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Phytochemical Screening of *Sargassum sp* and *in Vitro* Seed Germination Test

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ABSTRACT

The aim of the present paper is to evaluate the phytochemical screening of *Sargassum sp* and also to investigate the effect of its crude extracts on seed germination and seedling development of *Capsicum annum*. The samples were dried and made to coarse powder and stored at -20°C for further analysis. Crude extracts were prepared using three different solvents (DCM, methanol and hexane). Different concentrations of crude extracts were prepared in solid medium using MS basal media and germination tests were carried out *in vitro*. High concentration (50 uL/mL) of hexane extract exhibited the most promoting effect on seed germination and seedling development of *Capsicum annum* followed by methanol (50 uL/mL) and DCM (50 uL/mL). The presence of micro and macro nutrients, vitamins, growth hormones and other constituents in the seaweed extract might be very much useful to the crop but their concentration should be appropriate to enhance growth and productivity. It may be concluded that *Sargassum* extracts could serve as cost effective and eco-friendly product for sustainable agriculture.

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1. INTRODUCTION

Seaweeds are marine macro algae, which form an important component of the marine living resources of the world. They are available largely in shallow coastal waters of sea, estuaries and backwaters (Erulan *et al.*, 2009). Brown seaweeds are the second most abundant group comprising about 2,000 species which reach their maximum biomass levels on the rocky shores of the temperate zones. Seaweeds, mainly brown

species, have long been used as soil fertilizers because they have several advantageous effects of spraying their crude extracts on plant growth (Chandía and Matsuhiro, 2008; Abdel-Mawgoud *et al.,* 2010). Improved seed germination, higher yields and increased resistance to diseases upon treatment in various crops have been reported (Mercier *et al*., 2001). Among them, *Fucus* spp., *Padina* spp., *Laminaria* spp., *Sargassum* spp., and Turbinaria spp. are used as biofertilizers in agriculture (Hong *et al.,* 2007). The use of seaweed in farming and agriculture has a long history. Even though seaweed has been researched for over 50 years now, the exact mechanism through which it exerts its positive influence is still not fully known. Initially the thought was that the benefits derived from seaweed use were mainly caused by the trace elements (Van Hoffen, 2013). Thus, this present study tries to investigate the effect of seaweed (*Sargassum sp*) extract on in vitro seed germination and seedling development of *Capsicum annum*.

Based on previous study (Fatimah *et al.*, 2018), this present study also was aimed to do some qualitative phytochemical screening Analysis of *Sargassum sp.* Sargassum species has been chosen in this research because of an adequate amount of potassium, nitrogen, growth promoting important chemicals, such as hormones, micronutrients, humic acids, and so on present in this species of seaweeds that has potential as excellent biofertilizers. Unlike chemical fertilizer, biofertilizers derived from seaweeds or other organisms are usually biodegradable, non-toxic, non-polluting and non-hazardous to human, animals and birds (Dhargalkar and Pereira, 2005), which will benefit agriculture sector.

2. MATERIALS AND METHODS

2.1. Sampling Collection

Seaweed (*Sargassum* species) was collected from Port Dickson, Negeri Sembilan within 20 - 200 meters of seashore. Sampling of seaweed was carried out at littoral and sub-littoral zones during early months where the low seawater level provided easier condition to collect the samples. The collected seaweed will be cleaned with seawater to remove sand and epiphytes. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in an ice box containing slush ice and transported to the laboratory. The samples were washed throughly using tap water to remove the salt from the surface of the sample.

All samples collected will be stored at the laboratory in Universiti Pendidikan Sultan Idris for further investigation .

2.2. Preparation of Seaweed Liquid Extracts

The seaweed were dried for a week in a drying oven with controlled temperature (50.0 ± 0.5°C). Then the sample were prepared as coarse powder with a mixer grinder. Then the extraction process was carried out using methanol, Dichloromethane (DCM) and hexane under reflux for three day for each sample. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent. The extracts were concentrated and freed of solvent under reduced pressure at 45°C using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan). The dried crude concentrated extracts were stored in a refrigerator (- 4°C) until used for analyses.

2.3. Qualitative Phytochemical Screening Analysis of Seaweed

Preliminary phytochemical analysis of the *Sargassum sp* was conducted for tannin, alkaloids, flavonoids, saponins, and terpenoids were made by following standard procedures. This step is important to be done, and the result influence the total product be obtained.

