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Made in Ghana Agarose for gel electrophoresis of DNA

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Abstract
The study explored the potential of local Ghanaian seaweeds in producing agarose, a substitute for imported agarose, for gel electrophoresis in DNA fragment separation. Seaweeds like *Gracilaria cervicornis* and *Hydropuntia dentata*, collected from Kpone and Labadi, were processed to extract agarose using methods like polyethylene glycol, diethylaminoethyl cellulose, and dimethyl sulfoxide. These red algae showed high agar content, with *Gracilaria cervicornis* yielding more agarose with better gel strength and temperature properties than *Hydropuntia dentata.* No agar was obtained from *Ulva fasciata* and *Caulerpa taxifolia*. The study demonstrated the effectiveness of locally produced agarose in deoxyribonucleic acid separation, suggesting potential for commercial production of 'Ghanaian agarose' for molecular work.

Keywords: Agar, agarose, agaropectin, seaweed, gel

1. Introduction
The use of biodegradable and biocompatible materials is turning into a true necessity of the current era due to growing environmental concerns and the global effort to construct a sustainable future. In this regard, seaweed polysaccharides have long been investigated for use in the production of biomaterials spanning a broad range of industries as diverse as food, biotechnological, pharmacological, and biological fields [1].

Seaweeds are found along seashores, in salt water and fresh water and they come in various varieties; red, green, and brown marine algae [2]. A number of these seaweeds are edible and are used for commercial purposes [3]. Use as food, cosmetics, fertilizers, and for extraction of industrial chemicals [4]. Seaweeds are mostly exploited in areas like China, Indonesia, and the Philippians: these countries have aquatic areas such as ponds, streams, etc. as we have in Ghana [5]. However, in Ghana, much attention has not been given to this industrious aquatic plant and therefore its importance has not been exploited enough to help the country economically [6]. *Ralfsia expansa, Ulva flexuosa, Hydropuntia dentata, Hypnea musciformis, Lithothamnion bisporum, Ulva fasciata, Centroceras clavulatum, Ulva lactuca, Chaetomorpha linum,* and *Caulerpa taxifolia* are the most abundant seaweed in Ghana and they all play key roles in affecting the spatial community organization [7].

Among the various species of seaweed, species of *Gelidium, and Gracilaria* which are red seaweed are purposely used for the preparation of agarose due to the high content of agar found in them [8]. Agar is found in the cell walls of red seaweeds [9]. The most utilized polysaccharides in biotechnological applications are the seaweed compounds agar and agarose [10]. Agar has two main components: agarose and agaropectin [11]. The majority of agar is composed of agarose, a neutral gelling heteropolysaccharide. It is a linear polymer containing glycosidic bonds, as shown in Fig. 1 below. The two monosaccharides are 3, 6-anhydro-a-L-galactopyranose and β-D-galactopyranose, which are joined by glycosidic linkages (1-4) between β-D-galactopyranose and 3, 6-anhydro-α-L-galactopyranose, resulting in the disaccharide basic unit known as neoagarobiose and (1-3) between 3,6-anhydro-α-L-galactopyranose and β-D-galactopyranose.

Many procedures and investigations have been conducted on the manufacture of agarose from high-quality agars and low-grade agarose employing complicated or multi-step purification procedures. In the extraction of agarose, it is directly extracted from the seaweeds, or it is extracted from agar which is composed of 70% agarose and 30% agaropectin but agaropectin

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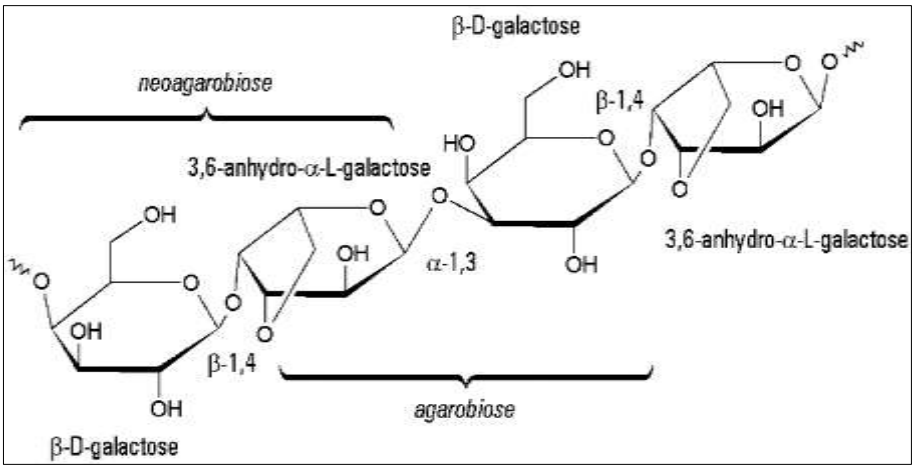
has high sulfate content and therefore has a low gelling strength compared to agarose making agarose the best substance for gelling [12]. The goal of this study was to explore agarose extraction from seaweeds found growing along the coasts of Ghana using different extraction methods.



