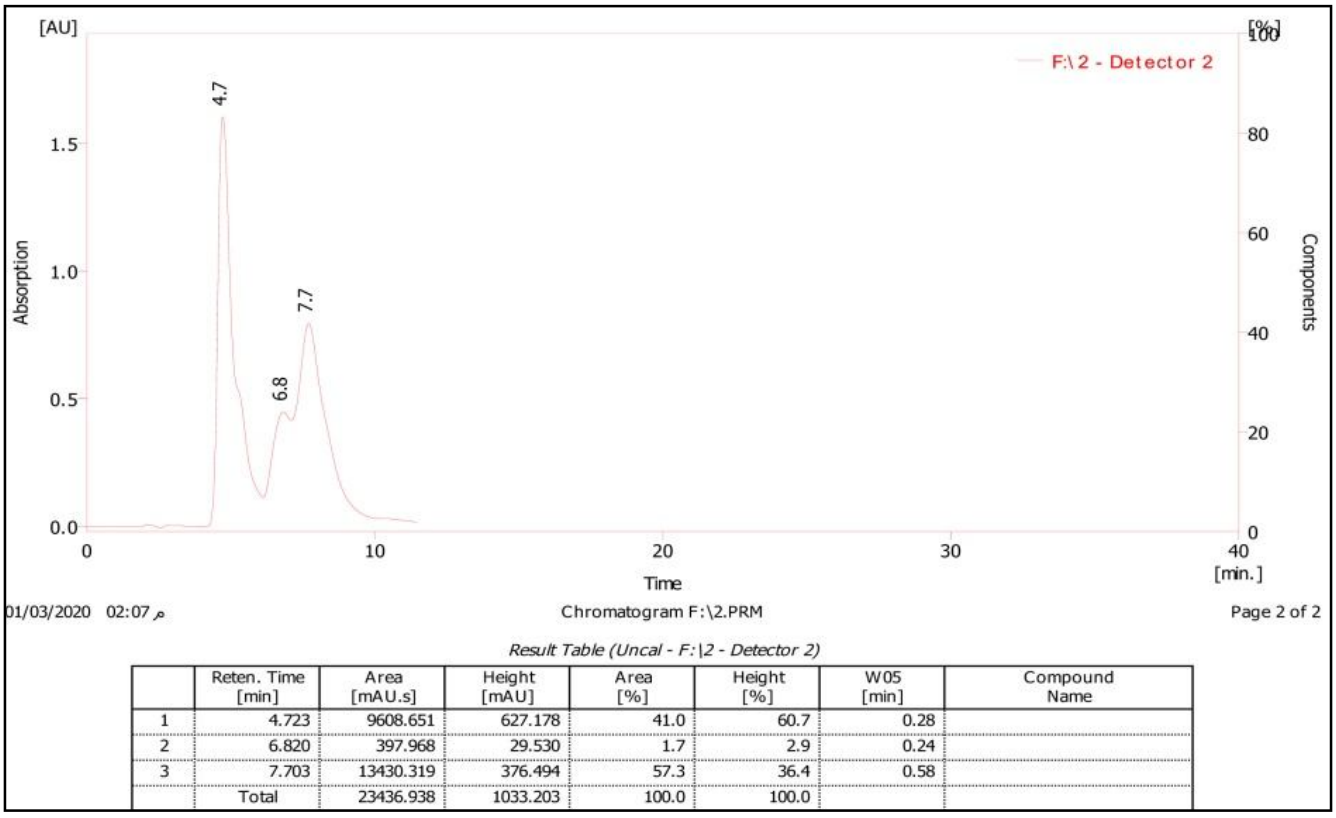
Fig. 4: *Gloeocapsa* Raw acetone extract (RG).

Purification and diagnosis of beta- carotene pigment

After conducting the extraction, some steps and methods for the purification of the beta- carotene pigment were performed

- Thin layer chromatography technique (TLC):

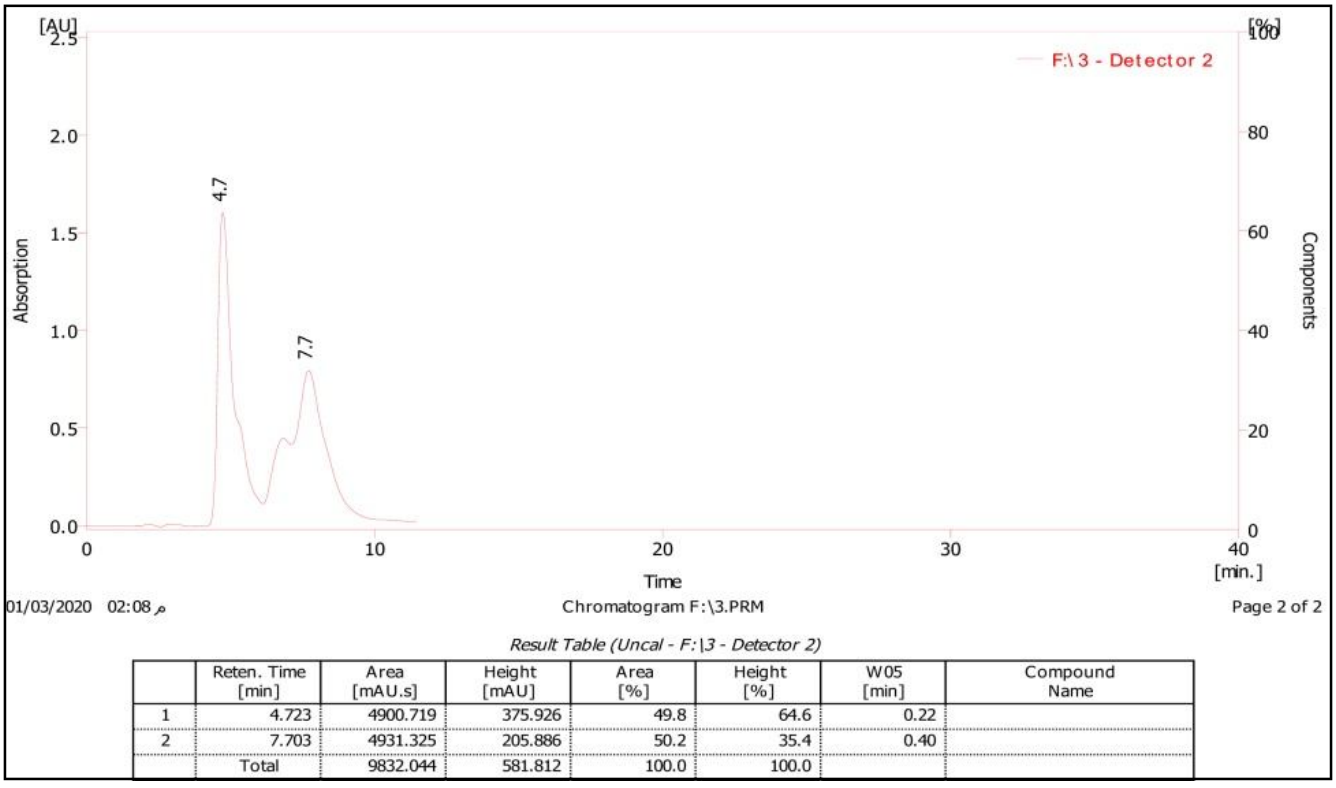
In this stage thin layer chromatography technique



