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The Economic Importance of the Egyptian Alga *Gracilaria* sp.

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ABSTRACT

Gracilaria sp. is a red macro-alga isolated from the marine environment of the Red Sea in Egypt. Unfortunately, this alga has not been well exploited yet in Egypt, thus the author preferred to highlight the economic importance of this alga. In this study, the chemical composition of the edible red seaweed *Gracilaria* sp. was evaluated via quantitative and qualitative tests using distilled water and two solvents (ethyl acetate and benzene) different in their polarity. The results showed that *Gracilaria* sp. contains a protein content of 22.75 %, fat of 1.01%, carbohydrate of 37.58% on dry weight basis, as well as pigments such as phycoerythrin, phycocyanin, allophycocyanin, β-carotene and chlorophyll a with yields of 3.8± 1.3, 1.8± 0.7, 0.9± 0.6, 7.7± 1.4, 1.6± 1.5mg/ ml D.W., respectively. Moroever, polysaccharide agar had a yield of 23.93% and total phenolic compounds of 54.5mg GAE/ g extract. Tests of the chemical composition of this alga showed the availability of many chemical compounds that have great economic importance in the field of medicine, pharmaceuticals, cosmetics, and food industries as an antioxidant IC_{50} 27 ± 1.68mg/ ml, a bactericidal and fungicidal agent and as a bio pesticide in the field of agriculture. Egypt should also benefit from polysaccharide agar from *Gracilaria* sp. in biotechnology laboratories as growth media for bacteria, yeast, and fungi as well as the use of *Gracilaria* sp. pigments in the food and cosmetic industries as good natural colors.

INTRODUCTION

It is constantly noticed that, especially due to the global crisis resulting from climate changes, people are keen to use products of natural origin that are safe for their health and can be used in the food, pharmaceutical and cosmetic industries using biotechnology. Historically, the Egyptian pharaohs added natural extracts to improve the appearance of their sweets **(Burrows *et al.*, 2009)**.

Algae were used in medicine in Egypt (1,550 BC) **(Kumar *et al.*, 2021)**. The marine environment of Egypt contains a wide range of organisms such as marine macro-algae or seaweeds, multicellular photosynthetic eukaryotic organisms. These organisms are used as natural products in food in many countries of Asia continent, in fertilizers, pesticides, and as biofuel. Algae have a major role in the food and cosmetics industries as well as in the medical field such as medicines. Marine macro-algae are classified into

three categories according to their pigments such as brown algae (Phaeophyta), green algae (Chlorophyta) and red algae (Rhodophyta). The red algae are generally found in the coastal environments, while they are relatively rare in freshwaters. They are rich in protein and dietary fiber (soluble fiber than Phaeophyta and Chlorophyta families). They contain beneficial essential fatty acids, essential amino acids, polysaccharides, pigments, phenolic compounds, flavonoids, alkaloids, terpenes and phytosterols, hence their biological activities were administered as drugs, antioxidants, antimicrobial, antitumor and as animal feed and in human food, pharmaceutical and cosmetics industries. Moreover, algae might be employed in several industrial applications, such as the application in wastewater treatments. The chemical composition of seaweeds varies with environment conditions such as temperature air conditions, season, geographical origin, and physiological maturity. Algae are a good source of phycocolloids such as the agar of *Gracilaria* sp. **(Francavilla *et al.*, 2013; Silva *et al.*, 2020; Kumar *et al.*, 2021; Long *et al.*, 2021)**.