***Source*:** Zucca, Fernadez-Lafuente and Sanjust (2016) [13]

Fig 1: Chemical structure of agarose

2. Materials and methods

2.1 Material

Weighing scale (OHAUS), Water bath (Grant JB series), Thermometer (Thermo-fisher), Centrifuge (Thermo-fisher), Hot plate (Delron), Saucepan, UV Transilluminator (Lab-compare) were some of the equipment used in this study.

2.2 Reagent/Chemicals

Quick load Purple 100bp DNA ladder N0551S (New England Biolabs), Acetic acid (AnalaR), Agarose powder (Sigma Aldrich), PEG 6000(Sigma Aldrich), DEAE cellulose (Cellex D), (Bio-Rad), DMSO (Sigma Aldrich), HCl (Sigma Aldrich), NaCl (Sigma Aldrich), NaOH (Sigma Aldrich) were the chemical and reagents used

2.3 Collection of seaweeds

Seaweeds; *Gracilaria cervicornis*, *Ulva fasciata*, *Caulerpa taxifolia*, and *Hydropuntia dentata* were collected from natural source in two locations, Labadi and Kpone Beach along the coast of Ghana (5°33'51.0"N 0°07'50.9"W and 5°40'51.3"N 0°03'15.5"E).

Fig 2: Pictures of the seaweed collected for the study from different source along the coastal line of Ghana.

2.4 Treatment prior to extraction

The seaweeds collected were transported to the Laboratory and thoroughly washed with water before drying in a cold-dried room for one week. After drying, bleaching was done by weighing 100g of each of the dried seaweeds in 10% Hydrogen peroxide in a transparent bowl and left overnight at room temperature [12]. The samples were then washed thoroughly with deionized water and treated with alkaline (5% NaOH) as reported by Xiao and colleagues [14].

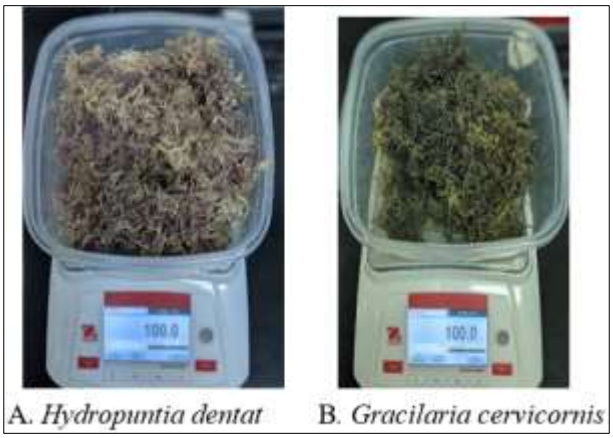


Fig 3: The Fig. show pictures of the dried weighed samples before the bleaching process

2.5 Agar extraction

The seaweeds were washed with deionized water after the alkali treatment to remove excess NaOH. Alkaline-treated seaweeds were transferred into 1000ml of fresh deionized water and heated at 90 °C for one and half hours. The extract from the seaweed was filtered using a 0.8 µm filter unit and allowed to gel at room temperature. The gelled agar was frozen overnight and thawed to remove all the excess water from the agar [15].

2.6 Agarose extraction

2.6.1 Agarose extraction using Polyethene Glycol (PEG)

A concentration of 6% PEG (molecular weight, 6000) and 1%

NaCl were added to 21.5g of agar from *Gracilaria cervicornis* and 17.2g from *Hydropuntia dentata*. The suspension was heated briefly at a temperature of 70 °C with constant stirring for 10 minutes. The agar suspension was then centrifuged at 1500rpm for 15min to obtain a precipitate from the supernatant liquor. The precipitate was separated from the supernatant and the remaining PEG was washed out of the precipitate with 0.1 mol/L NaCl solution. Deionized water was repetitively used to scrub the precipitate and finally washed with absolute ethanol and centrifuged again to obtain the agarose. The agarose was later freeze-dried using a Labconco Freeze Dryer Machine [15].

2.6.2 Agarose extraction using DEAE Cellulose

The extraction method used is as described by the group of Zhang Yu [15]. Briefly, 21.5g of agar from *Gracilaria cervicornis* and 17.2g from *Hydropuntia dentata* were added to 50ml anionic exchange resin DEAE-cellulose suspension and agitated at 80 °C until homogenous mixture was obtained. The anionic resin which adsorbs the sulfate galactan in agar was then removed by centrifugation at 1500 rpm for 15 minutes. The supernatant was allowed to gel, freeze-thawed, and later dehydrated in alcohol. The final product was freeze-dried to obtain white solid agarose.

