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Antioxidant and Photoprotective Activity of Brown Seaweed from North Sulawesi Coast

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Abstract : Marine ecosystems have a high diversity of living organisms compared to terrestrial ecosystems and provide abundant resources for human nutrition and health. The aims of this research were to determine total phenolic content, examine antioxidant and photoprotective activity, and characterize brown seaweed *Sargassum* sp, *Turbinaria* sp, and *Padina* sp from North Sulawesi coast component. The total phenolic content was determined using folin ciocalteau method, antioxidant activity was evaluated using total antioxidant, photoprotective was evaluated using Sun Protector Factor (SPF), and characterization of brown seaweed extract using UV-vis and IR Spectrophotometry. The results showed that total phenolic content from *Sargassum* sp 5,20 mg GAE/g, *Turbinaria* sp 1,47 GAE/g, and *Padina* sp 27,45 GAE/g. Total antioxidant of *Sargassum* sp 0,496 mg AAE/g, *Turbinaria* sp mg AAE/g, and *Padina* sp 0,295 mg AAE/g. The SPF value of *Padina* sp extracted with hexane 20.530, ethyl acetate 28.505, butanol 11.040, ethanol 13.705, and acetone 3.403. Characterisation of brown seaweed extract using spectrophotometer UV-vis consists of phenolic compound, carotenoid, and chlorophyll. FTIR spectra of brown seaweed extract showed that *Sargassum* sp contain (C-halide), (N-O), (-C=O), (N-H), (C-H) and (OH-) groups, *Turbinaria* sp (C-halide), (C=C), (N-H), (C-H) and (OH) groups, and *Padina* sp (C-halide), (C=C), (N-H), (C-H) and (OH-) groups. Brown seaweed possesses antioxidant and photoprotective activity.
Keywords : brown seaweed, total phenolic content, antioxidant, Sun Protector Factor, North Sulawesi.

Introduction

Marine ecosystems have a high diversity of living organisms compared to terrestrial ecosystems and provide abundant resources for human nutrition and health[1]. Marine invertebrates are a diverse group with habitats in all ocean ecosystems, ranging from the intertidal zone to the deep sea environment. Marine invertebrates can be classified into several major phyla, namely, Porifera (sponges), Cnidaria (corals, sea

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anemones, hydrozoans, jellyfish), Annelida (Polychaetes, marine worms), Bryozoa (moss animals or sea mats), Mollusca (oysters, abalone, clams, mussels, squid, cuttlefish, octopuses), Arthropoda (lobsters, crabs, shrimps, prawns, crayfish), and Echinodermata (sea stars, sea cucumbers, sea urchins)[2]. This diverse group also includes macroalgae, microalgae, bacteria, cyanobacteria, certain fish species and crustaceans that produce secondary metabolites as an adaptation to their hostile marine environment.

Seaweed (macroalgae) is one of marine organism. It called thallus because seaweeds are primitive non-flowering plants without root, stem, and leaves[3]. About 6000 species of seaweeds have been identified and are grouped into three major groups based on it pigment namely Chlorophyceae (green algae), Rhodophyceae (red algae) and Phaeophyceae (brown algae)[4]. The three classes of seaweed were significantly important because of economic value and chemical composition[5].

In general, *Sargassum* sp, *Turbinaria* sp, and *Padina* sp are brown seaweeds that grow in shallow waters of tropical regions. They are harmless, very easy to collect, and a potential source of phytomedicines. These organisms are largely exposed to a combination of sunlight and oxygen that leads to the formation of free radicals. However, the absence of oxidative damage on the structural components of seaweeds and their stability to oxidation during storage indicate that their cells should have potent protective antioxidative defense systems[6].

Tropical seaweeds are expected to develop a very effective antioxidant defense system due to the strong UV radiation in the tropical environment[7,8]. Antioxidant compounds play an important role against various diseases (e.g., atherosclerosis, chronic inflammation, cardiovascular disorders, and cancer) and aging processes[9]. In fact, previous studies have demonstrated that UV radiation induces the promotion of antioxidant defense in macroalgae[10,11].

