Comparative Study of Agarophytes - *Gracilaria edulis* and *Gelidiella acerosa* as Biostimulant and Application of Agar for Water-holding in Soil and Plant Growth Promotion

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**ABSTRACT**

**Background:** Seaweeds and its derivatives are extensively used as biostimulants in horticulture and agriculture as a replacement for chemical fertilizers. *G. edulis* and *G. acerosa* are easily cultivable and economically important seaweeds. They are a rich source of phytohormones, amino acids, antibiotics, vitamins, micro, macro elements and agar. Such natural products have great demand and been commercialized these days to promote sustainable agriculture. Dried and finely powdered algal biomass is used directly as a biostimulant. Algal polysaccharides such as agar can be an innovative alternative to synthetic polymers used in horticulture as they contain active biostimulant compounds and also reported to hold water in the soil that aids plant growth with minimum water consumption than usually required. *A. aritis* being one of the most consumed leafy vegetables throughout the world can be harvested indoors with added nutrients and minimal water utilization.

**Methods:** The field trial is a comparative evaluation of the two selected species of agarophytes for promoting plant (*A. aritis*) growth and the extracted agar tested for germination tests, bio-stimulatory property under water stress. Growth parameters were recorded after three weeks. The agarophytes were also qualitatively screened for phytochemicals and WD-XRF analysis.

**Result:** The present work will be a supplementary contribution for assessing agarophytes with biostimulant properties and the characteristic agar gels that expand plant tolerance to abiotic stresses, thus constituting an alternative to synthetic plant protection products.

**Key words:** Agar, Biostimulant, *G. acerosa*, *G. edulis*, Water holding capacity.

**INTRODUCTION**

Seaweeds are eukaryotic organisms thriving in marine environment and are called as macro algae. Marine algae are mass producers of biologically active natural products that have been used as food, fodder, biofertilizer, medicine and valuable raw materials in industries for the production of agar, alginate, carrageenan (Kolanjinathan et al., 2014). Global demand for food production has led to the increased application of chemical fertilizers for enhancing crop production but the toxic chemicals can cause health problems if consumed (Hansa, 1993). This scenario sets a challenge to develop eco-friendly innovative methods that increase agricultural yields, with minimum input (Tilman et al., 2002; Foley et al., 2011) by adopting sustainable production practices that could reduce or substitute the use of chemical inputs like chemical fertilizers and pesticides with natural or biological substances. The development of natural substances promoting plant growth called plant biostimulants and water-holding polymer gels has received increased attention (Hicham, 2016; Salim, 2019).

A biostimulant is an organic material, when used in small quantities can enhance the plant growth and development such that the response cannot be assigned to the application of traditional plant nutrients. The use of macro algae as agricultural biostimulants on crop plants has several benefits such as enhanced rooting, higher crop and fruit yields, freezing, drought and salt tolerance, enhanced photosynthetic activity and resistance to microbial pathogens (Sharma et al., 2014).

Macro-algae contains important plant growth hormones like auxins, abscisic acid, cytokinins, gibberellins, trace elements, vitamins, amino acids, antibiotics, micro and macronutrients. Cytokinins are one of the main active ingredients in seaweed extracts used as plant biostimulant (Booth, 1965; Tarakhovskaya et al., 2007). The nutrients and trace elements can be readily absorbed by plants and...
and other organic matter present in the seaweeds promote plant growth and improve the moisture retaining capacity (Crouch and Van Staden, 1993).

Gracilaria edulis and Gelidiella acerosa are the members of red algae with high commercial value, found in the subtidal areas in many parts of India and other countries (Sharma et al., 2017). Various species of red algae are used as food and as sources of important hydrocolloids: agar and carrageenan. These are water soluble carbohydrates used to thicken gels, jellies of varying degree of firmness, to form water soluble films and to stabilize some products like ice cream for retaining a smooth texture (Kaladharan et al., 1998, McHugh, 2003). The commercial value of seaweed is judged by their agar content and gel quality (Wei-Kang Lee et al., 2016).