2.6.3 Agarose extraction using dimethyl sulfoxide (DMSO)

This method was used as reported by Jeon *et al* [16]. About 21.5 g of agar from *Gracilaria cervicornis* and 17.2g from *Hydropuntia dentata* were added to DMSO to create 10% [w/v] while being stirred at 70 °C for one hour. The solution was later centrifuged at 2500 rpm for 20 min at 25 °C to obtain sediment. The sediment was repeatedly washed using deionised water to remove the remaining DMSO. The final product was freeze-dried to obtain agarose.

2.7 Physicochemical properties

2.7.1 Agar and agarose yield

Agar yield was calculated as a percentage of dry weight using the equation below;

$$\text{Agar yield} = \frac{agar\ dry\ weight}{seaweed\ dried\ weight} \times 100\%\ 1$$

Agarose yield was calculated as a percentage of dry weight of agarose extracted using the equation below;

$$\text{Agarose yield} = \frac{Agarose\ extracted}{Agar\ weight\ used} \times 100\%\ 2$$

2.7.2 Agarose gelling temperature

Gelling temperature was determined for agarose obtained from the three different extraction methods by immediately putting a thermometer into the hot agarose solution of 2% concentration in 15ml centrifuge tubes. The reading was taken immediately when the content was observed gelling.

2.7.3 Agarose melting temperature

Melting temperature was determined for agarose obtained from the three different extraction methods by immediately putting a thermometer into already gelled agarose of 2% concentration in 15ml centrifuge tubes. The tubes were then secured in a water bath, and the temperature was raised steadily from 50 to 100 °C. The reading was taken immediately when the content was observed melting.

2.7.4 Agarose gel electrophoresis

To a volume of 25ml, a concentration of 1.5% of the native agarose from each of the extraction methods was prepared by dissolving 0.375g in an Erlenmeyer flask using TAE buffer. The mixture was microwaved for 5min and swirled for a while to cool. A 0.16ul of Ethidium bromide was added to the agarose mixture and mixed thoroughly. It was then poured into a casting tray with the combs inserted and allowed to solidify for 20min. The casted gel was placed in TAE buffer in a gel Tank and 3ul of Quick load Purple 100bp DNA ladder N0551S (New England Biolabs) was loaded in the wells. The loaded gel was run at 100 V for 30-40min in a standard horizontal electrophoresis unit. The results from the electrophoresis were observed under a UV transilluminator and images were taken. Commercial agarose was used as a control of the native agarose.

2.7.5 Microscopic appearance of local and commercial agarose gel under an Inverted Microscope

An inverted microscope was used to observe the texture of the locally made-agarose and commercial agarose. Part of both the locally made gel and commercial agarose gel was cut and placed in a petri dish and then place under the inverted microscope and observe. A picture of the observed differences in the properties was captured.

3. Results

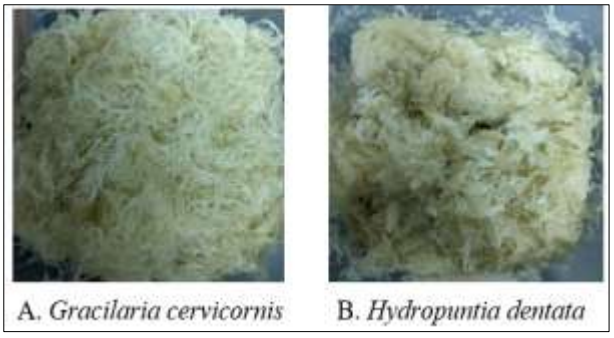


Fig 4: A picture of A. *Gracilaria cervicornis* and B. *Hydropuntia dentata* after bleaching with 10% hydrogen peroxide.

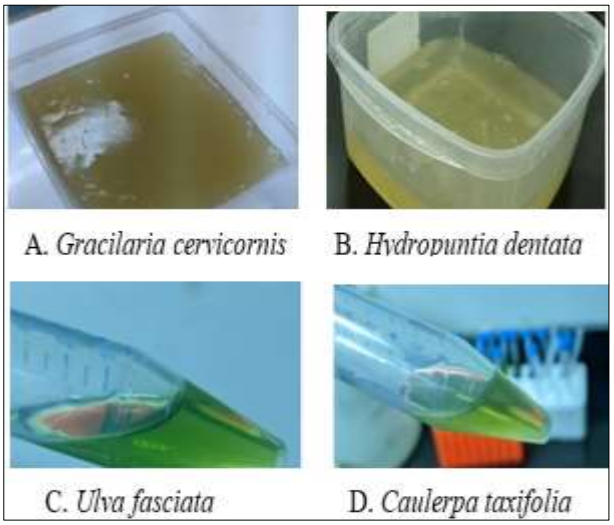


Fig 5: Agar extracts obtained from various seaweeds after 1.5h heating at 80 °C: A. *Gracilaria cervicornis* and B. *Hydropuntia dentata* formed a gel at room temperature whiles C. *Ulva fasciata* and D. *Caulerpa taxifolia* did not form a gel at room temperature

Table 1: Agar yield from *Gracilaria cervicornis* and *Hydropuntia dentata* seaweeds.