Recently, the potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.). Those compounds are widely distributed in plants or seaweeds and are known to exhibit higher antioxidant activities. Seaweeds are noted to contain not only labile antioxidants (i.e. ascorbate, glutathione) when fresh[12], but also, more stable molecules such as carotenoids[13], mycosporine-like amino acids[14], and a variety of polyphenols (e.g. catechins, phlorotannins)[15]. More reports that are recently revealed seaweeds to be a rich source of antioxidant compounds[16-19].

The brown seaweeds contain a lot assemblage of species that predominate in the coastal shelf areas of Manado, North Sulawesi. Among various seaweeds, *Sargassum* sp, *Turbinaria* sp, and *Padina* sp are abundantly available in this area throughout different season, and therefor these species has been short listed for the present study. Pharmacological properties of several seaweed species are still unexplored and unidentified. The present study is focused to analyze the in vitro antioxidant and photo-protective activities of three selected seaweeds from North Sulawesi coast.

Experimental

Standard and Chemicals

Gallic acid and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). The chemicals were acetone, butanol, ethyl acetate, ethanol, hexane, sodium carbonate, sulfuric acid, sodium phosphate, ammonium molybdate, folin-Ciocalteu reagent were pro analysis grade and purchased from Merck (Darmstadt, Germany).

Sample collection and preparation

Live and healthy samples of edible brown seaweed *Sargassum* sp, *Turbinaria* sp and *Padina* sp were collected between June-July 2017 from the intertidal region of North Sulawesi coast (latitude 1°28’55”N and longitude 124°50’56”E). Collected samples were immediately brought to the laboratory in new plastic bags containing natural sea water to prevent evaporation and some of seaweed samples were identified by the Department of Fisheries and Marine Science, University of Sam Ratulangi, Manado. Plants were washed

thoroughly with tap water to remove extraneous materials and shade dried. Dry plant material was ground in an electric mixer and stored at 4°C until future use.

Extraction

Fine powder of dry seaweed (20 g) was extracted with 60 mL of ethyl acetate at room temperature for 2 h. The extraction procedure was repeated thrice and the extract was filtered through Whatmann No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator (crude extract). The ethyl acetate phase (crude) was stored at 4°C until future use.

Determination of Total Phenolic content

The total phenolic content was determined using Jeong et al.[20] method. The seaweed extract (0.1 mL) was diluted with deionized water (7.9 mL). Folin-Ciocalteu phenol reagent (0.5 mL) was added, and the contents were mixed thoroughly. After 3 minutes, 2 mL of 20% sodium carbonate solution was added, and the mixture was mixed thoroughly. The mixture was allowed to stand for 30 minutes. After incubation at 37°C, the absorbance of the blue color produced was measured at 750 nm, using the Shimadzu 1800 UV VIS Spectrophotometer. Phenolic content was expressed in milligrams per gram of dry weight (seaweed extract) based on a standard curve of gallic acid (GA), which was expressed as milligrams per gram of gallic acid equivalent (GAE).

Determination of total antioxidant

Total antioxidant activities of the all the extracts were determined according to the method of Prieto et al.[21]. Briefly, 0.3 mL of sample was mixed with 3.0 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min under water bath. The absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid. A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid and was expressed as mg ascorbic acid equivalents per gram of sample on a dry weight (DW) basis.

Determination of Sun protection factor (SPF)

Determination of photoprotective activity was conducted by examining SPF in vitro value using spectrophotometer (Spectrophotometer UV-Vis Shimadzu 1800)[22,23]. Brown seaweed was extracted with ethanol, acetone, butanol, ethyl acetate, and hexane. Each extract was made into concentration 1 µg/mL in ethanol. The absorbance of extract solution in 1 cm cuvette was read using Shimadzu 1800 UV VIS spectrophotometer at λ 290-320 nm with 5 nm interval. The absorbance of the solution shows the effect of substance which absorbs or reflect UV light in solution. Mansur et al.[22] develop a simple mathematical equation to calculate SPF value. SPF = CF x I (λ) x absorbance Note: CF: Correction factor (10), EE: erythemal efficiency, λ: wavelength, I: sunlight spectrum simulation and Abs: sunscreen product absorbance.

