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Macroalgae Phytochemical Profile and Antioxidant Screening using the Thin Layer Chromatography

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ABSTRACT

Thin-layer chromatography, TLC is a method for screening phytochemical components in a sample that allows all chemicals and samples on a plate to be recognized at the same time. The goal of this study was to determine the chemical composition fingerprint of *Sargassium* spp., *Turburinaria* spp., *Gracilaria salicomia, Undaria pinnatifida, Hydrilla verticillate, Limnophila heterophylla, Ceratophylllum demersum, Utricularia aurea,* and *Vallisneria americana,* as well as the antioxidant response to DPPH on the TLC plate. The chemical components of macroalgae were separated on TLC plates by using a mobile phase containing toluene, acetonitrile, ethyl acetate, and glacial acetic acid in a ratio of 35: 5: 15: 0.15, and oxidative reaction on TLC was calculated using integrated density ImageJ. The ROI intensity of the DPPH reaction in *U. pinnatifida* was 140404.7, 133925.0, 596163.9 at R_f 0.4, 0.3, and 0.2, respectively; it was 162003.25 at R_f 0.3 in *V. americana,* and it was 693004.5, 120965.34 at R_f 0.4 and 0.3 in *L. heterophylla*. The antioxidant activity of green algae would be high in intensity, and it would be particularly effective in the *U. pinnatifida* fingerprint. The results showed that the TLC approach may be used to screen compounds and their responses to DPPH in macroalgae to determine early antioxidant activity.

Keywords: TLC, antioxidant, brown algae, green algae, red algae, secondary metabolite

INTRODUCTION

Algae have grown in prominence as a result of their unique qualities in a variety of ways. An alga is a collection of marine producers that function as a support system for other marine species and as a source of biomass in the marine food chain [1]. Algae can be found in a range of sizes and colors, and their abundance can be dispersed according to the environment [2]. Algae can develop without a root, stem, or leaves and can grow in a wide range of ecosystems, from the deep sea to oceans with a depth of less than 100 cm, in swampy areas, freshwater areas, and estuaries [3]. A vast variety of species distribution depended on the geographical feature of their habitats [4]. The procaryotic Cryptophyta, also known as blue-green algae, as well as the eukaryotic algae, often known as macroalgae, are categorized into three divisions: Rhodophyta (red), Phaeophyta (brown), and Chlorophyta (green) based on pigment content [5]. Macroalgae had been employed in the diets and traditional healers of Oriental and Southeast Asian communities for generations [6]. Macroalgae is also a rich dietary staple since it provides significant elements, vitamins, protein, iodine, and polyunsaturated fatty acids, and even some considerable quantities of soluble and insoluble dietary fibers, which is low in calories [7]. Macroalgae, in addition to being a primary metabolite food source, has been found to contain a variety of secondary metabolites. Sulfated galactans like agars, carrageenans, and porphyrans can be obtained from red algae, whereas ulvans

can be gotten from green algae. Alginates and other sulfated polysaccharides like ascophyllan, laminaran, and fucoidan can be obtained from brown fucoidan [8]. Algal extracts produce secondary metabolites with bioactivities that have been commercially used throughout the medical, pharmaceutical, agricultural, food, and cosmeceutical industries. Antimicrobial, antioxidant, anti-inflammatory, antiproliferative and anti-angiogenesis, and anticoagulant properties are some of the bioactive properties in algae evaluated from pre-clinical through clinical studies. Antioxidant activity in brown and red algae have been widely acclaimed for their ability to scavenge free radicals [9,10]. The antioxidant potential of seven sargassum species, *Ulva lactuca* L., and *Undaria pinnatifida* was investigated utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) to assess scavenging activity and a spectroscopic-based assay to evaluate total phenolic content (TPC) [11].

As the season progresses, algae will begin to bloom on the coastlines of the Gulf of Thailand, and residents of Nakhon Si Thammarat (NST) will gather and use the algae in a variety of ways, including food processing and feed. Screening for phytochemical profiles and examining the bioactivity of algae using scientific techniques may provide useful information to those who want to employ algae in their products [12]. Thin-layer chromatography (TLC) is a technique that is currently widely utilized in phytochemical fingerprint screening from plants and other creatures [13]. TLC, a technique for screening the phytochemical profile of multiple samples at once [14], has been approved and is currently used as a standard modern research technique for metabolite screening in plants and living organisms. There is a need for and value in a research effort that leads to a more in-depth understanding of the chemical constituents inside and how they behave, as well as the development of a bioactivity assay on a TLC plate. The goal of this study was to use the TLC technique to screen phytochemicals of macroalgae from the Gulf of Thailand in Nakhon Si Thammarat (NST) province for antioxidant capabilities as preliminary information for developing and extending into a commercial product [15].