Seaweed	Agar Yield (%)
Gracilaria cervicornis	21.5%
Hydropuntia dentate	17.2%

Table 2: Agarose yields from *Gracilaria cervicornis* and *Hydropuntia dentata* seaweeds using the three different extraction methods previously described.

Agarose	Different extraction methods		
	DEAE	PEG	DMSO
% Yield from *Gracilaria cervicornis*	10.1	7.2	4
% Yield from *Hydropuntia dentate*	8.2	6	3.2

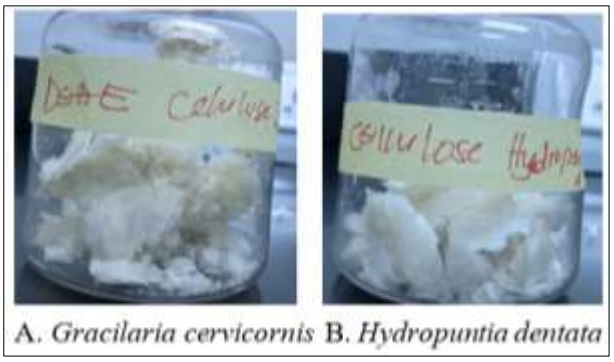


Fig 6: Agarose flakes from A. *Gracilaria cervicornis* and B. *Hydropuntia dentata* obtained from DEAE cellulose extraction method after freeze drying.

3.1 Freeze dried agarose

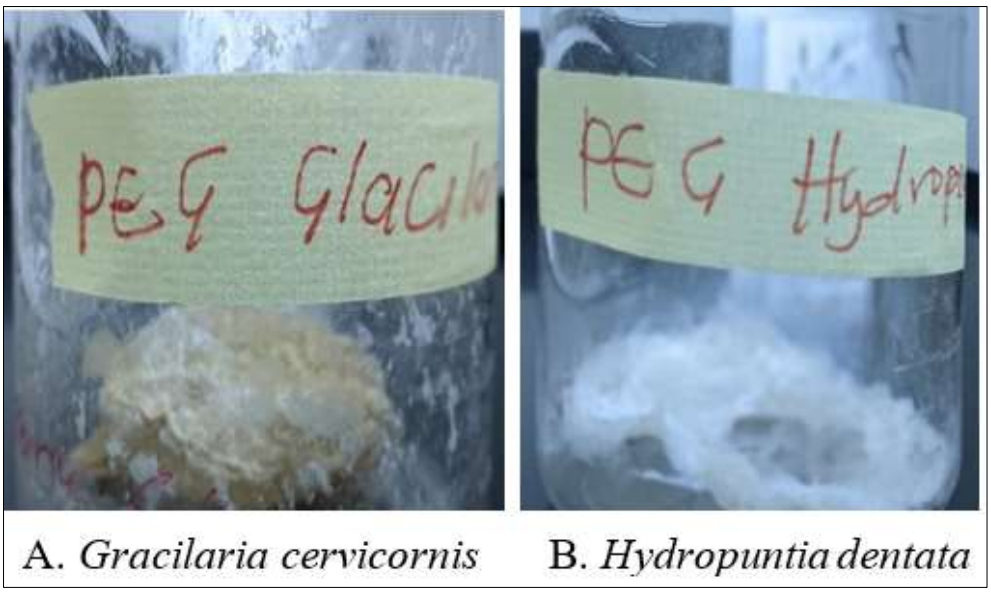


Fig 7: Agarose flakes from A. *Gracilaria cervicornis* and B. *Hydropuntia dentata* obtained from the Polyethylene glycol (PEG) extraction method after freez drying.