Characterization

Brown seaweed *Sargassum* sp, *Turbinaria* sp, and *Padina* sp were characterized using Ultraviolet and Visible (UV-Vis) Spectrophotometry and Fourier Transform-Infrared (FT-IR). Firstly, the UV-Visible spectra of brown seaweed extracts were recorded on a Shimadzu 1800 UV VIS spectrophotometer equipped with 1.0 cm quartz cells. The width of excitation slits was set to 1.0 nm. The spectra collected with subsequent scanning spectra from 200 to 800 nm at 1.0 nm increments. Secondly, spectra of brown seaweed extracts were determined using an FTIR spectrophotometer (Shimadzu, Japan.) with KBr pellets in the range 4000–400 cm^{-1}.

Statistical analysis

All experiments were performed in triplicates. Experimental data presented as mean values ± standard deviations and analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range test using SPSS version 23.

Result and Discussions

Total Phenolic Content

The total phenolic content of brown seaweed was determined using Folin-Ciocalteu reagent. The mechanism is based on the reduction of phosphomolibdate-phosphotungstate complex in Folin-Ciocalteu reagent by phenolic compound in an extract. The complex is then turned into molybdenum, which can be identified qualitatively by the formation of blue color and thus detectable with a spectrophotometer at 750 nm.

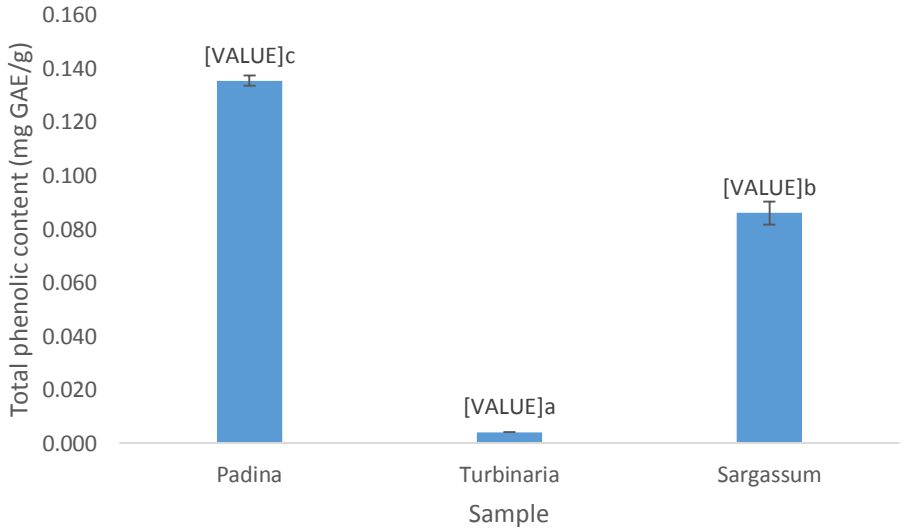

Fig. 1: Total Phenolic Content. Data are expressed as mean of triplicates with standard deviation. Values with the same superscript letter are not statistically significant at the 5% level

According to Huang et al.[24], all phenolic substances including simple phenols can react with Folin-Ciocalteu reagent. Figure 1 shows the total phenolic content of *Sargassum* sp, *Turbinaria* sp, and*Padina* sp. The highest total phenolic was found in *Padina* Sp 0,135 mg GAE/g followed with *Sargassum* sp 0,084 mg GAE/g, and the lowest *Turbinaria* sp 0,004 mg GAE/g. Statistical analysis revealed that total phenolic of the three species of brown seaweed was significantly different ($p<0,05$).

Phenolic compound generally easy to be extracted with semi polar and polar organic solvent[25]. Brown seaweed possesses high total phenolic content compared with red seaweed[8]. Polyphenolic content in seaweed varies depent on the season, harvest time, geographical location, and seaweed species[26].

Phenolic compounds exhibit their antioxidant activity by several mechanisms such as donating hydrogen atoms to free radicals, scavenging other reactive species such as $OH^{\bullet}$, $NO_2^{\bullet}$, N_2O_3, ONOOH, and HOCl. Some phenolic, mostly di and polyphenolic, can react with $O2^{\bullet-}$ or by binding transition metal ions (especially iron and copper), often resulting in forms poorly active in promoting free radical reactions and hence can also interfere with the uptake of metals from the diet[27,28].

Total Antioxidant

The total antioxidant is a better way of depicting of the combined effect of phenolic, flavonoids, and other reduction compounds in a plant extracts and expressed in terms of ascorbic acid equivalents (AAE). The Phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the action of antioxidant compounds and the formation of green phosphate-Mo(V) complex with a maximal absorption at 695 nm. Figure 3 shows total antioxidant of *Sargassum* sp, *Turbinaria* sp and*Padina* sp.