Result Table (Uncal - F:\2 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	9608.651	627.178	41.0	60.7	0.28	
2	6.820	397.968	29.530	1.7	2.9	0.24	
3	7.703	13430.319	376.494	57.3	36.4	0.58	
	Total	23436.938	1033.203	100.0	100.0		

Fig. 5: *Gloeocapsa* Upper layer ether (F1G).



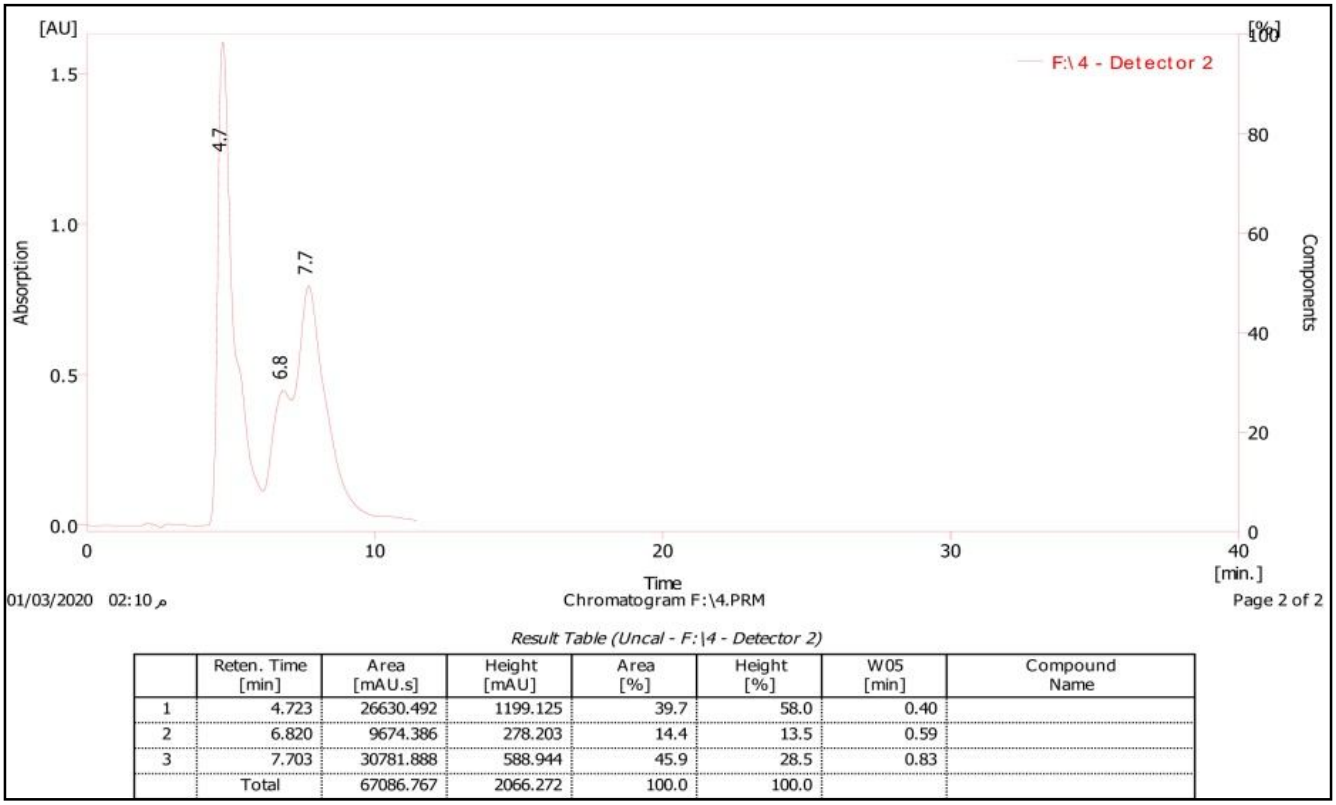
Result Table (Uncal - F:\3 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	4900.719	375.926	49.8	64.6	0.22	
2	7.703	4931.325	205.886	50.2	35.4	0.40	
	Total	9832.044	581.812	100.0	100.0		

Fig. 6: *Gloeocapsa* Lower layer methanol (F2G).

was used with the beta- carotene pigment extracted from cyanobacteria and green algae, and the tables below show the (R_f) values for the extracted pigment:

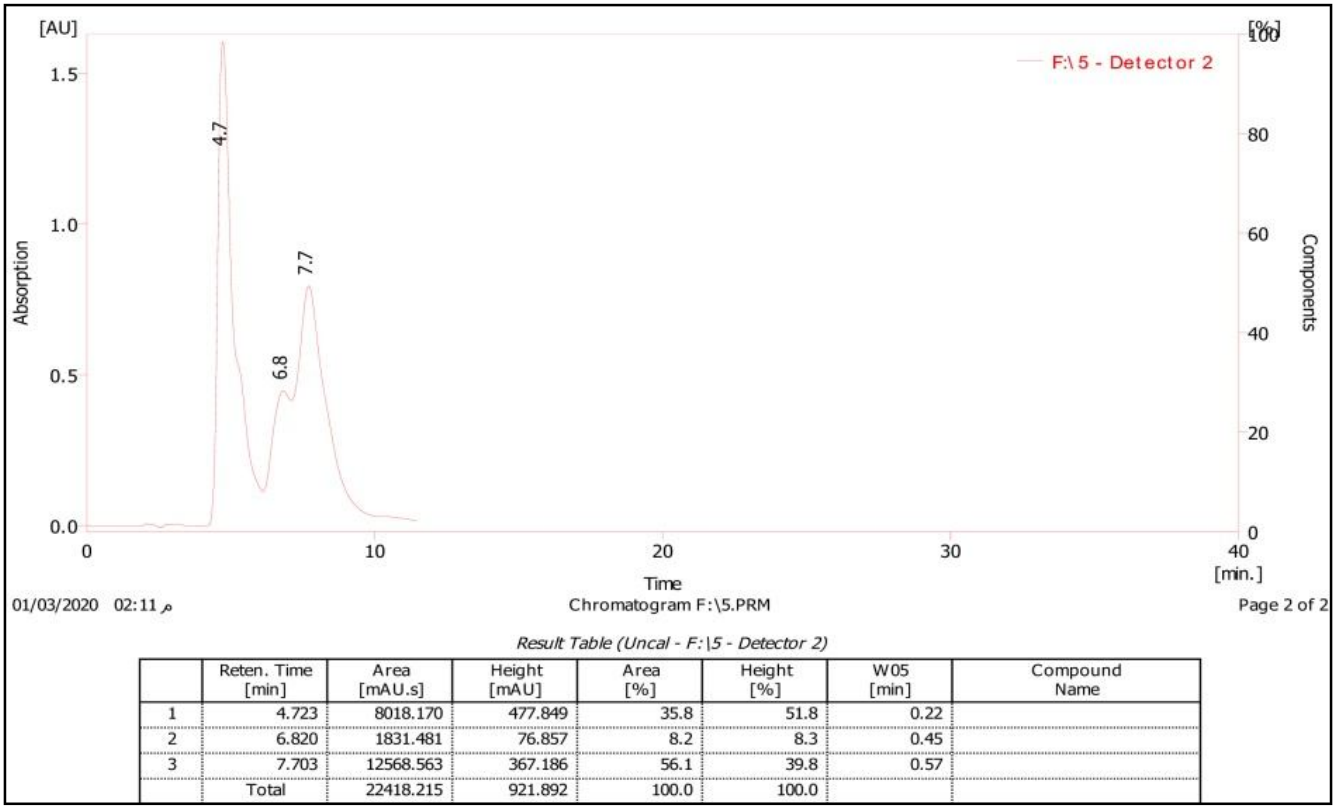
From the results in table 4 we find that the retention time for the standard pigment was (7.767 minutes) and this matches the retention time for the extracted samples



Result Table (Uncal - F:\4 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	26630.492	1199.125	39.7	58.0	0.40	
2	6.820	9674.386	278.203	14.4	13.5	0.59	
3	7.703	30781.888	588.944	45.9	28.5	0.83	
	Total	67086.767	2066.272	100.0	100.0		

Fig. 7: *Fischrella* Raw acetone extract (RF).