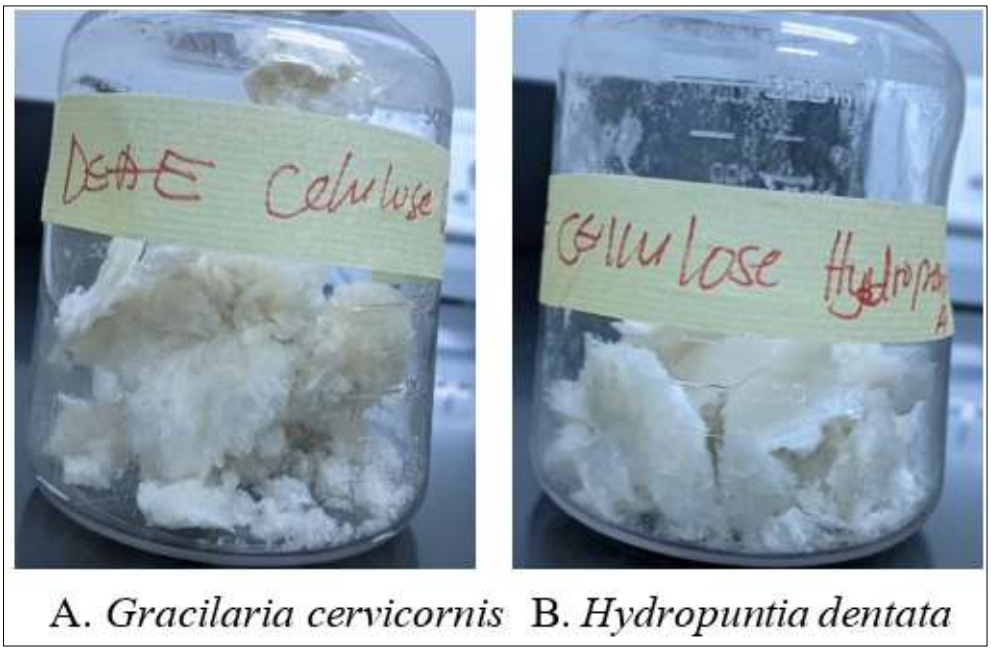


Fig 8: Agarose flakes from A. *Gracilaria cervicornis* and B. *Hydropuntia dentata* obtained from the DMSO extraction method after freez drying.

3.2 Physicochemical properties

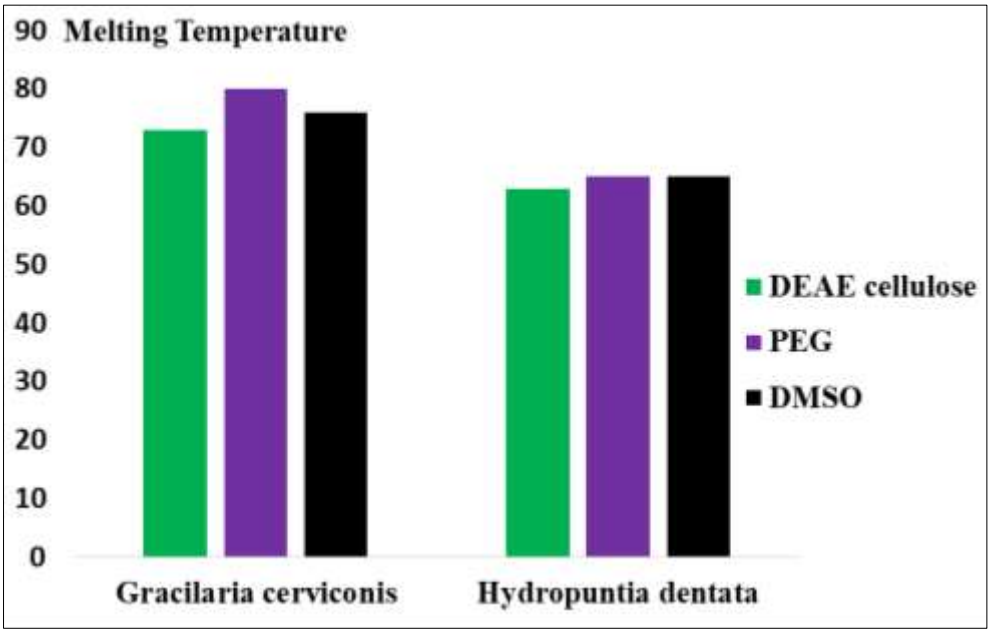


Fig 9: indicates the melting temperature of agarose prepared from *Gracilaria cervicornis* and *Hydropuntia dentata* using DEAE Cellulose, PEG, and DMSO.

