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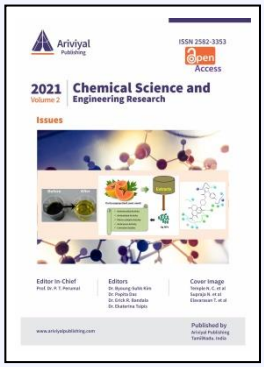

First Record for Cultivation of *Asparagopsis Taxiformis* Red Macroalga using Tissue Culture Technique

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Abstract: The discovering, development and enhancement of new biological activities agents with diverse kinds of antioxidant actions is required. The search for new active ingredients agents focuses on not only synthetic compounds but also natural products such as algae, the aim of the current work was to evaluate the potential use of red macroalga *Asparagopsis taxiformis* as a new source of antioxidant and biologically active compounds by cultivation it using tissue culture technique. The obtained preliminary results gave indicators for success cultivation of Asparagopsis algae using tissue culture method with production of antioxidant compounds especially with ethyl acetate extract when compared with extract from the raw cells. Also, there are great variation in total lipid contents and its composition from fatty acids and hydrocarbons list in both raw alga and callus.

Keywords: *Asparagopsis Taxiformis*; Tissue Culture; Active Ingredients; Antioxidant Activity

1. Introduction

The macroalgae industry worldwide uses around 11 million tonnes of wet seaweeds annually with a majority of it derived from cultivated farms,[1] Oceans and Seas represent a large store for different algae species, which are considered as a good source for foods, feeds and therapeutically useful drugs. The tissue culture technique used to enhance the biological and economic values of marine macroalgae.[2]

Marine macroalgae such as Asparagopsis sp are widely recognized as ecosystem engineers and/or foundation organisms in many habitats or environments since they convert the simple surfaces into structured environments that support hundreds of species.[3]

Asparagopsis taxiformis, (limu kohu) formerly A. sanfordiana, is a species of red alga, with cosmopolitan distribution in tropical to warm temperate waters, with different biological activities such as antioxidant, anticancer, antimicrobial and antiviral activities.[2]

Thus, the main aim of this study is to evaluate the potential use of tissue culture technique for cultivation of *Asparagopsis taxiformis* red macroalga and its chemical and antioxidant changes when compared with raw alga.

2. Collection of Alga

The alga was collected from El-Garam beach at Marsa Matrouh City. The alga sp belong to Bonnemaisoniaceae (Asparagopsis sp, super littoral and intertidal zones, 11-13 cm), Thallus of algae was cleaned from sand and foreign materials by washing with sea water followed by fresh water. After prepared of herbarium specimens of the algae, the algal sp was identified by Prof. Dr. Fathy M. Soliman, Professor of Phycology, Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

3. Tissue Culture Technique

Asparagopsis taxiformis was cultivated with tissue culture technique. In brief: After collection, explants were excised from apparently epiphyte and sterile thalli. Explants were epical secondary branches 1 cm long; explants were cylinder shaped fragments (0.5 cm long) excised from the middle zone of the long primary branches, explants were cleaned following the methods described by Garcia-Rrina.[4] After cleaning, explants were cut again to obtain cylinder-shaped explants. The culture medium was MS adjusted to different Solidities (7.5 and 15 g/l). Cultures were placed in a growth chamber at 20 ± 2°C with a day length of 18 hours and 27 $\mu molm^{-2}s^{-1}$.