Gracilaria sp., a red macro-alga which is called sea hair vegetable, asparagus, and thread vegetable, is composed of carbohydrates, proteins, lipids, and vitamins A, B1, B2, B6, B12 and C, in addition to minerals viz. calcium, phosphorus, potassium, sodium, iron, and iodine, fibers, phytohormones, phenolic compounds, flavonoids, alkaloids, terpenes and phytosterols. Consequently, its biological activities were determined as antioxidants, antimicrobial, antitumor, anticoagulant, anti-inflammatory, anti-hyperlipidemia, hypocholesterolemic, hepatoprotective, as animal feed and as prebiotic in food industry. Additionally, it is used as an agarophyte containing agar polysaccharide. Photosynthetic pigments are classified into three groups of light harvesting such as chlorophylls (a, b, c, and d), carotenoids (Carotenes and xanthophylls) and phycobiliproteins or phycobilin (Phycoerythrin, phycocyanin and allophycocyanin) found in the chloroplasts of the red algae and cyanobacteria. *Gracilaria* sp. contains phycoerythrin, phycocyanin, allophycocyanin, carotenes, carotenoids, and chlorophyll a. These pigments are valuable in the food industry as natural colorants, and they have significant importance in the food, cosmetics, and pharmaceutical industries. They are recognized for their safety and beneficial properties, including anti-inflammatory, antitumor, and antioxidant effects **(Elumalai *et al.*, 2013; Francavilla *et al.*, 2013; Ananthi *et al.*, 2016; Sudhakar *et al.*, 2018; Silva *et al.*, 2020; Kumar *et al.*, 2021; Long *et al.*, 2021)**.

Agar is a polysaccharide extracted from the red algae, primarily *Gelidium* sp. worldwide and *Gracilaria* sp. particularly in the developed countries, where it is widely used in biotechnology laboratories. Moreover, agar is a water-soluble gel-forming polysaccharide extracted from the cell wall of the agarophyte algae such as the Rhodophyta. it is a polysaccharide that accumulates in the cell walls of *Gracilaria* sp. Its content in particular seaweed varies depending on the season. Furthermore, agar is a mixture of agarose and agaropectin where agarose is a linear chain of polymer consisting of 1, 4-linked α-3,6 anhydro-L-galactose and 1,3-linked β-D-galactose repeating units,

while agaropectin is a sulphated polysaccharide composed of agarose and other components such as D-glucuronic acid, ester sulphate, and a small amount of pyruvic acid. The main importance of agar lies in being used as a cell culture medium in biotechnological laboratories. Agar exhibits a biological activity acting as an antitumor agent and reducing the oxidative stress in the human body. Whereas, in food industries, different applications have been associated with the use of agar either for the improvement of the texture or for thickening food. Agar and the pigments of *Gracilaria* sp. play a major role in food and cosmetics industries as natural and safe colors,while a radical role has been detected in the fields of medicine and pharmacy, serving as an anticoagulant, antioxidant, and antitumor **(Francavilla *et al.*, 2013; Silva *et al.*, 2020; Kumar *et al.*, 2021; Long *et al.*, 2021)**.

Microbes such as fungi, bacteria, and viruses are responsible for diseases in vegetables, fruits, crops, fish, rabbits, poultry, animals, and humans. Antimicrobials are the substances that inhibit the growth of microorganisms. The chemical components of *Gracilaria* sp. are responsible for its antimicrobial activity **(Amin, 2019)**.

Antioxidants are the substances that scavenge the reactive oxygen species as hydroxyl radical (OH) and free radicals which increase the oxidative stress in the human body and lead to DNA, proteins, and nucleic acid damage beside various harmful diseases, such as cancer and Alzheimer's. Therefore, antioxidant compounds play an important role in reducing oxidative stress and protecting health from different diseases. Algae can reduce oxidative stress and prevent other diseases since seaweeds contain numerous bioactive compounds with a higher antioxidant activity compared to land plants owing to the rich biochemical composition, such as chlorophyll, phycobiliproteins and carotenoids pigments, phenolic compounds, vitamins, polysaccharides, and other phytochemical components. Marine algae have shown a strong natural antioxidant activity, with no side effects aligned with the use of some synthetic antioxidants **(Amin, 2020).**

The research aimed to detect the beneficial phytochemical components found in algae inhabiting the waters of the Mediterranean Sea and the Red Sea of Egypt. In this work, I aimed to shed light on the alga *Gracilaria* sp., determine its chemical composition through quantitative and qualitative tests, and address its biological activities as antioxidant, bactericidal, and fungicidal against pathogens and, on the other hand, as an agarophyte to explore cheap, safe, and available alternative sources with a natural feature and antimicrobial impact. This study would encourage those in charge to start producing agar from *Gracilaria* sp. in Egypt.

