Extraction of Agar from the Libyan marine red algae *Gracilaria Verrucosa* and *Scinaia Forcellata* present at Sabratha City Cost

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ABSTRACT

Agar is a high content compound of polysaccharides, possesses an industrial and medical applications for human benefits, and it is derived from the cell wall of certain species of algae belong to *Rhodophyta* (red algae) phylum, and many Mediterranean countries have extracted it by different methods. Although the Libyan coast has a variety of marine resources, including red algae, the agar was not produced and tested in Libya, so this study was aimed to extract the agar from red Libyan algae. The red algae were collected from Sabratha/ Libya beach during two different seasons one at autumn 2017 and second at summer 2018, and isolated agar was from two varieties of red algae *Gracilaria Verrucosa* and *Scinaia Forcellata.* The yield of agar from this isolation were 81% per 34.5 g of *Gracilaria Verrucosa* and 84% per 23.8g of *Scinaia Forcellata.* Prepared native agar plates were appeared transparent and smooth. The media were stable on incubation at room temperature (23-25°C) and when incubated at (30 - 37°C /72hr). The native agar medium prepared from *Gracilaria Verrucosa was* form a duple microbial air born colonies after 48 hours at room temperature, and 9 colonies after 72 of incubation at 37C°. Beside the characteristics (sulphat content, melting temperature and gel temperature) of isolated agar from this research were similar with standard agar. Finally, Based on the results of this study, the isolation of agar from Libyan red algae in was successful, and the characteristic of extracted agar was suitable for application in microbial culture media.

Key word: agar, red algae, *Gracilaria Verrucosa, Scinaia Forcellata*.

1. Introduction

A hydrophilic colloid agar is commonly used in food industries by accounting of 80% of its Consumption, while the remaining 20% is used for biotechnological applications [1]. More than 9,000 tons of agar is produced currently worldwide, and gel with strengths greater than 700g/cm^2 in a 1.5% solution is considered as a high quality product demand at the international markets [2]. Agar is a main constituent of the red alga cell wall [3] and it isolated from red algae species of *Pterocladia*, *Gelidium*, *Gracilaria*, *Phyllophora*, *Ahnfeltia*, *Campylaephora*, *Acanthopeltis* [4], *Gelidiella* and *Gracilariopsis* [2, 5] *Gracilaria species*

and *Scinaaia forcellaia* are the major agar sources worldwide [6], and numerous studies have been conducted on their agar yield and quality from different geographical areas [7, 8, 9]. Originally, and even in the present times, it was made and sold as an extract in solution (hot) or in gel from (cold), to be used promptly in areas near the factories; the product was then known as tokoroten. It's industrialization as a dry and stable product started at the beginning of the 18th century and it has since been called kanten. However, the world agar- agar, has a Malayan origin and agar is the most commonly accepted term [10]. It is known as water-soluble, gel-forming polysaccharide composed of disaccharide-repeating unit of 3-linked D-galactose and 4-linked 3,6- anhydro-L- galactopyranosyl (3,6-AG) residues, with possible occurrence of sulfate, methoxyl and/or pyruvate substituent at various positions in polysaccharide chain. Moreover, the pattern of substitution group depends on the species, various environmental and physiological factors were it used in extraction and isolating of agar [11, 12]. Agar has many applications in food processing, pharmaceutics, cosmetics, microbiology, molecular biology, biotechnology [8] and as intestinal regulator [13]. Also, agar is used as a gelling agent in solid media in much smaller quantities, and at a much higher price, another type of agar called purified agar (agarose) is also available. These are bacteriological agar that could also be used in biochemistry for electrophoresis or immunodiffusion [10]. Most Agar production in the world is derived from the seaweed genera *Gracilaria* (53%) and *Gelidium* (44%), a small Quantity (3%) is produced from other agarophytes like *Gelidiella* and *pteroocladia*. *Gracilaria sesquipedale* was reported in Morocco, Corsica, Italy and Algeria [14, 15, 16, 17, 18]. In Egypt the Agar was Extracted from *Gracilaria* and *Gelidium* in the period from July 2003 to Jun 2004 [19], in Algeria, the interest to develop marine algae is very new as compared to other countries, and the first study was in 2011 on the algae at Mostaganem coast, Western Algeria. Another work was applied in Algeria in 2016, with interest to valorization of *Gelidium sesquipedale* from Southwestern Mediterranean basin [3]. As in Morocco, the agar was extracted firstly from *Gelidium* in1984 [10], in Libya no previous extraction of agar from native red algae, and no information on suitability for the production of good quality Libyan agar. Until now, diagnostic microbiological laboratory in Libya uses imported agar, which costs about ~ $150 per kg. The use of locally produced agar is likely to be less cost and save a money and will enable to distribute Libya agar in world market. The aim of this study was to extract the pure. agar from red Libyan algae.