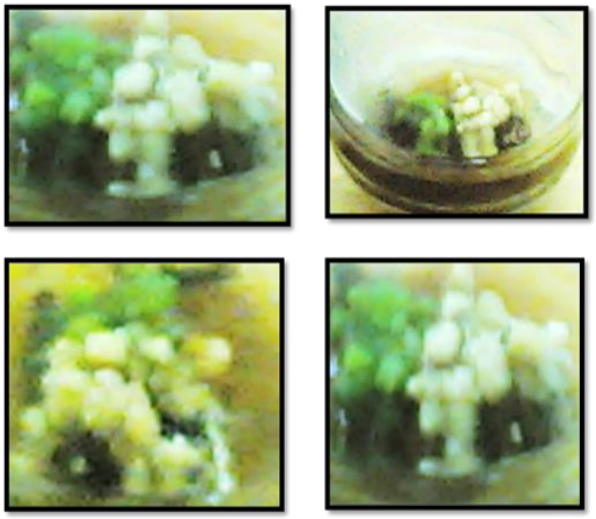

Fig. 1. Tissue Culture (Callus) of the Red Macroalga *Asparagopsis Taxiformis.*

4. Determination of Total Lipid

Lipids were extracted by a modified method described by Xu et al.[5] Five gram air dried algal sample was extracted twice with a mixture of distilled H_2O, chloroform and methanol (8:10:20, v/v/v) and sonicated for 10 min using a microtop of Microson Ultrasonic cell disrupter and then, the extract was filtered through GF/C whatman glass microfiber (47mm). Chloroform (10 ml) and distilled water (10 ml) were added sequentially to the filtrate and sonicated again for 10 min. The resultant solution was filtered under vacuum through whatman glass filter microfiber. The filtrate was washed several times by 5% NaCl solution (30 ml, each), and then the chloroform layer was separated and dried over anhydrous sodium sulfate. The chloroform extract was evaporation at 40°C under reduced pressure to dryness and the total lipids were weighed and stored at -20°C until analysis.

5. Separation of Fatty Acids and Unsaponofiable Matter

A 0.2 g of algal extracted lipids was saponified with methanolic KOH (30 ml, 1N) containing BHT (1 mg) at 60°C for 1 h. under reflux. The unsaponifiable matter was extracted with ethyl ether, washed several times with distilled water and dried over anhydrous sodium sulphate and the solvent was evaporated.

6. Identification of Fatty Acids and Hydrocarbons

Fatty acids methyl esters (FAME) were analysed by gas liquid chromatography (GLC) and the unsaponofiable compounds were also identified by GLC using an instrument equipped with a flame ionization detector (FID).

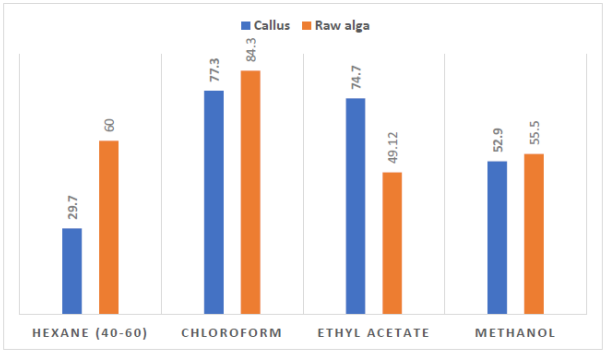

Fig. 2. Comparison of the Antioxidant Activity between Callus and Raw Algae of *Asparagopsis Taxiformis.*

7. Extraction and Determination of Chlorophyll and Carotenoides

The amount of chlorophyll and carotenoids were defeminated according to Holden[6] method.

8. Antioxidant Activity using DPPH Method

The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) tests were carried out as described by Burits and Bucar.[7]

9. Results and Discussions

Asparagopsis taxiformis is considered to be one of the most important sources of raw materials for industrial agar production and one of another fun chemical. Tissue culture technique has increasingly been applied to seaweeds because they facilitate development and propagation of genotypes of commercial importance. This assay reports callus formation in the red algal *Asparagopsis taxiformis*, collected from Marsa Matrouh., Preliminary experiments poorly on solid media. Accordingly, the 2 agar media mentioned before, the callus develops of the explants 6-8 weeks after inoculation. (Fig. 1) The callus was pale yellow in color, and its volume gradually increased with time until 9 weeks of inoculation on an initial agar plate. The poor growth of callus in solid media may be due to the difficulty of diffusion of elements in solid phase instead of semi-solid media which led to a decrease in the growth of callus (9 weeks) and it may also be due to the presence of non-suitable condition for production of callus from red macroalgae.