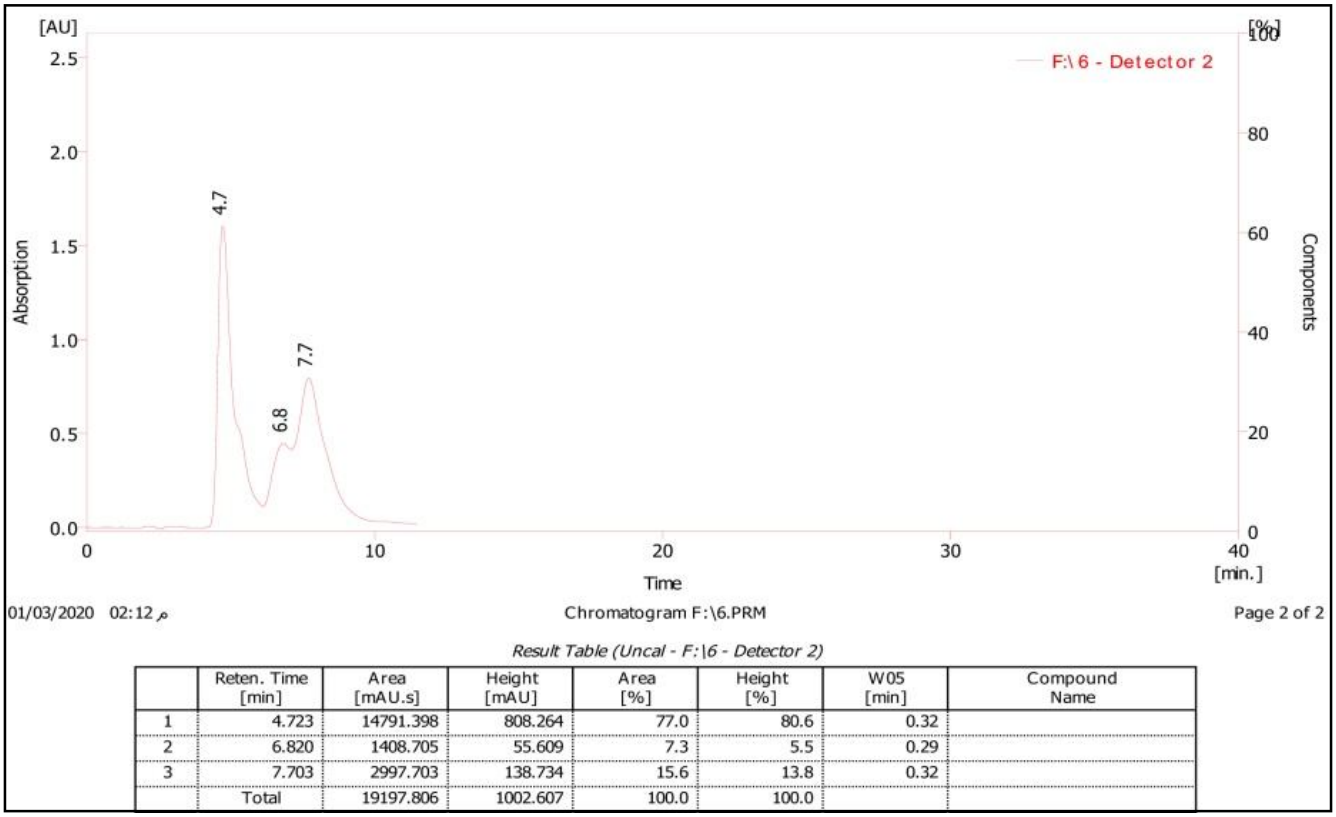


Result Table (Uncal - F:\5 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	8018.170	477.849	35.8	51.8	0.22	
2	6.820	1831.481	76.857	8.2	8.3	0.45	
3	7.703	12568.563	367.186	56.1	39.8	0.57	
	Total	22418.215	921.892	100.0	100.0		

Fig. 8: *Fischrella* Upper layer ether (F1F).

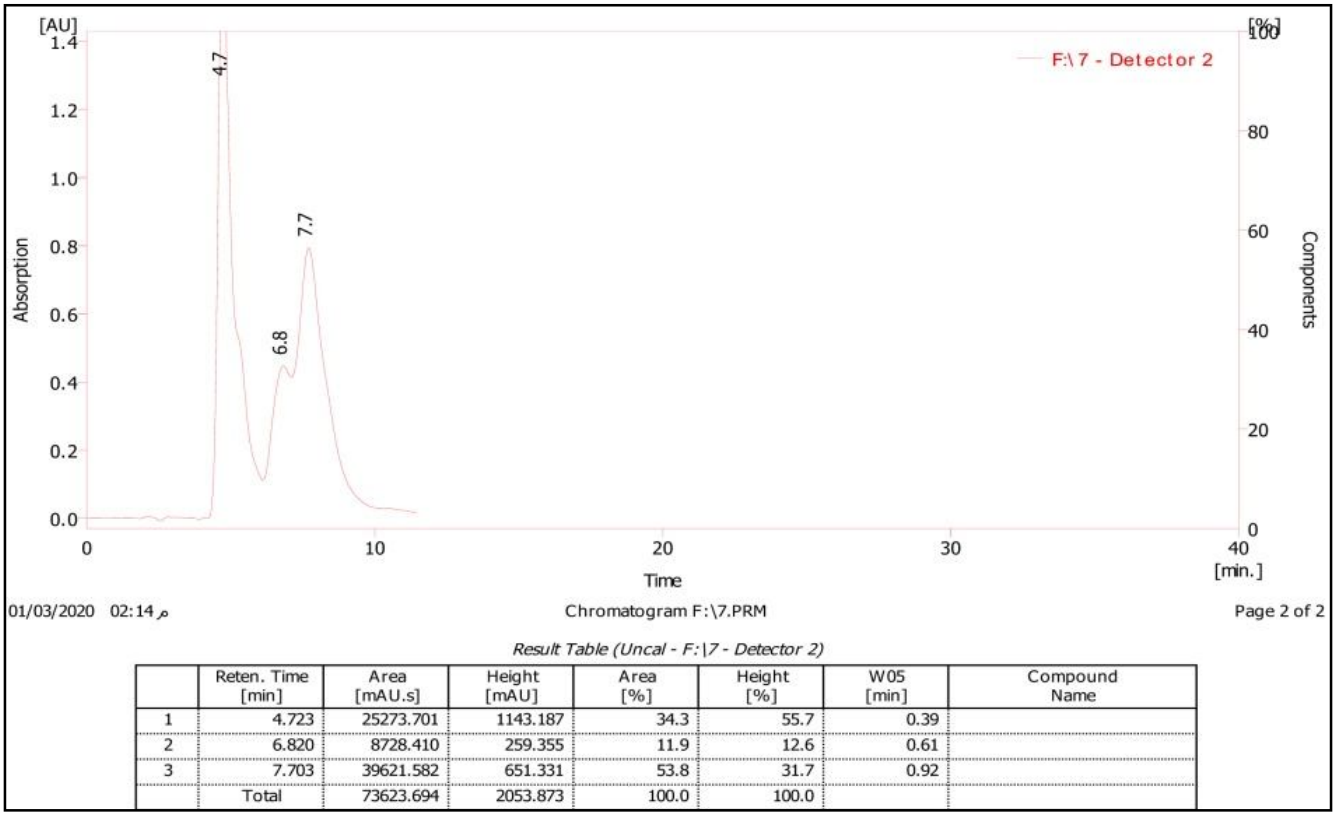
of the pigment in the studied isolates. Sathya (2017) mentioned that beta- carotene extracted from the small green algae appeared at a retention time (10.76 minutes). Aluc *et al.,* (2018) mentioned that beta- carotene pigment separated from the green algae *Chlorella vulgaris* and *Scendesmus regularies* appeared at retention times



Result Table (Uncal - F:\6 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	14791.398	808.264	77.0	80.6	0.32	
2	6.820	1408.705	55.609	7.3	5.5	0.29	
3	7.703	2997.703	138.734	15.6	13.8	0.32	
	Total	19197.806	1002.607	100.0	100.0		

Fig. 9: *Fischrella* Lower layer methanol (F2F).