MATERIALS AND METHODS

1. Collection of algae (*Gracilaria* sp.)

Algae were collected along the coast of the Red Sea of Egypt (Fig. 1) **(Abdel-Latif *et al.*, 2012)**. Subsequently, the algae were washed with sea water to remove sand pebbles,

epiphytes, and shells. Then, they were shipped to the laboratory in ice boxes, washed with diluted solution of sodium chloride followed by distilled water. The algae were shade dried, grounded in an electric mixer, and stored in a refrigerator at 4ºC for further use.

Fig. 1. Localities of alga sampling along the coast of the Red Sea, Egypt

2. Identification of *Gracilaria* sp. (Table 1)

Gracilaria sp. was identified by the professor of microbiology and phycology, Faculty of Science, Zagazig University (Table 1 & Fig. 2) as follows:

Table 1. Classification of *Gracilaria* sp.

Domain	**Eukaryota**
Kingdom	**Plantae**
Division (Phylum)	**Rhodophyta**
Class	**Rhodophyceae**
Order	**Gracilariales**
Family	**Gracilariaceae**
Genus	**Gracilaria**
Species	***Gracilaria* sp.**

Fig. 2. *Gracilaria* sp.

3. Reagents

All chemicals and reagents used in the experiments were of an analytical grade.

Methods

4. Extraction of *Gracilaria* sp. phytochemicals

One gram of *Gracilaria* sp. was dissolved in 10ml of the solvent (distilled water, ethyl acetate and benzene). The solvent was stirred for 1 hour at 6000rpm followed by filtration, and the extracts were kept in dark bottles in the refrigerator for further usage **(Amin, 2019)**.

5. Determination of chemical composition of *Gracilaria* sp.

***5.1. Qualitative and quantitative phytochemical analyses by chemical tests as mentioned by* Amin (2019)** (Tables 2, 3, 4 & Figs. 3, 4, 5)

5.1.1. Quantitative phytochemical analyses of primary metabolites by chemical tests, as mentioned by Amin (2019).

***5.1.1.1. Primary metabolites (% of DW)** (Table 2).*

Determination of carbohydrates

Total carbohydrate was estimated according to the phenol-sulphuric acid method of **Dubois *et al*. (1956)** using glucose as standard.

Determination of proteins

Total protein was calculated using the elemental N determination by the nitrogen-protein conversion factor of 6.25 according to **AOAC (1995)**.

Determination of lipids

Total lipids were estimated according to **AOAC (2000)**.

Determination of moisture

The moisture content was determined by the oven method at 105°C until their constant weight was obtained.

Determination of ash

Ash content was acquired by heating the sample overnight in a furnace at 525°C, and the content was gravimetrically determined.

5.1.2. Qualitative phytochemical analyses of secondary metabolites by chemical tests (Table 3)

5.1.3. Photosynthetic pigments determination of *Gracilaria* sp. (Kumar *et al.*, 2011) (Table 4)

The photosynthetic pigments were estimated as follows: the phycobiliproteins (100 mM phosphate buffer, pH 6.5), chlorophyll and carotenoids (100% acetone) were extracted by using 1g of freeze- dried algae, followed by centrifugation at 3000×g at 4°C for 20min. The amount of chlorophyll and carotenoids pigments was calculated according to the method of **Balamurugan *et al.* (2013)**. Phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) contents were estimated using the equations described by **Francavilla *et al.* (2013)**.