Agar is a gelatinous polysaccharide present in the cell wall of many red algal species. It is used as a gelling, thickening and stabilizing agent. Agars are usually composed of repeating agarobiose units alternating between 3-linked β-D-galactopyranosyl (G) and 4-linked 3,6-anhydro-α-L-galactopyranosyl (LA) units. It has good gelling power in aqueous environment, can hold water, retains moisture, rich in nutrients and often used in orchid nurseries and plant tissue culture studies to cultivate plants (McHugh, 1987).

Seaweed extracts, its purified compounds like polysaccharides laminarin, alginates, carrageenans and their derivatives can be used as biostimulant (Craigie, 2011; Khan et al., 2009). They can be applied on soils, in hydroponic solutions or as foliar treatments. In soils, their polysaccharides contribute to gel formation, water retention and soil aeration (Patrick du Jardin, 2015). Algal polysaccharides are biologically active compounds with several potential applications (Kulshreshtha et al., 2016). These properties of polysaccharides suggest that agar can be used to promote plant growth with minimal use of water in a nutrient rich environment.

The objective of the present study is to ascertain the comparative study on the use of agarophytes 6 G acerosa and G edulis as biostimulant and application of agar in water-holding capacity and promotion of plant growth and evaluation of potential species by qualitative phytochemical screening and WD 6 XRF analysis.

**MATERIALS AND METHODS**

**Sample collection**

The red algae 6 G. edulis and G. acerosa were collected from the coast of Kiliakarai (Lat. 9.2343’S, Lon. 78.7836’E), Gulf of Mannar, Tamilnadu, India. The collected seaweeds were washed with fresh water to remove salts and other impurities, sundried, powdered and stored.

**Qualitative phytochemical screening**

Qualitative phytochemical screening of the aqueous extracts of the seaweeds was carried out as per the standard methods (Harborne, 1998; Khandenwel, 2002).

**Detection of alkaloids**

**Mayer’s test**

The extract was treated with Mayer’s reagent and observed for the formation of cream coloured precipitate.

**Detection of phenolics**

**Lead acetate test**

A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

**Detection of flavonoids**

**Sulphuric acid (H2SO4) test**

A fraction of the extract was treated with concentrated H2SO4 and observed for the formation of orange colour.

**Detection of coumarin**

1 ml of 10% NaOH was added to 10 ml of the seaweed extract. Formation of yellow colour indicates the presence of coumarins.

**Detection of terpenoids**

Chloroform (2 ml) and concentrated H2SO4 was added to 0.5 ml of extract. Formation of red-brown colour at the interface indicates the presence of terpenoids.

**Detection of quinone**

Concentrated H2SO4 (1ml) was added to 1 ml of seaweed extract. Formation of red colour indicates the presence of quinones.

**Detection of anthraquinones**

Few drops of 2% HCl were added to 0.5ml of seaweed extract. Appearance of red colour precipitate indicates the presence of anthraquinones.

**Detection of tannins**

**Ferric chloride test**

The extract (5 mg) was dissolved in 5ml of distilled water and few drops of neutral 5% FeCl3 solution were added. Formation of blue green colour indicates the presence of tannins.

**Detection of phlobatannins**

Few drops of 10% ammonium solution were added to 0.5 ml of seaweed extract. Appearance of pink colour precipitate indicates the presence of phlobatannins.

**Detection of anthraquinones**

Few drops of 10% ammonium solution were added to 0.5 ml of seaweed extract. Appearance of pink colour precipitate indicates the presence of phlobatannins.

**Detection of phenolics**

**Benedict’s test**

The extract (5 mg) was dissolved in 5ml of distilled water and few drops of neutral 5% FeCl3 solution were added. Formation of blue green colour indicates the presence of phenolics.