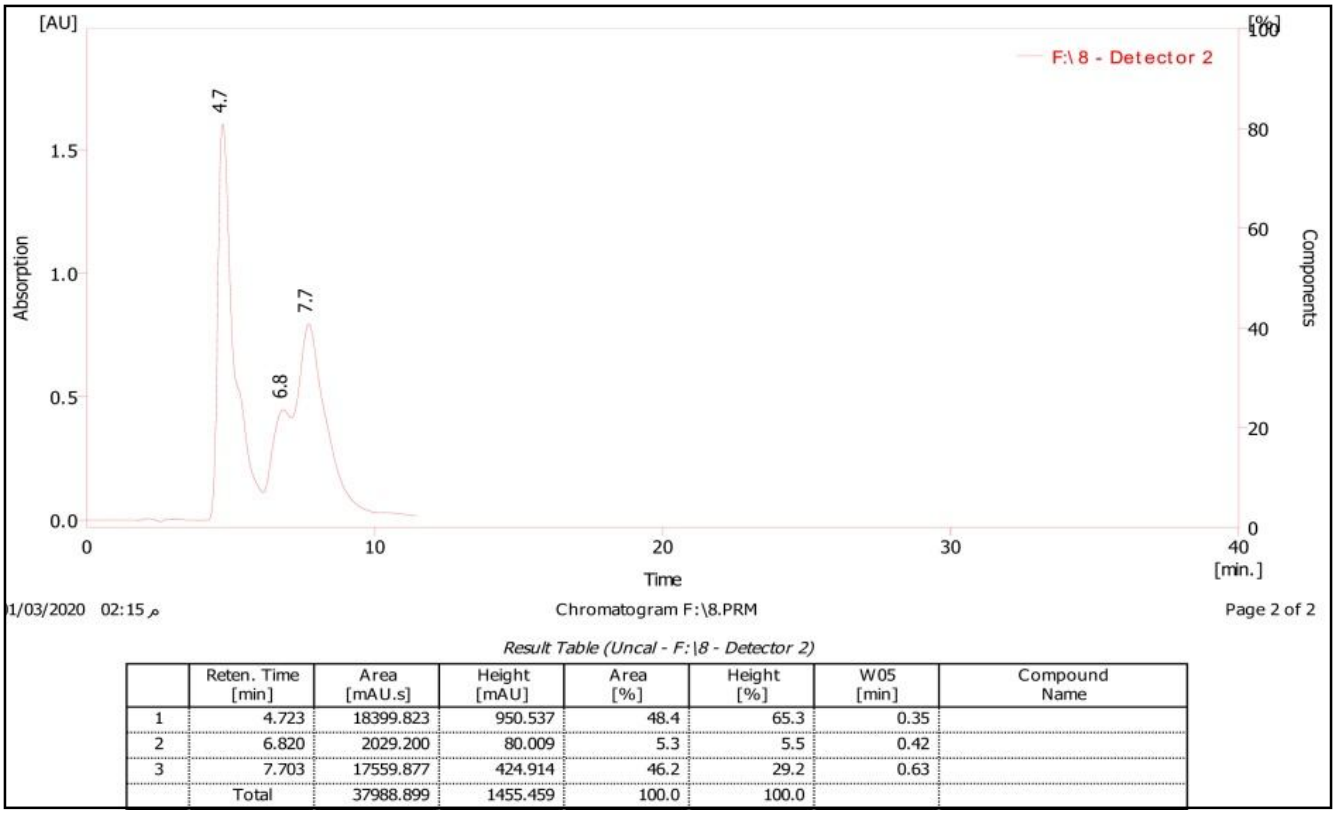
Result Table (Uncal - F:\7 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	25273.701	1143.187	34.3	55.7	0.39	
2	6.820	8728.410	259.355	11.9	12.6	0.61	
3	7.703	39621.582	651.331	53.8	31.7	0.92	
	Total	73623.694	2053.873	100.0	100.0		

Fig. 10: *Chlorella* Raw acetone extract (RC).

(41.32 minutes) and (41.31 minutes) respectively.

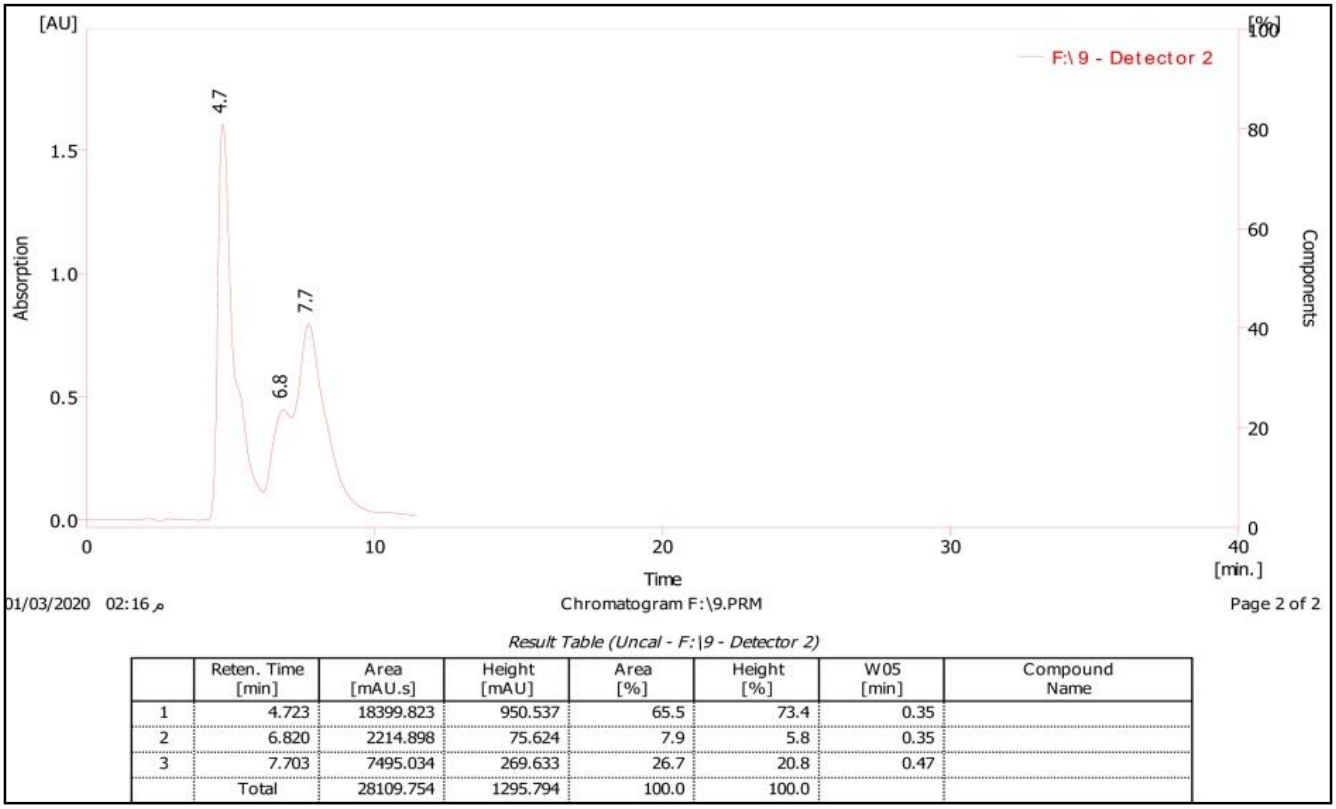
Results of the quantum estimation showed that the maximum concentration of the pigment was (1179.1 ppm) in the acetone extract for the *Chlorella* algae (RC) and