Robaina et al.[8] studied the effects of solidity and osmolality of explants culture medium and reported that the solidity of the culture medium plays an important role in the development of either bud or callus formation in Gelidium versicolor (Gmelin) Lamouroux, Grateloupi doryphora (Montagne) Howe and Laurencia Nageli. All Gelidiella explants cultured in 0.5 and 1.0 % agar showed 100% bud formation, whereas the same organism cultured in 0.8-3.0% agar showed callus development under identical culture conditions. The osmolality of the culture medium seems to play a crucial role in determining bud or callus development.

Table 1. Comparison between Callus and Raw Alga of *Asparagopsis Taxiformis* in Lipophilic Matter.

Chemical Constituents (Lipophilic matters)	Callus	Row algae
Chlorophyll a (mg/g)	0.17	0.07
Chlorophyll b (mg/g)	0.08	0.41
Total chlorophyll (mg/g)	0.25	0.48
Total carotenoids (mg/g)	0.28	0.04
Total lipid%	2.1	4.7

Table 2. GLC Analysis of the Unsaponifiable Matters of *Asparagopsis Taxiformis* (Delile) Trevisan

RR_t^a	Percentage*	Name
00.46	0.125	n- Tetradecane (C 14)
00.48	0.25	n-Octadecane (C 18)
00.50	7.28	n- Eicosane (C 20)
00.71	33.52	n- Decosane (C 22)
00.72	11.47	n-Tetracosane (C 24)
00.73	9.48	n- Hexadecane (C 26)
00.74	9.83	n- Octacosane (C 28)
00.76	1.55	n- Tricosane (C 30)
01.00	11.79	Cholesterol
01.180	14.69	Stigmasterol

RR_t^a = Relative Retention Time

Table 3. GLC analysis of the saponifiable matters of *Asparagopsis taxiformis* (Delile) Trevisan

RR_t^a	Percentage	Name
00.254	00.982	Caproic acid (C 6)
00.314	00.109	Caprylic acid (C 8)
00.346	00.013	Nonanoic acid (C 9)
00.390	00.223	Capric acid (C 10)
00.414	00.076	Caprioleic acid (C 10:1)
00.457	00.018	Undecanoic acid (C 11)
00.526	00.417	Lauric acid (C 12)
00.572	10.407	Laurioleic acid (C 12:1)
00.606	00.867	Tridecanoic acid (C 13)
00.647	03.585	Myristic acid (C 14)
00.673	02.000	Myristoleic acid (C 14:1)
00.680	10.157	Pentadecanoic acid (C 15)
00.769	11.357	Palmitic acid (C 16)
00.885	00.636	Palmitoleic acid (C 16:1)
00.894	02.380	Heptadecanoic acid (C 17)
00.899	01.031	Stearic acid (C 18)
00.947	04.984	Oleic acid (C 18:1)
00.950	08.785	Linoleic acid (C 18:2)
01.00	19.477	Linolenic acid (C 18:3)
01.10	10.301	Eicosenic acid (C 20:1)
01.20	02.742	Arachidonic acid (C 20:4)
01.80	01.436	Behenic acid (C 22)

RR_t^a= Relative Retention Time of Linolenic Acid

Table 1 showed that the green pigments were different in both materials. Chlorophyll a and b was more pronounced in wild algae than callus, in spite of the accumulation of carotenoids in callus than in row algae. These results may be due to the change in environment growth condition in addition to the variations between compositions of saponifiable and unsaponifiable contents of algae.

DPPH assay was used to compare between the antioxidant activity of successive extract from the callus and row algae. The obtained results (as shown in (Fig. 1)) indicated that the row algae have high antioxidant activity in non-polar extracts (hexane and chloroform) reaching 60 and 84.3% respectively; while callus have the highest antioxidant activity in ethyl acetate fraction by 74.7%.