**Detection of alkaloids**

**Mayer’s test**

The extract was treated with Mayer’s reagent and observed for the formation of cream coloured precipitate.
Detection of cardiac glycosides
Glacial acetic acid (2 ml) and few drops of 5% ferric chloride were added to 0.5% of the extract. This was under layered with 1 ml of concentrated H$_2$SO$_4$. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Detection of proteins
The extract was dissolved in 10 ml of distilled water and filtered by Whatmann No.1 filter paper and the filtrate was subjected to tests for proteins and amino acids.

Millon’s test
Few drops of Millon’s reagent were added to 2 ml of the filtrate. A white precipitate indicates the presence of proteins.

Detection of amino acids
Ninhydrin test
Two drops of ninhydrin solution was added to 2 ml of the filtrate. A characteristic purple colour indicates the presence of amino acids.

Detection of steroids and phytosteroids
0.5 ml of seaweed extract was treated with equal volume of chloroform and few drops of concentrated H$_2$SO$_4$. Appearance of brown ring indicated the presence of steroids and bluish brown ring indicates the presence of phytosteroids.

Detection of acids
0.5 ml of the seaweed extract was treated with sodium bicarbonate solution. Formation of effervescence indicates the presence of acids.

Detection of saponins
Froth test
Extracts were diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of gums and muclilages
2.5 ml of the extract was added to 5 ml of absolute alcohol, stirred and filtered. The precipitate was air dried and examined for its swelling properties.

Agarophytes as biostimulant
Seed pretreatment
The algal powder: water was boiled in water bath at 60°C for 30 minutes. The extract was used to soak the spinach seeds (Amaranthus antis) to promote germination and fast sprouting of the seeds.

Plant culture
In the present study, polythene bags were used for raising the crops. They were kept in the net house to prevent damages caused by birds, rats, squirrels and other animals. The plants were grown by two methods using G. edulis and G. acerosa.

Direct mixing with soil
The dried algae powder was mixed with soil in the ratio of 1:100 (10 g in 1 kg of soil) and 2:100 (20 g in 1 kg soil) labeled as A$_1$, A$_2$.

Contact placement
The seaweed powder was spread on the furrow in the proportion of 0.5g and 1 g in 1 kg of soil. The seeds were sown and covered with soil B$_1$, B$_2$.

Aqueous extract of the seaweed was used as a starter solution. Controls consisted of the soil without algae powder and seeds without pretreatment and starter solution.

Extraction of agar and use in plant growth
The washed seaweeds were soaked overnight, treated with HCl, washed, cooked in the digester. The agar gel was collected and freeze-dried (Kaliaperumal and Uthirasivan, 2001). The various steps involved in the processing of seaweed for agar extraction is given in the Fig 1. The extracted agar was used to check its effect on the growth of plants with minimal use of water. A preliminary study was carried out in a cell culture plate. The extracted agar gel was mixed with soil in different concentration (2%, 4%, 6%, 8% and 10%) and labeled as A, B, C, D, E and Control. Five seeds were sown into each well. Control consisted of soil with seeds. About 200 µl of water was sprayed into each well and observed for growth. The same procedure was followed for growing plants in transparent cups by spraying 2 ml of water for the soil: agar mixture and control.

WD-XRF analysis
Wavelength Dispersive X-ray Fluorescence Spectroscopy (WD-XRF) analysis was performed at CECRI - Karaikudi to analyze the micro and macro elements present in G. edulis and G. acerosa respectively.

RESULTS AND DISCUSSION
Qualitative phytochemical screening
Qualitative phytochemical screening indicated the presence of carbohydrates, proteins, alkaloids, flavanoids, cardiac glycosides, coumarins, quinones, anthraquinone and terpenoids, in G. edulis and G. acerosa which added to its potentiality as a biostimulant. The results are summarized in the Table 1.