Result Table (Uncal - F:\8 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	18399.823	950.537	48.4	65.3	0.35	
2	6.820	2029.200	80.009	5.3	5.5	0.42	
3	7.703	17559.877	424.914	46.2	29.2	0.63	
	Total	37988.899	1455.459	100.0	100.0		

Fig. 11: *Chlorella* Upper layer ether (F1C).



Result Table (Uncal - F:\9 - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.723	18399.823	950.537	65.5	73.4	0.35	
2	6.820	2214.898	75.624	7.9	5.8	0.35	
3	7.703	7495.034	269.633	26.7	20.8	0.47	
	Total	28109.754	1295.794	100.0	100.0		

Fig. 12: *Chlorella* Lower layer methanol (F2C).

the minimum concentration of the pigment was (2712 ppm) in the lower layer of the cyanobacteria *Gloeocapsa* (F2G). As for the acetone extract of the *Fischrell* and *Gloeocapsa*, the concentrations of the pigment were (1092 and 924.2 ppm) respectively, and the concentrations of the beta- carotene in the algae *Chlorella, Fischrella* and *Gloeocapsa* were (780.3, 891 and 638.6 ppm) respectively. And finally for the lower layer of the algae *Chlorella* and *Fischrella,* the concentrations of the pigment were (410.3 and 364.8 ppm) respectively.

The results of research and studies, one of which was the study of Damergi *et al.,* (2017), showed that at the extraction of carotenes pigment from *Chlorella*