Table 4. GLC Analysis of the Saponifiable Matters of *Asparagopsis Taxiformis* (Delile) Trevisan (Callus)

RR_t^a	Percentage	Name
0.58	6.63	Myristic acid (C 14)
0.60	0.84	Myristoleic acid (C 14:1)
0.68	28.21	Palmitic acid (C 16)
0.72	3.85	Palmitoleic acid (C 16:1)
0.96	16.14	Stearic acid (C 18)
1.0	36.03	Oleic acid (C 18:1)
1.10	7.76	Linolenic acid (C 18:3, n3)

RR_t^a = Relative Retention Time of Oleic Acid

Table 5. GLC Analysis of the Unsaponifiable Matters of *Asparagopsis Taxiformis* (Delile) Trevisan (Callus)

RR_t^a	Percentage	Name
0.72	0.67	n- Eicosane (C 20)
0.75	1.15	n-Heneicosane (C21)
0.77	3.45	n- Decosane (C 22)
0.80	3.40	n-Tricosane (C23)
0.82	1.58	----------
0.83	1.69	----------
0.85	4.36	n-Tetracosane (C 24)
0.92	21.21	n- Pentacosane (C25)
0.96	7.13	n-Hexacosane(C26)
1.0	40.45	n-octacosane (C28)
1.07	4.07	n-Nonacosane (C29)
1.14	10.56	n-Tricontane (C30)

RR_t^a = Relative Retention Time of n-octacosane

This result may be due to the difference in contents of these cells from antioxidant active compounds as show in (Fig. 2).

The data of the crude lipid extracts obtained from the algal cells are presented in Table (1). The obtained results indicated that the total lipid patterns of the 2 cells studied (row and callus cells) were totally different. The algal cells had the maximum percentage of total lipid (4.7%) while the algal callas showed the lowest percentage (2.1 %).

The relative percentages of total fatty acids are presented in Tables (2 and 3). The total fatty acids patterns from algae shows relativity high level of unsaturated fatty acid compared with the saturated components (67.06 and 32.94% respectively) while algal callus showed the opposite pattern. Among saturated fatty acids myristic, stearic acids and in all algae cells palmetic acids were almost common. For the two types of algal cells palmetic acid and alfa-linolenic acid occurred in large quantity for callus (28.21 and 7.8%) than for row algae (11.36 and 8.78%). However, row algae cells have alfa- linolenic acid as the highest amount of fatty acids (19.5%). Meanwhile, for the callus oleic acid was found to occur in the largest portion of fatty acid present (36.03%).

The relative percentages of total unsaponifiable substances are presented in Table (4) for row algae and Table (5) for callus. Relatively high levels of hydrocarbons as compared to the sterols were recorded in both algal types (row and callas), C22 and C24 occurred in the large quantity among all the hydrocarbons isolated (33.5, 11.47% and 3.5, 4.4% respectively). In addition, row algae had the highest amount of sterol as hydrocarbon. From these sterols (cholesterol and stigmasterol were 11.79 and 14.69 respectively), but the callus cells had no sterols. These results are in agreement with those of Kamenaeska et al.,[9], who reported the sterol composition of the brown algae Stilophora rhizodes (Turner) J. Agardh, Punctaria

latifolia Grev., Punctaria plantaginea (Roth.) Grev. From the Black Sea and recorded fifteen sterols which were identified in the sterol fractions. The main ones were cholesterol and 24-methylenecholestrol. The same author reported that the studied algae might be considered close to red algae in terms of their high content of cholesterol and its derivatives and to brown algae in terms of their content of methylated at C24 sterols.

10. Conclusions

From the above data, we can conclude that the tissue culture is very important technique for enhance the algae values for production of pharmaceutical compounds and their biological activities. Also, it's possible for use this technique for enhancement production of oriented biological compounds which difficult for production using the ordinary cultivation method.

Conflicts of Interest

The authors declare no conflict of interest.

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