2. Materials and Methods

2. 1 Red algae collection

The red algae samples *Gracilaria Verrucosa* and *Scinaia Forcellata* were collected during two seasons, from 9 to14 October/ 2017 and from 14 to16 august/ 2018, at Sabratha beach in

West Libya [figure 1]. Samples were tacked directly to laboratory of Molecular biology and Biochemistry Department at Science college/ Sabratha University, Libya. Samples were sorted and identified using a relevant taxonomic literature [20], then subjected to processing and analysis [figure 2, 3].

Figure 1: Libya map, Sabratha city **Figure 2: *Gracilaria Verrucosa* algae.** **Figure 3: *Scinaia Forcellata* algae.**

2. 2 Algae bleaching

Gracilaria Verrucosa and *Scinaia Forcellata* samples were washed out and soaked in (sodium hypochlorite 1%) for 1hour, Then, they were rinsed with tap water until sodium hypochlorite is lose. After, they spliced into bits and dried.

2. 3 Isolation of agar

The bleached samples were subjected to the direct sun light until became dry then, were dried at 50C° in the oven until reach to a stable weight [figure 4]. The dried *Gracillaria verrucosa* and *Scinaia Forcellata* algae, weighed approximate 34.55g and 23.8g in respectively were soaked in tap water for 1hour at room temperature, after that they were boiled for 5 hours in 2500 ml of distilled water, then filtered hot under pressure by using a cheese cloth. The filtrated was frozen at -20C° and thawed after that removing gel agar from the thawed liquid and dried in oven at 50C° until obtained dry agar [21].

2. 4 Determination of agar yield from *Gracillaria verrucosa* and *Scinaia Forcellata*

The weighed of dried *Gracillaria verrucosa* and *Scinaia Forcellata* algae were approximate 34.55g and 23.8g in respectively, and the weight and of the agar produced from it, were measured to calculate the agar content per algae *spp*.

2. 5 Determination of melting and gel temperature of agar

The 1 g of agar dissolved in 19 ml of distilled water in 50 ml glass tube and cooled in a night with vertical position at room temperature. A piece of coloured paper mark with diameter of 0.25 cm was put on surface gel agar and fixed thermometer in that glass tube. The glass tube was entered in the beaker that consist water, then boiled. Melting temperature gel agar was noted when the stick on the surface gel move down until reach base glass tube.

Whereas gel temperature was determined with allowing the agar solution to be cool at room temperature, and gel temperature was noted when the glass tube was moved oblique and the agar solution did not flow again.

2. 6 Determination of Sulphate content of agar

Approximate of 0.1g of agar was digested with 5ml HNO_3 (63%) and 5ml H_2O_2 30% at 150C° for 15 minute. The digested product was subjected to the test by using a Spectrophotometer (dr 1900, hach), on the stored program. In a test, 10 ml of the digested product used for blanket and 10 ml + sulfaver to take a read.

2. 7 Test of Media

The extracted agar were prepared by using 4 % (w/v) on plates. A non sterile loop and agar were used to prepare 40 ml of molten agar and poured onto sterile plate. plates were kept at room temperature for one night, then incubated at 37C° for 16-48 hours.