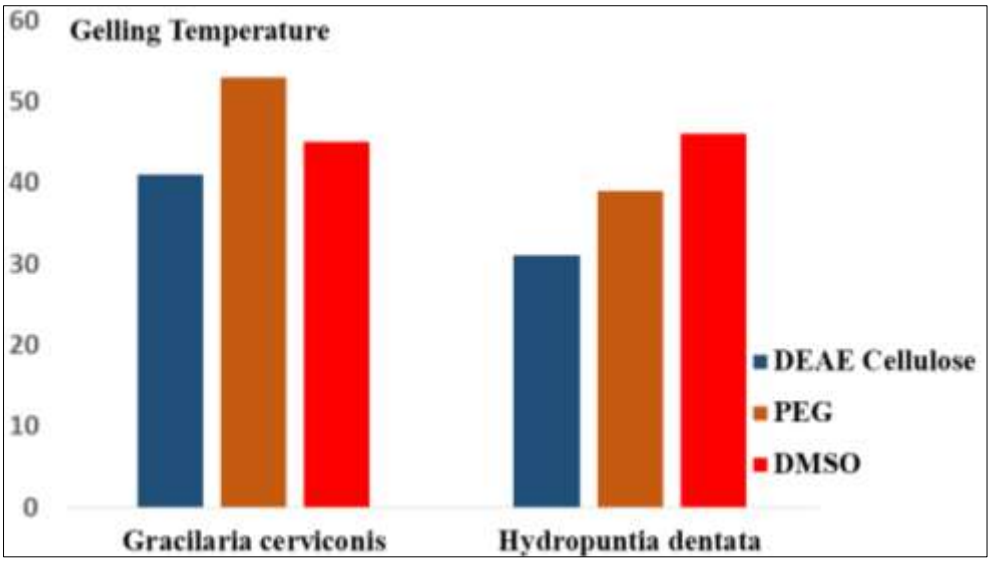


Fig 10: Shows the gelling temperature of agarose prepared from *Gracilaria cervicornis* and *Hydropuntia dentata* using DEAE Cellulose, PEG, and DMSO.

Table 3: This table summarizes the performance of the various tested agaroses based on visual evaluation.

Property	Commercial	Deae cellulose		PEG		DMSO	
	Agarose	G.C	H. D	G.C	H. D	G.C	H. D
Gel Strength	+++++	+++	++	+++	++	+++	++
Clarity	+++++	++++	+++	+	++	++	++
Separation	+++++	+++	++	+	+	+	+

Key: + = fair, ++ = average, +++ = good, ++++ = very good, +++++ =excellent, G.C (*Gracilaria cervicornis*), H.D (*Hydropuntia dentata)*

3.3 Texture of agarose under an Inverted Microscope

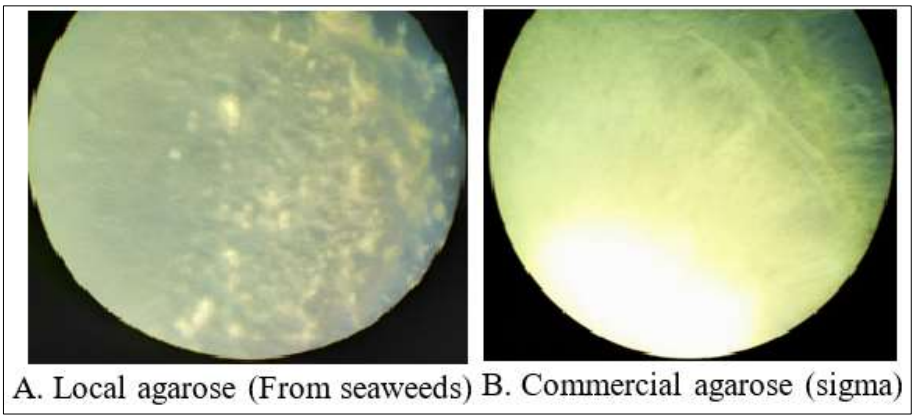


Fig 11: Typical appearance using an inverted microscope inverted microscope of (a) 2% (w/v) agarose gel prepared from the local agarose and (b) a 2% (w/v) agarose gel prepared from Sigma agarose

3.4 Gel Electrophoresis

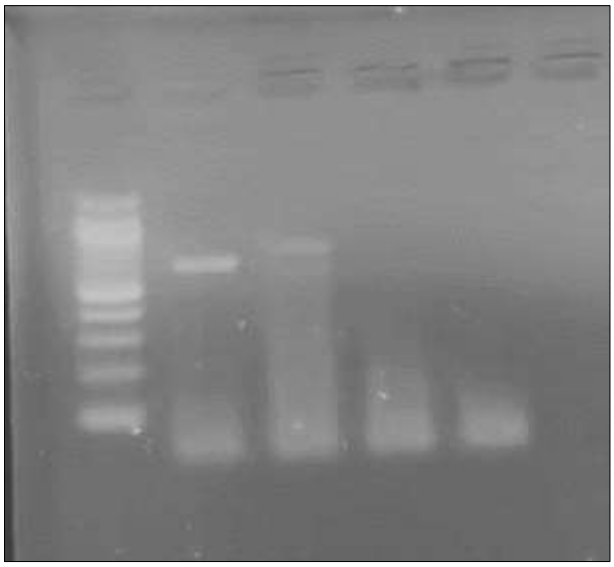


Fig 12: Electrophoregram showing DNA Ladder and DNA (pfhrp2) markers on agarose gels made using commercial agarose (Sigma) as a Standard control