Agarophytes as biostimulant
The use of seaweeds in horticulture and agriculture has increased in recent years (Dhargalkar and Pereira, 2005). Seaweeds as biostimulant are preferred because of the presence of N, P, potash content, trace elements and metabolites similar to plant growth regulators. Seaweed was reported to be superior to chemical fertilizer because of high level of organic matter which aids in retaining moisture and minerals in upper soil level available to roots (Wallenkkemp, 1955). The high fiber content acts as a soil conditioner, while the mineral content is a biostimulant.
Among the two methods, plants grown by contact placement showed increased growth than the seaweed mixed directly with soil. This may due to the fact that in direct mixing of the seaweed with soil, there is a slow release of nutrients into the soil and the soil gets fertilized over time. But in contact placement, the roots of the plant are in vicinity of the seaweed where the nutrients are readily absorbed by the roots promoting fast growth of the plants thereby fertilizing the soil. G. edulis enhanced plant growth than G. acerosa. The results are given in the Table 2.

**Use of agar for plant growth**

The bioactive polysaccharides and oligosaccharides derived from seaweeds aids in plant growth stimulation and plant defense (Vera et al., 2011; Gonzalez et al., 2013). Algal extracts protect plants from pathogens, insects and from abiotic stress such as drought, frost and salinity etc. Marine macro algal polysaccharides can trigger signaling cascades that activate plant defense response and resistance to infection and diseases. Algal polysaccharides act as chelating agents by binding cations of trace elements, a large number of chemical groups, especially carboxyl residues. These hydrocolloids possess good gelling and water-binding capacity. This property is directly related to a degree of sulfatation (Michalak et al., 2017).

Agar gel extracted from G. acerosa was checked for water holding capacity and promotion of plant growth. This method can be effective when facing water scarcity and arid areas. Good growth of plants was observed even in low concentrations (2% and 4%) of agar mixed with soil with very minimal water requirement (200 µl) in cell culture plate. There was no growth of seeds in control well as the seeds were unable to germinate under water stress. Plants grown in transparent cups also showed similar result with good growth at low concentrations of agar (2% and 4%). After 10

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**Table 1: Qualitative phytochemical screening of G. edulis and G. acerosa.**

| Phytochemicals            | G. edulis | G. acerosa |
|---------------------------|-----------|------------|
| Alkaloid                  | +         | +          |
| Phenolics                 | ++        | +          |
| Flavonoids                | ++        | +          |
| Coumarin                  | -         | -          |
| Terpenoid                 | +         | +          |
| Quinone                   | +         | ++         |
| Anthraquinone             | +         | +          |
| Tannin                    | -         | +          |
| Phlobatannin              | -         | -          |
| Carbohydrate              | +         | +          |
| Glycoside                 | +         | +          |
| Cardiac glycoside         | ++        | +          |
| Protein                   | +         | +          |
| Aminoacids                | +         | +          |
| Steroid and phytosteroid  | +         | +          |
| Acid                      | ++        | ++         |
| Saponin                   | ++        | +          |
| Gums and mucilages        | +         | +          |

(+) Present, (-) Absent.
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SUMMARY AND CONCLUSION

Marine algae are the richest source of a number of novel bioactive compounds. Red algae that are used for the extraction of agar are called agarophytes. There are many species of agarophytes but *Gracilaria edulis* and *Gelidiella acerosa* are the two most commercially important high-quality-agar yielding and easily cultivable species. The qualitative phytochemical screening and elemental analysis showed that these two agarophytes are rich in nutrients such as proteins, carbohydrates, other essential nutrients, micro and macro elements. These agarophytes are used as a biostimulant to enhance the crop production without the use of chemical fertilizers.
The agar extracted from agarophytes is tested to promote plant growth and water holding capacity in soil under water stress. The water holding capacity of the agar in soil was good which retained the moisture but the agar gel contracted over time which suggests the need to apply agar periodically. This natural polysaccharide can be engineered in future to absorb and hold significant amounts of water suitable to be applicable in horticulture and agriculture. Environmental factors also play an important role in the growth of plants which have to be optimized. The comparative study on the use of agarophytes revealed that G. edulis and its agar can be best utilized as a biostimulant in promoting plant growth under water stress and also can be used to grow A. aritis indoors.

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