MATERIALS AND METHODS

Sample collection and sample extraction

Algae samples were collected at the coast of the Gulf of Thailand during April-December, 2020. Samples were completely dry at 50°C in a hot oven for 48 h and ground into powdery. Three hundred milligrams of the algae sample were extracted in 10 ml methanol at 70°C with 200 rpm shaking for 2 h, the supernatant of the extracts was transferred into the new extracting tube and vacuum dried using the SyncorePlus (BUCHI). The extract was resuspended in 2 ml methanol and transferred into a 2 ml microtube, centrifuged at 10,000 rpm at room temperature for 5 min to discard the pellet, the clear supernatant of the extract was then transferred into a new 2-ml microtube. The extracts are kept at -20°C for the next step.

Thin-Layer Chromatography (TLC)

Ten microlitres of each extract were spotted on 10 x 20 cm TLC Silica gel 60 F_{254} Plate (Merck) using the Semi-automatic sample dispenser, CAMAG® Linomat 5 (CAMAG). The plate was then developed in a saturated TLC developing tank containing toluene, acetonitrile, ethyl acetate, and glacial acetic acid in a ratio of 35: 5: 15: 0.15 until the solvent front reached 80 mm. The generated TLC plate was air-dried to eliminate the solvent's capillary force and analyzed at 254 and 366 nm UV-Transilluminator and documentation (Documentation system with CCD camera TLC VISUALIZER 2, CAMAG).

Antioxidant activity test on TLC

On a TLC plate, the antioxidant activity of the algae's chemical fingerprint was examined by spraying 0.5 percent 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol and incubating at ambient temperature for 15 minutes without light. The area of the DPPH reaction to the constituent on the TLC plate was calculated using ImageJ's integrated density sums of all pixels.

Statistical analysis

The intensity was presented as the region of interest (ROI) intensity graphs were created using the statistical software Prism 9® (Graphpad, CA, USA), which includes a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was defined as a probability level of less than 0.05.

RESULTS

Sample collection

The algae were collected in the Gulf of Thailand, and the latitude and longitude were plotted on a Google map (Figure 1). Seasonal abundance caused species to spontaneously disperse. *Turbinaria* spp. and *Gracilaria salicomia* were collected from Koh Tamet in April and November, respectively. *Sargassum* spp. were collected from the coastal areas of Huasai and Pak Panang in November. In May, *Utricularia aurea, Vallisneria americana,* and *Ceratophylllum demersum* were discovered in the Klay canal, Tha Sala district; from May to December, *Limnophila heterophylla* and *Hydrilla verticillate* were discovered in the Thadee Irrigation canal. Assist. Prof. Dr. Supaporn Sutin gave the *Undaria pinnatifida*. In terms of habitat distribution, brown algae such as *Turbinaria* spp., *G. salicomia,* and *Sargassum* spp. were identified on the shoreline. Red algae such as *U. aurea, V. spiralis,* and *C. demersum* were widespread in a canal with high water flow, whereas green algae such as *L. heterophylla* and *H. verticillata* were found in a canal with low water flow.

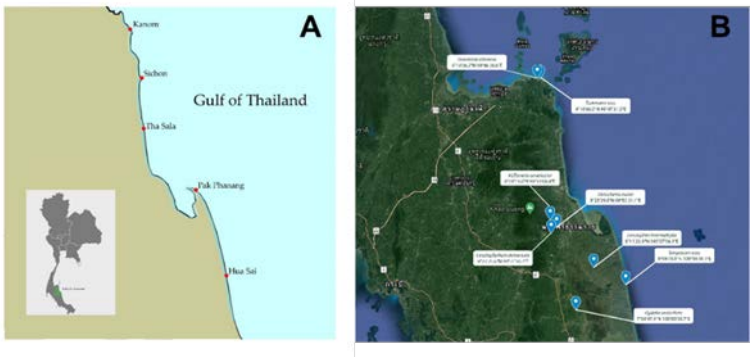

Figure 1. The sample collection sites of Nakhon Si Thammarat province, in the Gulf of Thailand (A). The distribution of macroalgae around Nakhon Si Thammarat (B).

The phytochemical fingerprint of algae on a TLC plate

Methanolic extracts of *Sargassum* spp., *U. pinnatifida*, *H. verticillate*, *Turbinaria* spp., *L. heterophylla, G. salicomia, C. demersum, U. aurea,* and *V. americana* were separated on a TLC plate employing a mobile phase solution of toluene: acetonitrile: ethyl acetate: glacial acetic acid at an each algal species' identifying chemical profile was supplied to the fingerprint, which was visible at both 254 and 366 nm (Figure 2A, 2B). On the TLC sheet, fingerprints of all nine algae species were identified at R_f 0.6, capable of absorbing light at 254 and 366 nm but with varying band intensity. It denotes the proportion of uneven substances. The light absorption fingerprints of

brown algae in Tracks 1, 4, 6, green algae in Tracks 2, 3, 5, 7, and red algae in Tracks 8 and 9 are comparable (Figure 2B).