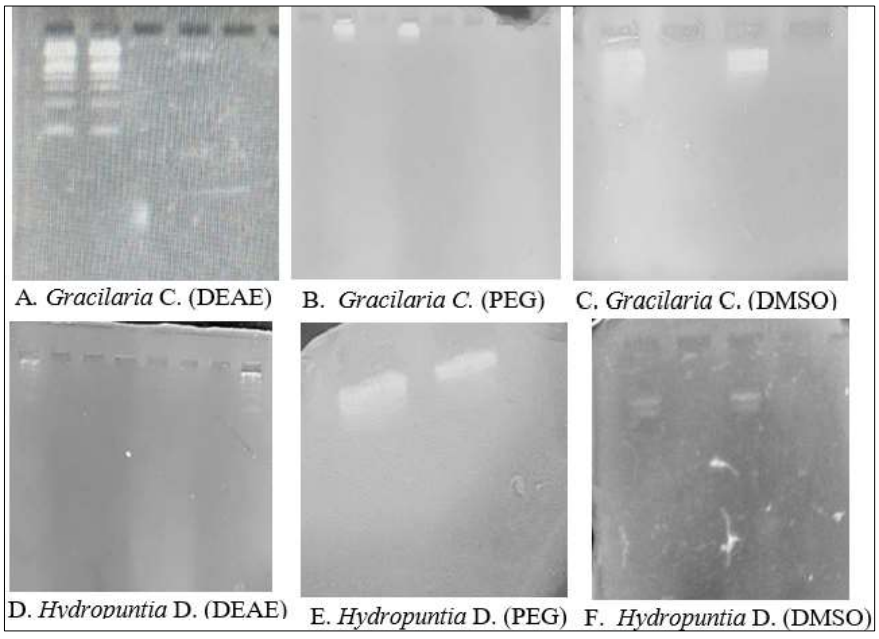


Fig 13: Gel electrophoresis results of DNA ladders prepared using *Gracilaria cervicornis* (A, B, C) and *Hydropuntia dentata* (D, E, F) agarose from DEAE Cellulose, PEG, and DMSO extraction methods.

4. Discussion

4.1 Exploration of agar and agarose from different seaweeds

From the results of this current study, the potential of local seaweeds and examination of their abilities in relation to the migration and separation of DNA fragments in gel electrophoresis indicates that local agarose could be used as a substitute for imported commercial agarose when further optimised. The agarose extracts from the seaweeds, *Gracilaria cervicornis* and *Hydropuntia dentata* which belong to the division Rhodophyta found to produce agar as reported by Araki (1966) [17]. The extract from green algae *Ulva fasciata* and *Caulerpa taxifolia* on the other hand did not produce agar for further extraction of agarose. This is because according to Usov [18], Rhodophyta (Red algae) are known to have an unusual source of sulfated galactans in their cell wall, such as agar, agarose, and carrageenans, whereas green algae's cell wall is thin, transparent and firm consisting of outer pectic and inner cellulosic layers and thus unable to produce agar.

4.2 Agar and agarose quality

The agar quality is influenced by a variety of factors, including species, extraction methods, postharvest storage, and some ecological parameters such as life cycle, season, and geographical features [19]. The type of pretreatment used in extraction influences the yield and other properties of agar. Preparations such as washing to remove sands, extraneous material, and other impurities such as seaweeds, mollusks, invertebrates, and crustaceans, exposure of seaweed to sunlight for drying, decolourisation with hydrogen peroxide and alkaline treatment influenced the quality of the agar and agarose [20].

Hydrogen peroxide was used as a decolourisation agent in this pretreatment method because of the nascent oxygen (oxygen atom which exists as a mono-atom) it liberates during decomposition. This nascent oxygen then combines with the seaweed's coloring matter (Chromophore), which is then oxidized at the carbonyl groups [21]. Though the same pretreatment methods were employed on both species, the results from the experiment indicated that *Gracilaria cervicornis* had a higher yield (21.5%) than *Hydropuntia dentata* (17.2%) which confirms that the yield of agar is dependent on the species. Moreover, visual assessment and feeling by touching proved that *Gracilaria cervicornis* had a higher gel strength and also gel faster than *Hydropuntia dentata* as observed in this present study.

Generally, both species (*Gracilaria cervicornis* and *Hydropuntia dentata*) had a lower agar yield compared to what has been reported in literature. This implies that the gel strength and yield of some species could primarily be determined by season, location, soaking temperatures, soaking times, extraction times, extraction temperature and other processing methods [22]. Other findings confirm that alkali treatments affect agar properties by reducing agar yield [23]. Hence, the decrease in agar yield in this current study could be related to polysaccharide decomposition and diffusion into the NaOH solution [24] or it could be because agar dissolves more easily in an alkaline solution than water [25].