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2.4. Dilution of Seaweed Extract

1 g/mL from each crude extract of *Sargasum sp* from three different solvent (methanol, DCM, and hexane) was diluted to make five different concentrations (10, 25, 50, and 100 uL) for eachs solvent. Each of them was tested on seed germination.

2.5. Experiment on Seed Germination

Chilli or *Capsicum annum* seeds with uniform shape, size, color and weight were selected to be tested with a different concentration of seaweed extracts in Murashige and Skoog (1962) . The seeds were surface sterilize in a microfuge tube with Tween 20 for 10 minutes .After that the seeds were soaked in a series of gradual descending concentration of sodium hypochlorite (100, 70, 50, and 25%) for about 3 minutes in each solution. Lastly the seeds were rinsed three times with sterile distilled water before placed onto the solid MS media for seed germination *in vitro* test. The seeds were incubated up to 30 days in a controlled room. The rate of germination was determined in 14 days after inoculation. For seedling development several measurement were taken including hypocotyle height, root length, and number of leaves.

3. RESULTS

The qualitative phytochemical screening results of Sargassum sp were shown in **Table 1.** Hexane extracts showed positive results for all phytochemical analyses. DCM extracts showed the absence of alkaloids while methanol extracts showed the absence of flavonoids and tannin.

Sargassum crude extracts were tested on seed germination and seedling development of Capsicum annum. The results in **Figure 1** showed that the length of chilli roots when inoculated in different crude extracts at different concentration. The highest root length result was found in 50 uL/mL crude extracts from hexane solvent. However, the root length reduced in a higher concentration (100 uL/mL). Similar results were found for the length of chilli hypocotyl. At 25 uL/mL crude extracts from hexane solvent induced the highest length of stem compared with other crude extracts (**Figures 2 and 3**).

Table 1: Qualitative phytochemical screening analysis of *Sargassum sp* extracted with Different Solvent

Phytochemicals	Methanol Extracts	Hexane Extracts	DCM Extracts
Alkaloids Mayer's test	White creamy precipitate (positive result)	White creamy precipitate (positive result)	Black green (original colour) (negative result)
Flavonoids H2SO4 test	Yellow (original) (negative result)	From yellow to colourless (positive results)	From yellow to colourless (positive results)
Terpenoids Salkowski test	Reddish brown colouration (positive result)	Reddish brown colouration (positive result)	Reddish brown colouration (positive result)
Saponin (Forming emul-sion)	Forming emulsion when add olive oil	Forming emulsion when add olive oil	Forming emulsion when add olive oil
Tannin Ferric chloride test	Yellow (negative result)	Greenish precipitate (positive result)	Greenish precipitate (positive result)

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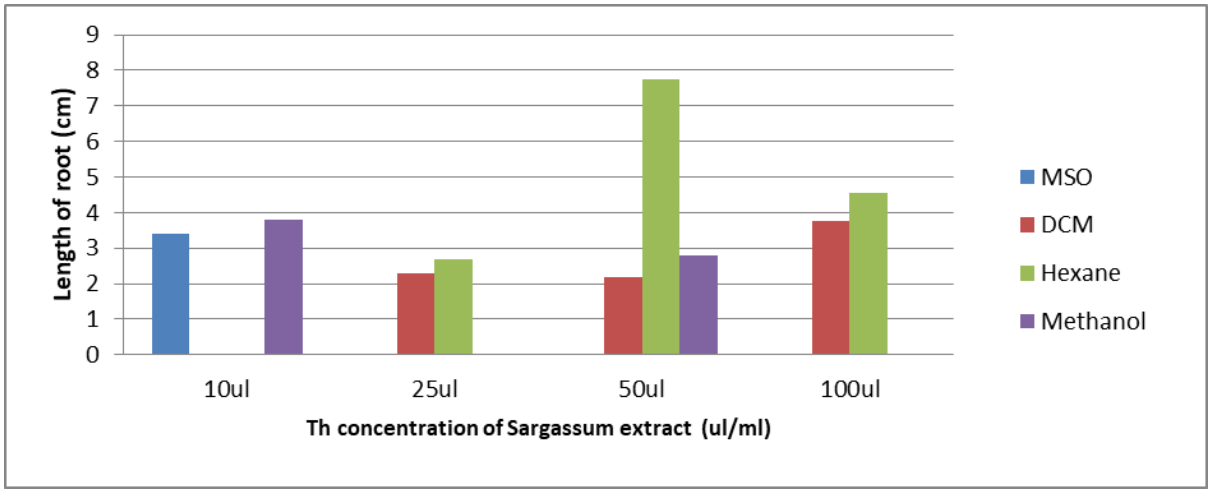

Figure 1. The comparison of root length of *Capsicum annum* when grown in different crude extracts of *Sargassum sp*.