For the chlorophylls and carotenoids, equations for calculating the concentration of pigments were determined as follows:

$$\text{Ca (chlorophyll a)} = 11.75\ [A_{662}] - 2.35\ [A_{645}].$$

$$\text{C b (chlorophyll b)} = 18.61\ [A_{645}] - 3.96\ [A_{662}],$$

$$\text{Total carotenoids} = (1000\ [A_{450}] - 2.270 \times \text{Ca} - 81.4 \times \text{C b})/227$$

The absorbance of the aqueous supernatants was determined at 498.5, 614.0, and 651.0nm, and their APC, PC, and PE contents were calculated as mg mL^{-1} using the following equations:

$$APC = 181.3\ A_{651} - 22.3\ A_{614}$$

$$PC = 151.1\ A_{614} - 99.1\ A_{651}$$

$$PE = 155.8\ A_{498.5} - 40.0\ A_{614} - 10.5\ A_{651}$$

5.1.4. Total phenolic compounds determination of *Gracilaria* sp. (Fig. 3)

The total phenolic compounds content of *Gracilaria* sp. extracts was assessed using three different solvents: distilled water, ethyl acetate, and benzene, each varying in polarity. The determination followed the colorimetric method described by **Shahidi and Naczk (1995)**. For each extract, 0.5ml was mixed with 0.5ml Folin reagent and 8ml distilled water, then shaken for 2 minutes. Following this, 1ml of Na_2CO_3 was added. The resulting blue color was measured after 1 hour at 725nm against a blank. The results were expressed as mg of gallic acid per gram of an extract of *Gracilaria* sp.

5.2. Agar extraction

Agar was extracted by using countless methods, such as autoclaving (heat under pressure) extraction, microwave extraction, ultrasonic extraction, enzyme extraction, acid / alkali extraction and hot / cold water extraction. However, the preferred method of extraction of the agar is the hot water extraction due to its safety, low cost, quick and easy operation, as well as it doesn't destroy the structure of agar and will not introduce impurities.

Agar was extracted from the dried *Gracilaria* sp. upon applying the hot water extraction method. Briefly, 50g of dried *Gracilaria* sp. was added to a beaker containing 1000ml of distilled water. The beaker was then placed in a hot water bath at 90°C for 1h. Afterward, the extracted agar was dried at room temperature (27°C) until gel formation and getting a constant weight. Finally, the dried agar was stored at 4°C till further analysis **(Vuai, 2022)**.

5.2.1. Evaluation of agar yield

The agar yield was calculated using the following equation:

$$\%\ \text{Agar Yield} = [(\text{Dry weight of agar (g)} / \text{Dry weight of seaweed (g)})] \times 100$$

5.3. Concluding the chemical composition of Gracilaria sp. *(Fig. 4)*

6. Determination of biological activity of *Gracilaria* sp.

6.1. Antioxidant activity of Gracilaria* sp. *(Fig. 5)

A volume of 1000µl of each of the three extracts of *Gracilaria* sp. was added to 4ml of DPPH (0.1mM of 2.2'- biphenyl picryl hydrazyl (DPPH). After 30min. of the incubation period at room temperature in the dark, the absorbance was read against the blank at 517nm. Inhibition of free radical DPPH was calculated according to the following equation **(Amin, 2019)**:

$$\% \text{ Scavenging activity} = (\text{A control} - \text{A sample} / \text{A control}) \times 100$$

6.2. Antimicrobial activity of Gracilaria sp. *by Kirby-Bauer Method (Table 5)*

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method (**Bauer *et al.*, 1966)**. Briefly, 100µl of the tested bacteria and fungi was grown in 10ml of fresh media until they reached a count of approximately 108 cells/ml for bacteria 105 **(Pfaller *et al.*, 1988)**. 100µl of bacterial suspension was spread onto agar plates corresponding to the broth in which they were maintained (Disc diffusion method for yeasts developed by using the standard method (M44-P) approved by **NCCLS (2003)**. Plates inoculated with the bacteria Desulfomonas pigra ATCC 29098T and were incubated at 35- 37^{o}C for 24- 48h, then the diameters of the inhibition zones were measured in millimeters **(Bauer *et al.*, 1966)**. Standard discs of Ampicillin (Antibacterial agent), served as positive controls for antimicrobial activity but filter discs impregnated with 10µl of solvent (distilled water, chloroform, DMSO) were used as a negative control. This area of no growth around the disc is known as a "Zone of inhibition" or" Clear zone". Agar-based methods, such as E-test and disk diffusion, can be good alternatives since they are simpler and faster than broth-based methods **(Liebowitz *et al.*, 2001; Matar *et al.*, 2003)**.