3. Results and Discussion

3.1 Quality of nutrient agar media

Native agar plates prepared from extracted agar of *Gracilaria Verrucosa* and *Scinaia Forcellata* harvested during autumn and summer seasons, were appeared transparent and smooth. It a just need to a more grounding to be more clear like a commercial agar [plate 1]. The media were stable on incubation at room temperature (23-25°C) and when incubated at (30 - 37°C for 72 hours). The native agar medium prepared from *Gracilaria Verrucosa* was form duple colonies after 48 hours at room temperature, and 9 colonies after 72 of incubation at 37C° [plate 2, 3].

Plat: 1

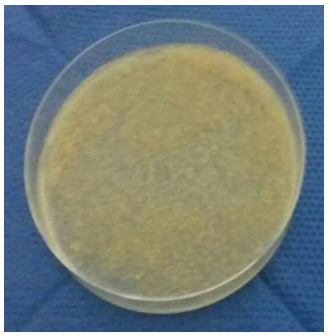

Plate 1: agar medium prepared With native agar extracted from *Scinaia Forcellata.*

Plat: 2 **Plat: 3**

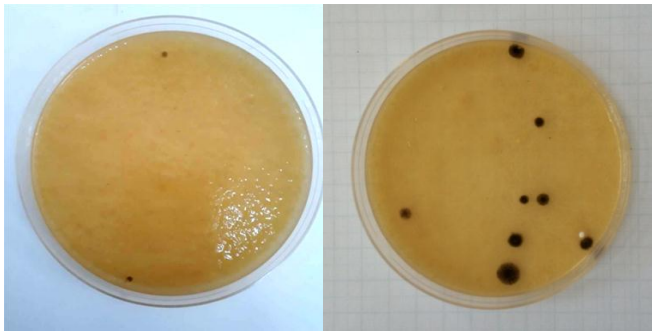

Plate 2 and Plate 3: native agar extracted from *Gracilaria Verrucosa*, plate 2: microbial growing after 24hr, plate 3: after 72hr.

The agar yield obtained in this study were calculated with formula as follow:

Yield= $\frac{A-B}{A} \times 100$

A: weight of dried algae, B: weight of produced agar

Yield of 34.5 g from *Gracillaria Verrucosa* $= \frac{34.5 - 6.56}{34.5} \times 100 = 81\%$

Yield from 23.8g from *Scinaia Forcellata* $= \frac{23.8 - 3.7}{23.8} \times 100 = 84\%$

The organoleptic tested of extracted agar from *Gracillaria Verrucosa* and *Scinaia Forcellata* could be seen in Table 1.

Beside the characteristics of sulphant content, melting temperature and gel temperature [Table 2], the isolated agar in this research were similar with standard agar [figure 4, 5].

Table 1 : *The organoleptic tested of isolated agar*

Material test	organoleptic test		
	Colour	Taste	Shape
Gracillaria Verrucosa	yellow	Immediately: tasteless After 2 month: bitter	Thin sheet
Scinaia Forcellata	yellow	tasteless	Thin sheet

Shape of agar isolated not powdered

Table 2 : *Characteristics of agar product*

Red Alga	Sulphate content %	Melting temperature (°C)	Gel temperature (°C)
Gracillaria Verrucosa	10.4	75°C	30°C
Scinaia Forcellata	9.2	65°C	25°C

Figure 4: Agar product from *Gracillaria Verrucosa*.

Figure 5: Agar product from *Scinaia Forcellata*.

4. Conclusion

Based on the results of this study, the isolation of agar from Libyan red algae was successful, and the characteristic of extracted agar was suitable for application in microbial culture media. Finally we recommend for: more future study in Libya on polysaccharides from red algae, more assessment of extracted agar from native algae, future study to isolate the purified agar (agarose) from same spp. of algae and producing of native agar.

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