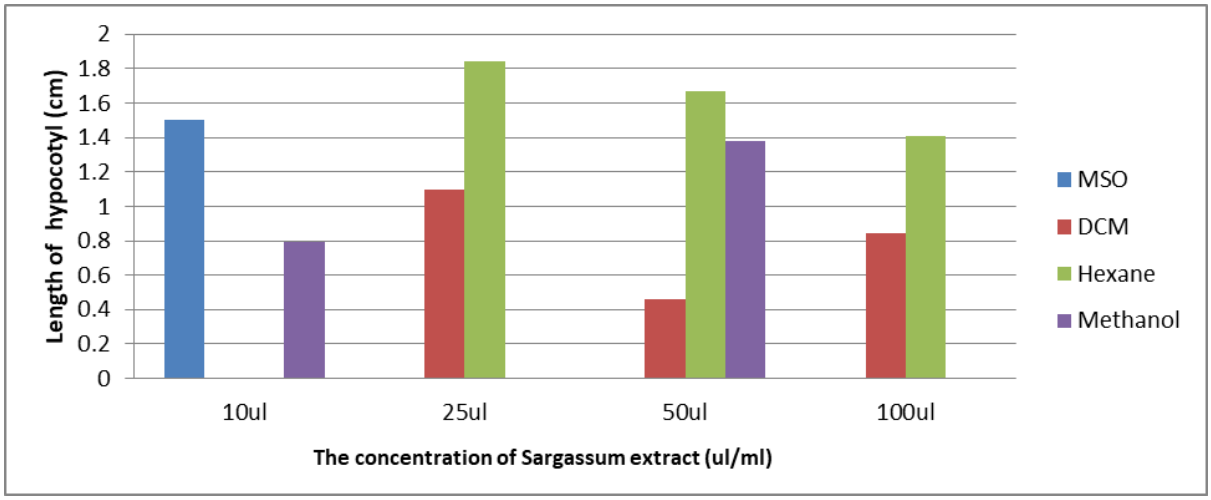

Figure 2. The comparison of hypocotyl length of *Capsicum annum* when grown in different crude extracts of *Sargassum sp.*

4. DISCUSSION

The present study showed the phytochemical screening of sargassum sp. They were mostly extracted from hexane (**Table 1**). This marine algae is rich in secondary metabolites such as alkaloids, flavonoides, saponins, tannins, and terpenoids which have been extensively used in the preparation of drugs and in medicinal industry (Kuda *et al.*, 2005). A high percentage chemical compound identified in hexane crude extract is usually chemically or biologically active (Hossain *et al.*, 2013). The important characteristics of seaweeds are basically synthesizing of important compounds. Seaweeds rarely show any photodynamic damage during metabolism, as they have efficient protective and oxidative mechanisms. These important characteristics also help in protecting commercially important plants from extreme sun heat when seaweed extract have been used as fertilizer (Matsukawa *et al.*, 1997).