4. Statistical analysis

The statistical package SPSS (version 20) was used for statistical analysis. Propit analysis was performed to calculate the medium effective concentration (EC_{50}) for determining the bactericidal and fungicidal activities in addition to the medium inhibition concentration (IC_{50}) for determining the antioxidant activity, all of which were calculated by the linear regression analysis.

RESULTS AND DISCUSSION

The marine environment is rich in marine organisms that should be addressed to explore new natural chemical components that may be used in agriculture as bio-stimulant, animal feed, biofuel in the food and cosmetics industries and in the pharmaceutical and medical fields. *Gracilaria* sp., a red macroalga, is safe for humans and the environment, with no known side effects. It is easy to obtain, and it contains numerous phytochemical components. These properties make it valuable for use in the food and cosmetics industries, as well as in pharmaceutical and medical applications.

1. Chemical composition of *Gracilaria* sp.

In this study, three solvents with various polarity for making extracts were used such as distilled water (polar solvent), ethyl acetate (semi-polar solvent), and benzene (non-polar solvent). The objective was to identify the solvent that extracts the most bioactive components from *Gracilaria* sp. through various qualitative and quantitative tests. The results indicated that distilled water and ethyl acetate were the most effective solvents.

Qualitative and qantitative organic tests are summarized in Tables (2, 3, and 4). The results indicate the presence of numerous phytochemical constituents in *Gracilaria* sp. (Fig. 4)

1.1. Quantitative phytochemical tests

Table 2. Chemical composition of primary metabolites of *Gracilaria* sp.

Chemical compound	%(w-w)
Moisture	6.7
Total carbohydrates	37.58
Proteins	22.75
Lipids	1.01
Ash	31.96
Agar	23.93

1.2. Qualitative phytochemical tests (Table 3)

Table 3. Qualitative phytochemical tests of secondary metabolites of *Gracilaria* sp.

Chemical constituent	Name of the chemical test	Prescence
Phytosterols and steroids	Salkowski test	+++
Triterpenoids	Salkowski test	+++
Alkaloids	Wagner's test	++
Tannins	Ferric chloride test	+
Saponins	Froth Test	+++
Gardiac glycosides	Legal’s test	++
Anthraquinones	Modified Borntrager's test	++
Flavonoids	Alkaline reagent Test	+++
Cardiac Glycosides	Legal’s test	+++
Anthraquinones	Modified Borntrager's test	++
Tannins	Ferric chloride test	-

1.3. Determination of pigments of Gracilaria sp. *(Table 4)*

Table 4. Pigments of *Gracilaria* sp.

Pigment	mg/g D.W.
Phycoerythrin	3.8 ± 1.3
Phycocyanin	1.8 ± 0.7
Allophycocyanin	0.9 ± 0.6
Total chlorophylls	1.6 ± 1.5
Total carotenes	7.7 ± 1.4

1.4. Determination of phenolic compounds of Gracilaria sp. *(Fig. 3)*

Phenolic compounds were evaluated using three extracts of *Gracilaria* sp. The extract that exhibited the highest percentage of phenolic compounds was the ethyl acetate (Fig. 3).