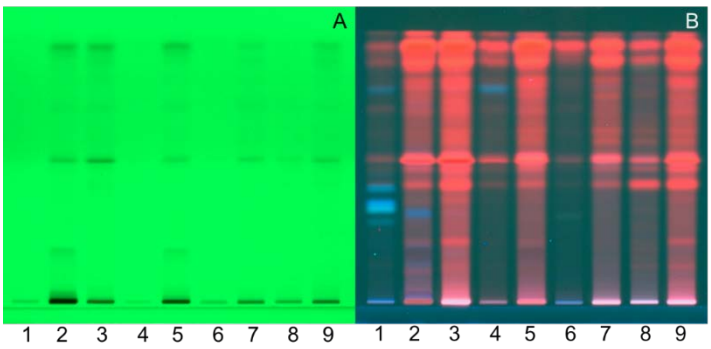

Figure 2. A phytochemical fingerprint of macroalgae; A: under 254 nm, B: under 366 nm. Track 1-9 represented the fingerprint of the seaweed extract, track 1: *Sargassum* spp., track 2: *U. pinnatifida*, track 3: *H. verticillate*, track 4: *Turbinaria* spp., track 5: *L. heterophylla*, track 6: *G. salicomia*, track 7: *C. demersum*, track 8: *U. aurea*, and track 9: *V. americana*.

Antioxidant activity

The three species of algae that responded with DPPH on TLC plates were *U. pinnatifida* (Track 2, Figure 3A), *L. heterophylla* (Track 5, Figure 3A), and *V. americana* (Track 9, Figure 3A). The ROI of the *U. pinnatifida* green algae at R_f 0.2, 0.3, and 0.4 (track 2, Figure 3A) had the highest intensity as 596163.9, 133925.0, and 140404.7, followed by the ROI of the *L. heterophylla* green algae at R_f 0.3 and 0.4 as 69300 and 12096 and the ROI of the *V. americana* red algae at R_f 0.3 as 16200. In addition, the ROI of the DPPH reaction region was not found in any of the other six species. DPPH did not affect any of the three brown algae species.

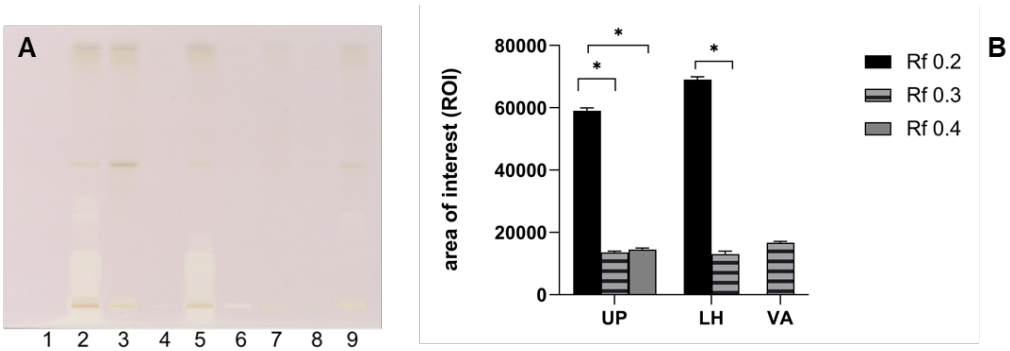

Figure 3. The ROI of the macroalgae phytochemical reaction to DPPH on a TLC plate (A) was illustrated by graph (B). UP is a *U. pinnatifida* green algae (Track 2), LH is an *L. heterophylla* green algae (Track 5) and VA is a *V. americana* red algae (Track 9). The star on the bar graph represents the statistical significant at *p-value* < 0.05.

DISCUSSION

The phytochemical patterns and absorption intensities are associated with each algal group's pigment production [16]. In antioxidant screening using TLC plates, the brown macroalgae group showed no DPPH reaction, indicating that the species discovered in previous investigations is not the same as the one studied here [17]. The region's sensitivity to DPPH could imply that algae are high in antioxidants. Based on the results of this experiment, it's unclear whether the response was

caused by algae's high antioxidant content. However, it was likely caused by algae's antioxidant-rich chemicals. By detecting a reaction to DPPH, a TLC screen can show whether or not a compound possesses antioxidant activity. This method can be utilized to assess antioxidant properties in various TLC discs in a quick format.

CONCLUSION

Researchers used TLC to screen for macroalgae phytochemical components and measure DPPH reactivity to the chemicals, which appeared as fingerprints on TLC plates, and the results could be interpreted from the experiment. It's simple to use and provides basic data for further investigation, such as antioxidant testing using spectroscopy.

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