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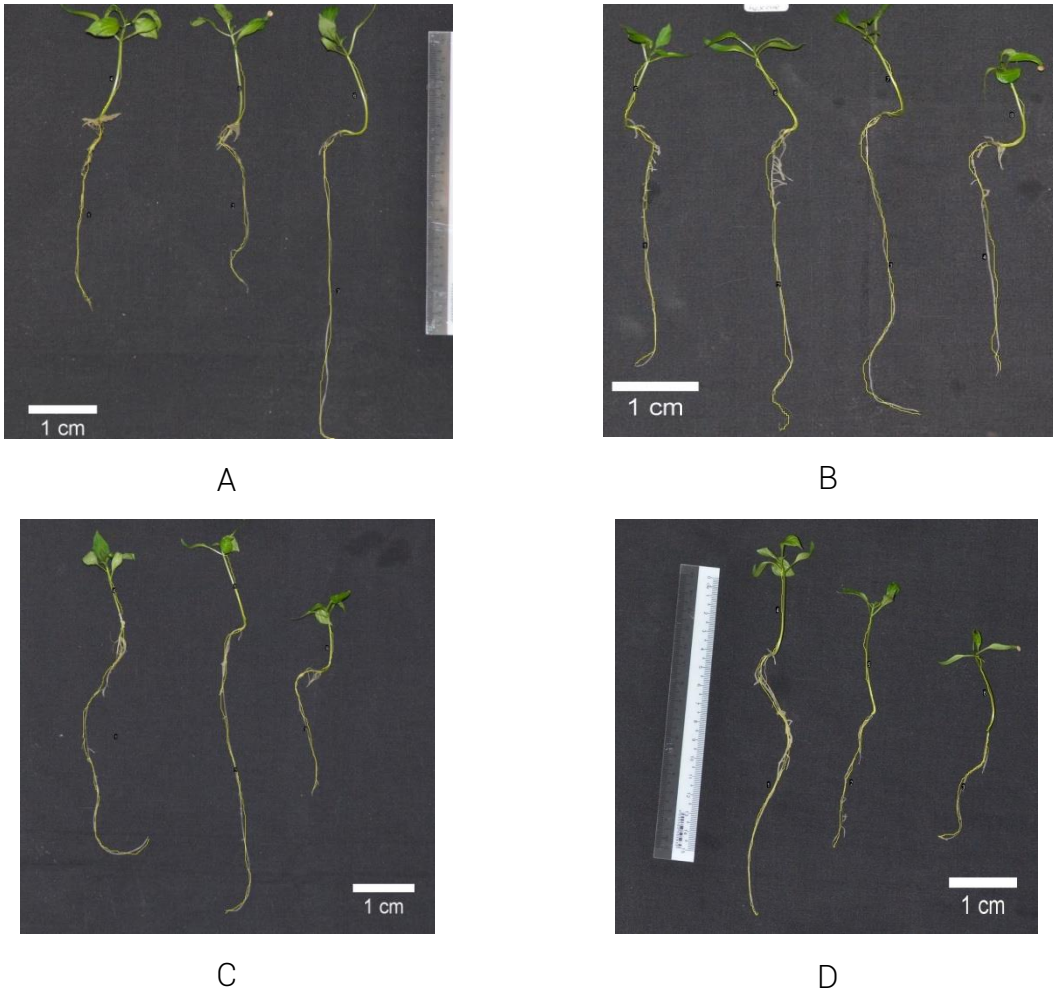

Figure 3. Diagram A (MSO as control) , Diagram B (50 uL/mL of *Sargassum* hexane extract), Diagram C (50 uL/mL of *Sargassum* methanol extract). Diagram D (50 uL/mL of *Sargassum* DCM extract)

The potential of Sargassum extracts in enhancing growth of chilli seedling has been explored in this present study. The beneficial effects of *Sargassum sp* extract obtained on growth of *Capsicum annum* have shown interesting results (see **Figures 1 and 2**) and proven some findings from previous research. The application of Sargassum wightii liquid extracts has increased seed germination percentage and growth of Triticum aestivum (Kumar and Sahoo, 2011). In other research by Sridhar and Rengasamy (2012), the aqueous extracts of S.wightii were found to promote the Capsicum annum growth and yield at 1% in concentration. In our study, the most potential result in promoting hypocotyl and root growth was observed in hexane extracts of *Sargassum*. In the phytochemical screenings, hexane extracts of *Sargassum sp.* contain alkaloids, flavonoids, saponins, tannins and terpenoids (**Table 1**), which no doubt could give better results in promoting the hypocotyl and root length. This situation may be due to the good amount of phosphorus in the seaweed extract which promote growth. Hernández-Herrera *et al.* (2014) found phosphorous in seaweed extracts facilitates root proliferation of *Solanum lycopersicum L*. However, seedling growth may also be influenced by hormones such as auxin and cytokinins as further analyzed by Sridhar and Rengasamy (2012), which showed that the material contained high amount of auxins

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and cytokinins. In conclusion, the effects of Sargassum extracts on seedling growth and root development indicates the presence of phytoconstituents that are important to meet the growth requirement. In the present study hexane extracts was found to be more effective than other extracts in promoting the seedling growth and root development of *Capsicum annum*.

5. CONCLUSION

These early findings are beneficial for further research in fully utilize the abundant resources of seaweed as organic fertilizer to replace the commercial chemical fertilizer. It may be concluded that *Sargassum* extracts could serve as cost effective and eco-friendly product for sustainable agriculture.

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6. AUTHORS' NOTE

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article. Authors confirmed that the data and the paper are free of plagiarism.

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