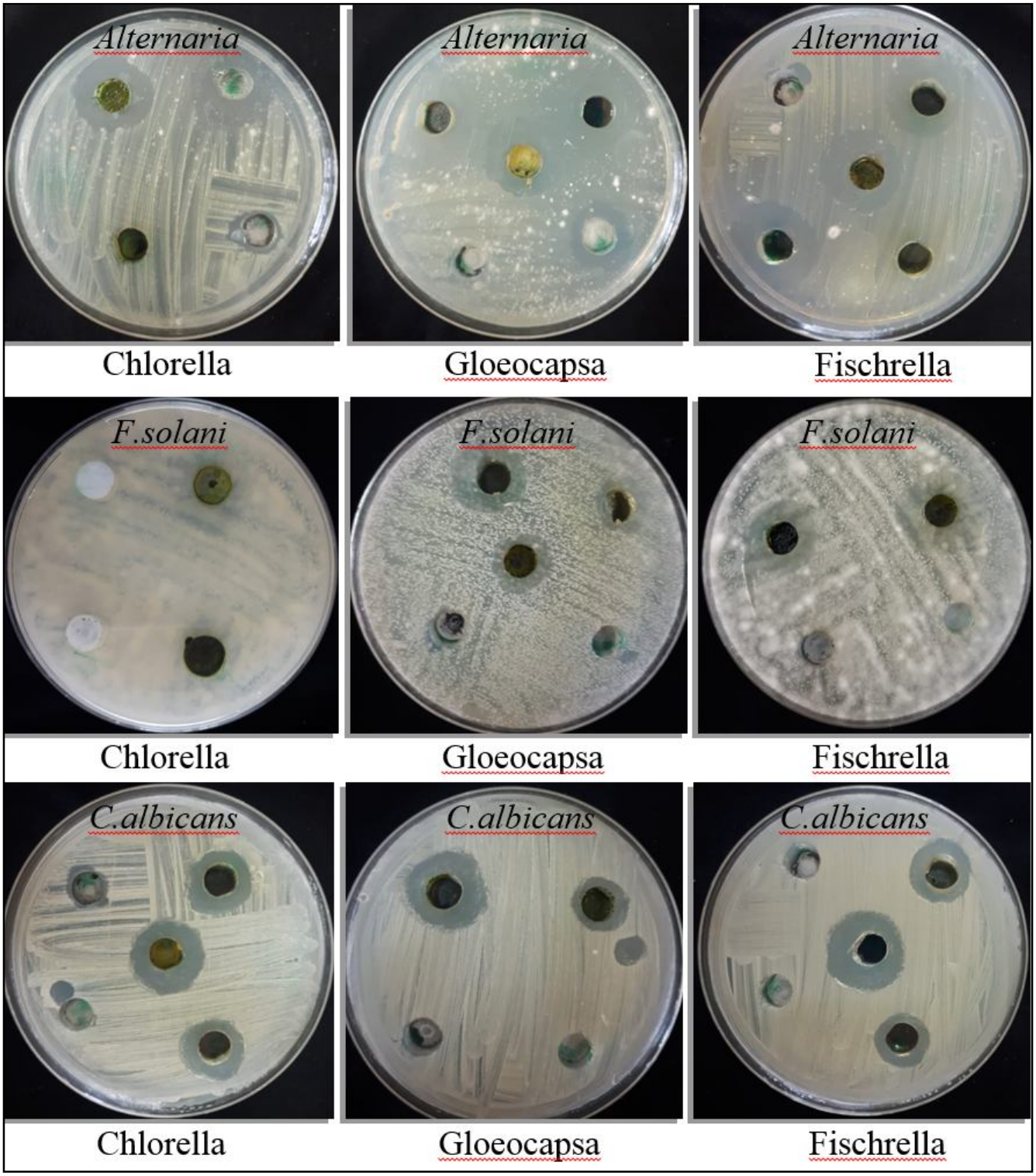


Fig. 13: Antifungal activity of b-carotene extract from micro algae.

vulgaris algae, the total concentration of the pigment was (417 g/µg) of the dry weight of the alga and the concentration of B- carotene pigment varied between (50- 45 g/µg). Emeish (2012) was able to isolate *Dunaliella salina* alga from the Dead Sea, and he extracted B- carotene from it with a concentration of (941.1).

Anti-microbial activity of beta-carotene extract from micro algae

The acetone extract of cyanobacteria had more effect on negative Gram bacteria than on positive Gram bacteria, whereas the acetone extract of green algae had more effect on positive Gram bacteria compared to

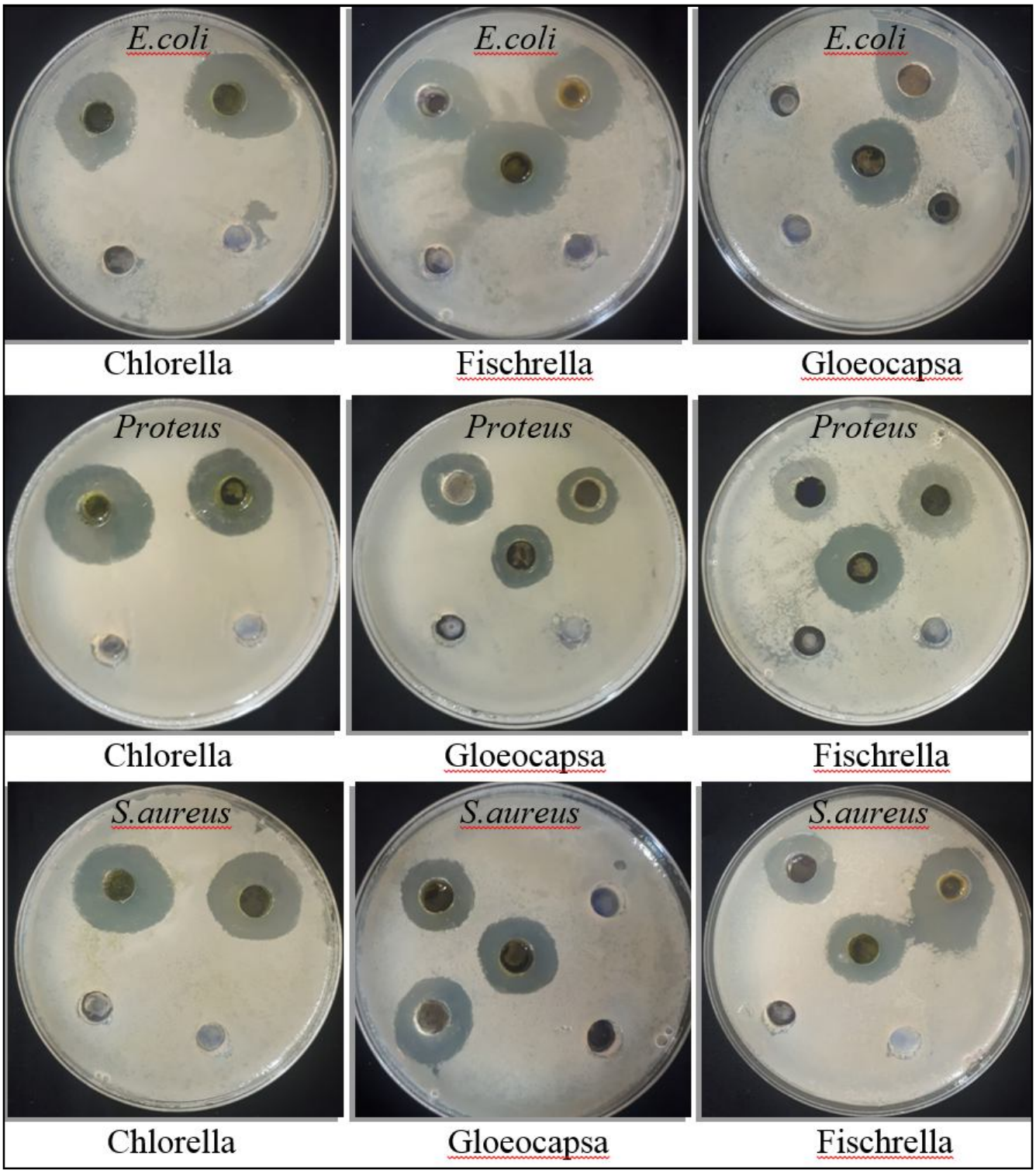


Fig. 14: Antibacterial activity of b-carotene extract from micro algae.

negative Gram bacteria. The acetone extract of *Chlorella* (green algae) markedly outmatched the other raw (acetone) extracts of cyanobacteria in its efficacy against bacteria and fungi. This also applies to the beta- carotene pigment F1 extracted from *Chlorella* and its anti- effect against bacteria and fungi.

As for the lower layer after separation F2 for all the genera, it showed lower efficacy against bacteria and fungi than the raw extract R, also the upper layer F1 the beta- carotene.