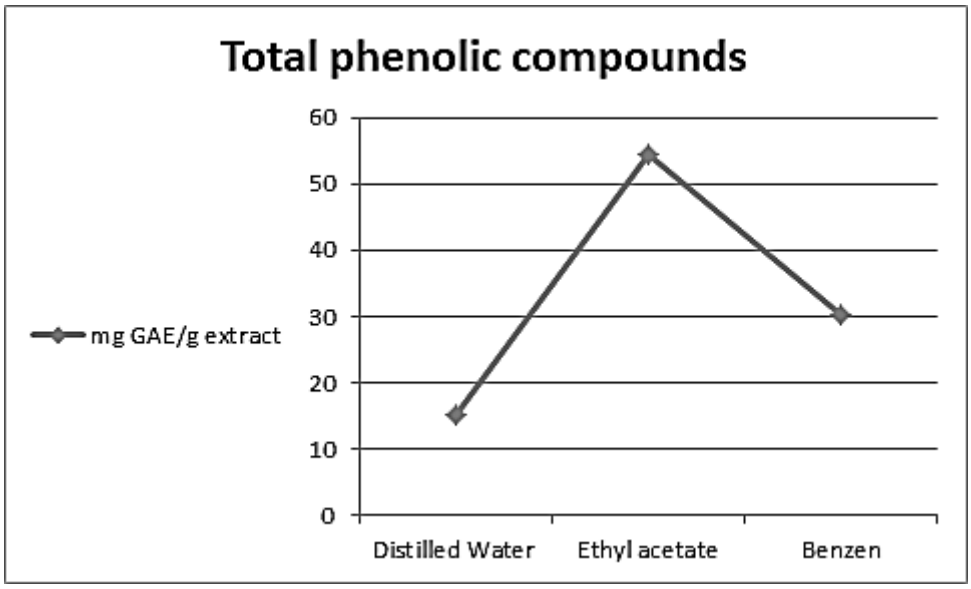

Fig. 3. Phenolic compounds of three extracts of *Gracilaria* sp.

From previous tests for the determination of the chemical composition of *Gracilaria* sp. it was deduced that the chemical composition of *Gracilaria* sp. (Fig. 4) is as follows:

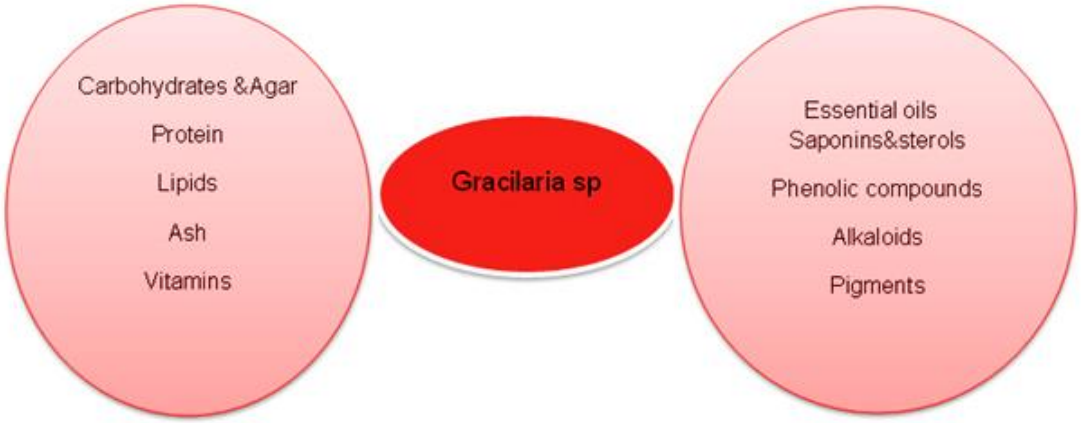

Fig. 4. Chemical composition of *Gracilaria* sp.

2. Biological activity of *Gracilaria* sp.

2.1. Antioxidant activity of Gracilaria sp. *(Fig. 5)*

Many people prefer natural antioxidants with less or no side effects as the case with most synthetic antioxidants. All extracts of *Gracilaria* sp. showed an antioxidant activity at 1000µl of each extract of each solvent of *Gracilaria* sp. (Fig. 5). However, the distilled water extract showed the highest antioxidant activity, with an IC50 value of 27± 1.68mg/ ml.