4.3 Performance of different extraction methods

From the exploration of the convenient method for the preparation of agarose, DEAE cellulose method had the best performance from Table 3 followed by PEG and DMSO. The DEAE Cellulose is a positively charged (anionic exchange) resin powder for separating and purifying proteins and nucleic acids [26]. DEAE Cellulose being positively charged works by adsorbing the negatively charged agaropectin in the agar. In the PEG extraction method, the separation is based on the reduced solubility of agarose in media containing PEG. This means agaropectin is more soluble in PEG than agarose and therefore agarose precipitates out and is removed by centrifugation. Similarly, the DMSO method is also based on differences in solubility.

4.4 Gelling and melting temperatures

The highest gelling and melting temperature were 53 °C and 80 °C respectively, and were observed in PEG-treated *Gracilaria cervicornis*. Also, DEAE Cellulose treated *Hydropuntia dentata* had the lowest gelling and melting temperatures of 31 °C and 63 °C respectively. The commercial agarose had a gelling temperature and melting temperature of 37 °C and 82 °C respectively. The deviation of the gelling and melting temperatures for the native agarose prepared by the three methods from the commercial agarose could be due to the difference in species and this is because the commercial agarose was prepared from *Gelidium* sp. [27]. The molecular weight patterns in agar may be responsible for the higher melting and gelling capabilities of the agar isolated from *Gracilaria* in this study compared to *Hydropuntia*. In contrast to *Hydropuntia*, *Gracilaria* sp. has a variety of structures with diverse replacements, including sulfate esters, methoxyl, and pyruvic acids, according to Vuai [28].

4.5 Gel electrophoresis

It was demonstrated in this study that both the local agarose genera *Gracilaria* and *Hydropuntia* were capable of separating DNA products. However, in terms of DNA markers (100 bp), the resolution of commercial agarose was brighter and sharper when compared to local agarose from *Gracilaria cervicornis* and *Hydropuntia dentata.* Generally, comparing the agarose from the two local species (*Gracilaria cervicornis* and *Hydropuntia dentata*), *Glacilaria* had the best performance in terms of separation and resolution of DNA products as compared to *Hydropuntia dentata*.

Within the various methods used in extracting agarose from agar, agarose from the DEAE Cellulose method showed the best separation and resolution followed by DMSO and PEG. The size of the DNA fragments, the voltage used, the extraction technique, the presence of ethidium bromide, and the electrophoresis could affect the DNA fragment migration [29]. It has been demonstrated repeatedly in other research that concentrations have an impact on the electrophoresis results and rely on the DNA molecular weight, which ranges from 0.5% to 1.5% [30].

However, an average concentration of 1% has been recorded for numerous applications [31] which was used in this study. The poor separation of DNA fragments as observed in this study could be due to the presence of undesired components (impurities) in the local agarose as observed by comparing the images of both the local agarose and the commercial agarose under an inverted microscope, the picture from local agarose depicted a rough texture while that of the commercial agarose revealed a very smooth texture [32].

These local agaroses offer a number of benefits, especially in

terms of price and accessibility. However, the separation quality and resolution from these agarose gels can still be improved by regulating the sulfur concentration, pH, and of course, the presence of some unwanted impurities. Future research should delve deeper into these issues.

4.6 Conclusion

Agar was prepared from *Gracilaria cervicornis* and *Hydropuntia dentata* seaweeds collected from Labadi and Kpone through to Prampram Beach, Ghana. The agar prepared from *Gracilaria cervicornis* had high gel strength compared with *Hydropuntia dentata*. Agarose was produced from extracted agar using DEAE Cellulose, Polyethylene glycol (PEG), and dimethyl sulfoxide (DMSO) extraction methods. The physicochemical properties of the resulting agarose indicated that *Gracilaria cervicornis* agar treated with DEAE cellulose had a better gel strength based on visual assessment and had higher melting and gelling temperature as compared to *Hydropuntia dentata*. DNA fragments separated by *Gracilaria cervicornis* treated with the DEAE Cellulose method were the best among the others and the bands were quite similar to that of the commercial agarose. Also, *Gracilaria cervicornis* seaweed was seen to be the best in agarose production than the other species collected for this work and therefore could be useful in large-scale agarose production in Ghana. In general, DNA electrophoresis proved that the local agarose prepared from both *Gracilaria cervicornis* and *Hydropuntia dentata* seaweeds have an electrophoretic property and can be further refined and used for molecular research work in Ghana and other African countries that rely heavily on agarose importation.

5. Acknowledgment

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6. Declaration of competing interest

This is to certify that the authors have no conflict of interest.

7. Funding

Not applicable.

8. Reference

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