All the samples for the different genera used (cyanobacteria and the green, and all their extracted parts) they showed efficacy against all pathogenic bacteria genera and fungi.

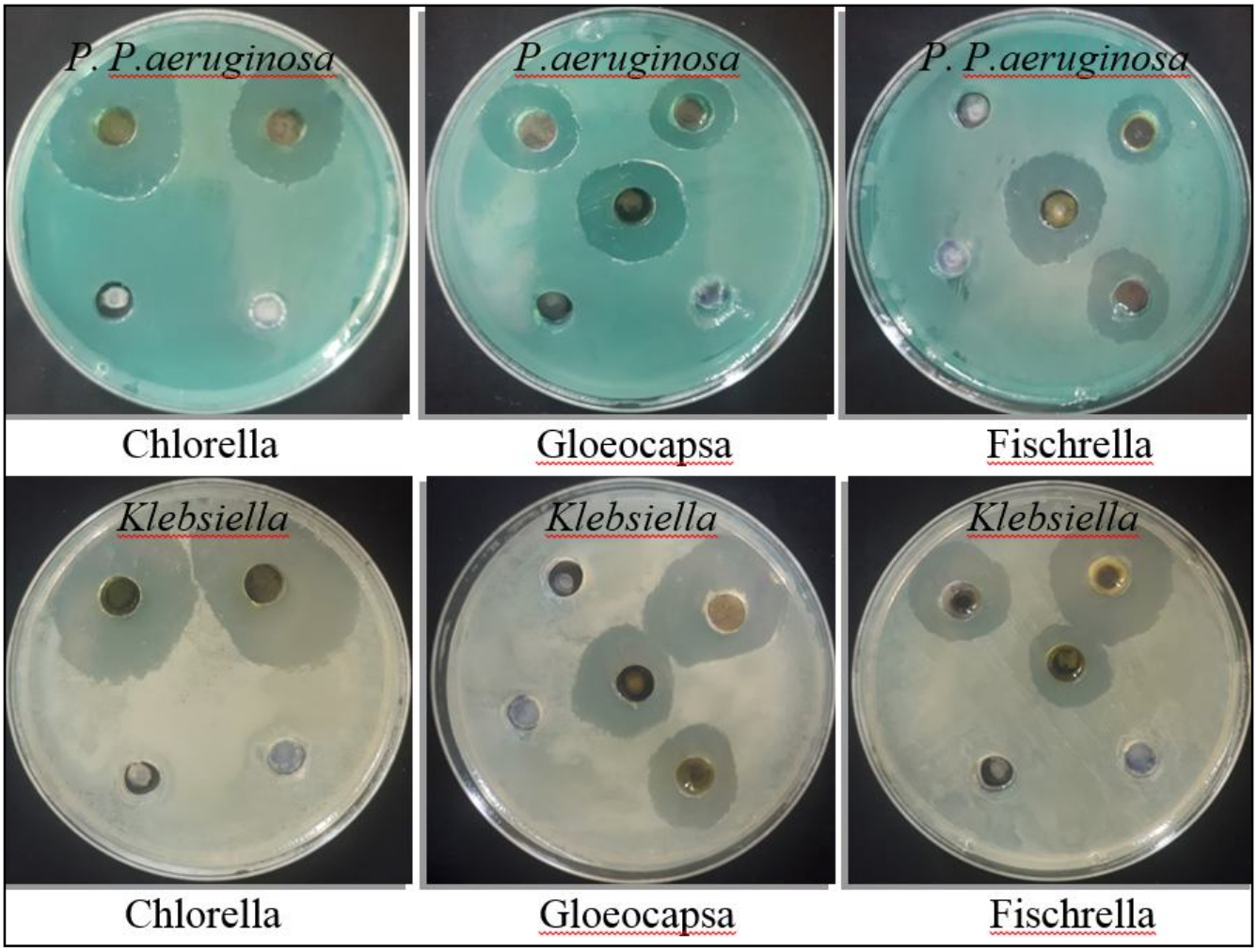


Fig. 15: Antibacterial activity of b-carotene extract from micro algae.

Table 5: Illustrating the anti- effect of beta- carotene pigment against bacteria and fungi.

-	-		19	26	-	-	17.5	20	24.5	-	-	16.5	17	20	
-	-		20	25.5	-	-	18	21	24	-	-	15.5	19	22	
-	-		21	34.5	-	-	17	17	24	-	-	17.5	20	21.5	
-	-		20	31.5	-	-	18	19	22	-	-	17.5	20	20	
-	-		18	29.5	-	-	20	20	23	-	-	18	19	21	
-	-		18	23.5	-	-	15.5	19.5	22	-	-	13.5	18	22	
-	-		20	24	-	-	16	19	23	-	-	15	17.5	20	
-	-		20	22.5	-	-	18	20	20	-	-	16.5	18	18	

R: Raw samples, F1: Fraction 1 (b-carotene) higher layer, F2: Fraction 2 (lower layer), C1: Control 1 (acetone), C2: Control 2 (ether: methanol), N: Non applied to it

The study of (Bhagavathy *et al.,* 2011) indicated that the acetone extract of *Chlorococcum humicola* showed an effect against positive Gram bacteria compared to the negative Gram bacteria, whereas regarding its effect on fungi, it showed an effect against *Asperigellus flavus* fungus, and showed no effect against *Candida albicans.* As for the B- carotene pigment it showed the best inhibition area against *E. coli*, but no effect against *P. aeruginosa* bacteria. Regarding fungi it showed its highest effect against *C. albicans,* and its lowest effect was against *Aspergillus flavus.*

Conclusions

Microalgae (cyanobacteria and green algae) produce beta- carotene pigment and the best concentration of the pigment was in the green alga (*Chlorella*) when diagnosed by high performance liquid chromatography (HPLC). The acetone extract (R) for the cyanobacteria, green algae, beta- carotene (F1) and the second layer after separation (F2) have anti- effect against bacteria and pathogenic fungi, the thing that offers a possibility of using them as antibiotics in the future.

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