The higher antioxidant activity of *Gracilaria* sp. is due to its high content of primary and secondary metabolites, as determined in the study of **Amin (2019)**, who argued that the presence of phycoerythrin, phycocyanin, allophycocyanin, polysaccharides, phenolic compounds, chlorophylls, carotenes, carotenoids, flavonoids, alkaloids, terpenes and phytosterols is responsible for the antioxidant activity of *Gracilaria* sp.

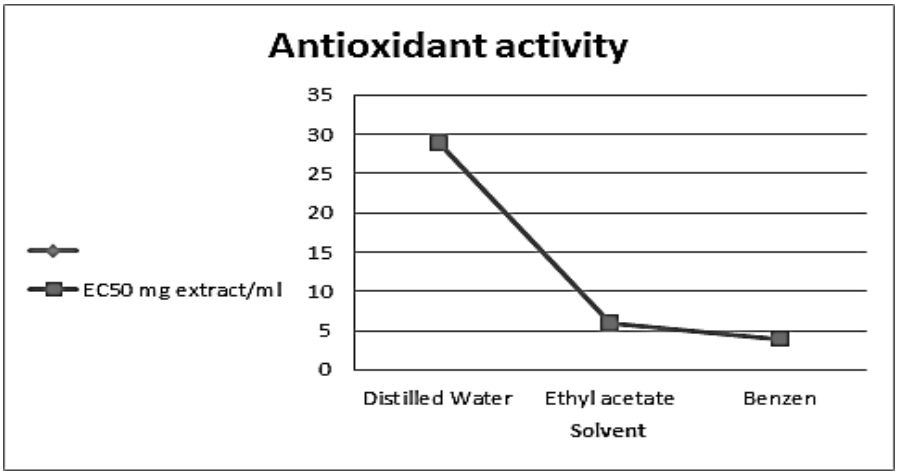

Fig. 5. Antioxidant activity of three extracts of *Gracilaria* sp.

2.2. Antimicrobial activity of Gracilaria sp. *(Table 5)*

Table 5. Antimicrobial activity of *Gracilaria* sp.

Microorganism		Standard	Inhibition zone diameter (mm/mg)
Bacillus subtilus	G+	32	39
Escherichia coli	G -	30	25
Klebsiella sp.	G -	18	17
Pseudomonas aeuroginasa	G -	28	38
Staphylococcus aureus	G+	26	29
Streptococcus faecalis	G+	30	32
Neisseria gonorrhoeae	G -	25	27
Alternaria alternate	F	-	55
Aspergillus fugigatus	F	14	60
Fusarium oxysporum	F	-	60
Penicellium sp.	F	-	50
Candida ablicans	Y	16	60

F = Fungus /G+ = Gram- positive bacterium/ G - = Gram- negative bacterium/ Y = Yeast/- = not determined

From previous tests examining the biological activity of *Gracilaria* sp. we can conclude the economic importance of *Gracilaria* sp. (Fig. 6), as follows:

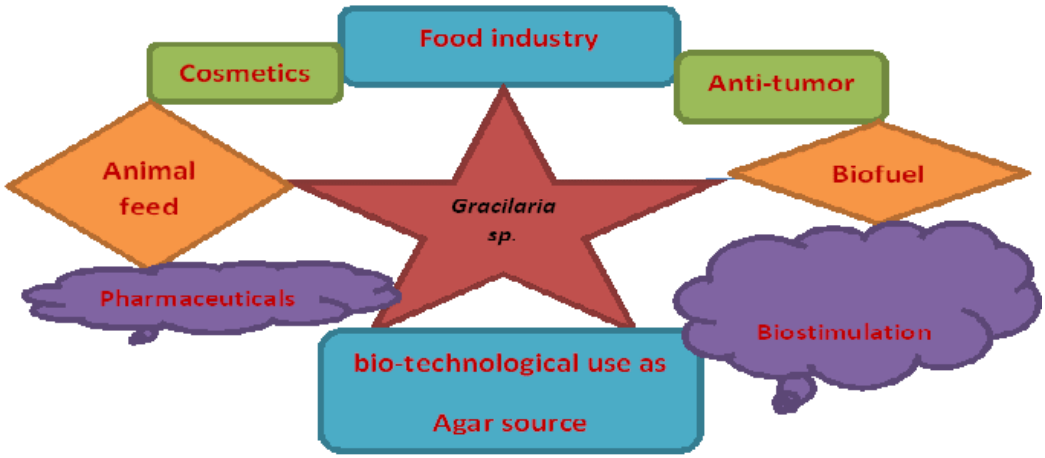

Fig. 6. The economic importance of *Gracilaria* sp.

Tables and Figures indicate the chemical composition of *Gracilaria* sp. proving that *Gracilaria* sp. is a macro-algal rich in many primary and secondary metabolites that have biological activity, especially in polar solvents and specifically in distilled water.

Distilled water and ethyl acetate were the most suitable solvents for the extraction phytochemical compounds of *Gracilaria* sp. such as carbohydrates, proteins, phenolic compounds, flavonoids and saponines, where carbohydrates and proteins are water soluble, while phenolic compounds, flavonoids and saponines are polar compounds. On the other hand, there were alkaloids and terpenoids in the extract of distilled water; however they are not soluble in water. The presence of alkaloids and terpenoides may be due to the moment dipole of polar solvents that make induction to the non-polar compounds that have no dipole, hence non-polar compounds can dissolve in polar solvents **(Prasetyo, 2013)**.

Phytochemical compounds such as phenolic compounds, viz. flavonoids, saponins, alkaloids, terpenes, phytosterols, photosynthetic pigments as chlorophyll a, carotenoids (carotenes and xanthophylls) and phycobilin (phycoerythrin, phycocyanin and allophycocyanin) are good examples of antioxidant compounds and responsible for the antioxidant activity of *Gracilaria* sp.

Bacteria and fungi are pathogens causing diseases for plants, animals, fish, and human. We should search for new alternative sources that could be used as anti-microbial. *Gracilaria* sp. has countless biological activities owing to its phytochemical constituents such as polysaccharides, protein, lipids, polyphenols, saponines, flavonoids, alkaloids, essential oils, natural colors such as phycoerythrin, phycocyanin, allophycocyanin, chlorophylls, and carotenoids that could be used as antimicrobial and antioxidant in medicine, food, cosmetics, and pharmaceutical industries. Antimicrobial substances play a crucial role in affecting the microbial cells through various mechanisms. They target the cell membrane's phospholipid bilayer, degrade enzyme systems, and disrupt the microorganisms' genetic material. Secondary metabolites from algae such as polyphenols can compromise microbial cell permeability, interfere with membrane function and cellular integrity, and ultimately cause cell death **(Francavilla *et al.*, 2013; Silva *et al.*, 2020; Kumar *et al.*, 2021; Long *et al.*, 2021)**. *Gracilaria* sp. should be utilized for agar production in Egypt and as a bio-pesticide to address pollution and climate change.

CONCLUSION

In this paper, the main target was to spotlight the *Gracilaria* sp., a red macroalga with a beneficial chemical structure. Its aqueous extracts should be explored as antimicrobial agents against pathogenic bacteria, fungi, and yeasts that cause diseases in humans, plants, animals, fish, and poultry, without any harmful effects. *Gracilaria* sp. has the potential to serve as an excellent substitute for wild plants in the food industry as a supplement to combat food spoilage, in cosmetics as a preservative, and in the medicine and pharmaceutical industries. Additionally, it could be used as a bio-pesticide in agriculture due to its valuable chemical components and natural colors. Egypt should consider exploiting its marine resources to boost its economy, following the trend ofthe

developed countries which produce agar from *Gracilaria* sp. The researcher is also looking forward to investigating other marine organisms with valuable phytochemical components, biological activities, and safety profiles.

Conflict of interests

There is no conflict of interests.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that he has read and approved the manuscript, and no ethical issues are involved.

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