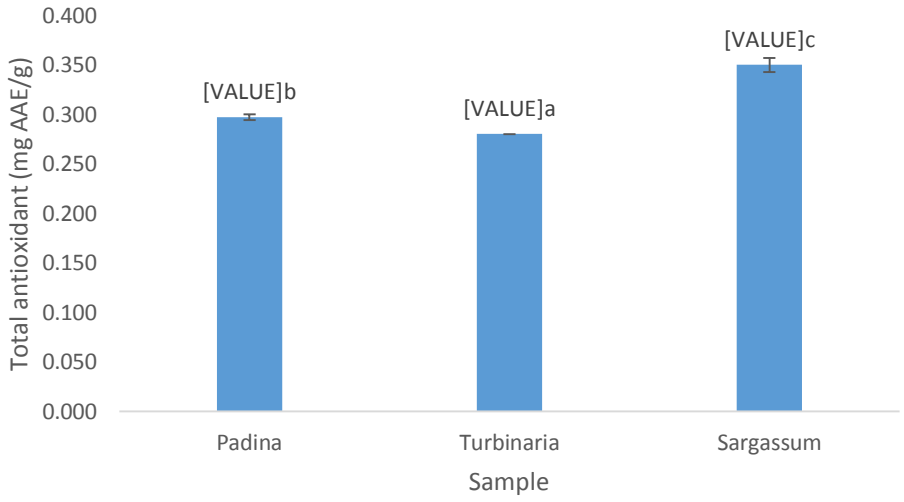

Fig. 2: Total antioxidant of brown seaweed. Data are expressed as mean of triplicates with standard deviation. Values with the same superscript letter are not statistically significant at the 5% level

The highest total antioxidant was found in *Sargassum* sp 0,3050 mg AAE/g followed by *Padina* sp 0,297 AAE/g, and the lowest *Turbinaria* sp 0,280 AAE/g. Based on statistic analysis, total antioxidant between the three seaweeds was different significant ($p<0,05$). Total antioxidant of sample was related with it electron donor ability. The total antioxidant capacity value follows the same order as that of phenolic content in the extracts respectively.

Boi et al.[29] reported that *Sargassum serratum* possess antioxidant activity. Antioxidant activities of *Sargassum serratum* was depended on the extracting conditions. Brown algae *Sargassum serratum* has high antioxidant phlorotannin content. Other findings reported that free radical scavenging activity of *Sargassum* extracts was affected by extracts types, seasons, sites and species. The tropical macroalgae developed an effective antioxidant defense system which might reflect an adaptation to high solar radiation[30]. This screening emphasized the great antioxidant potential, i.e., free-radical, superoxide anion radical scavenging, reducing activity and inhibition of lipid peroxidation[7].

Setha et al.[31] stated that *Padina* sp extract exhibit antioxidant activity against free radicals. The antioxidant activity of methanol crude extract of seaweed *Padina* sp to give IC_{50} value of 200.88 mg/L, crude extract of ethyl acetate to give IC_{50} value of 483.09 mg/L and extract n-hexane gave IC_{50} value of 900.00 mg/L. *Padina tetrastomatica* also show radical scavenging and singlet oxygen quenching activity[32].

Furthermore, Preethi et al.[33] have evaluated the methanolic extract of *Turbinaria ornata* for anti - oxidant activity. The result shows the presence of antioxidants and concluded the presence of antioxidant activity in *Turbinaria ornate*. The same result also founded by Parthiban et al.[34]. Acetone and ethanolic extract of *Turbinaria ornata* for antioxidant activity result show the presence of antioxidants and concluded the presence of antioxidant property.

Sun Protector Factor (SPF)

The SPF value is used as the world standard for sunscreen effectivity. *Padina* sp extract was used for SPF evaluation because of it high total phenolic content. SPF value of brown seaweed extract is presented in figure 3. Ethyl acetate extract exhibit the highest SPF value followed with hexane, ethanol, butanol, and acetone. The SPF value were 28.505; 20.530; 13.705; 11.040; 3.403, respectively. Based on statistic analysis, SPF value between the five Padina sp. extracts was different significant ($p<0,05$).

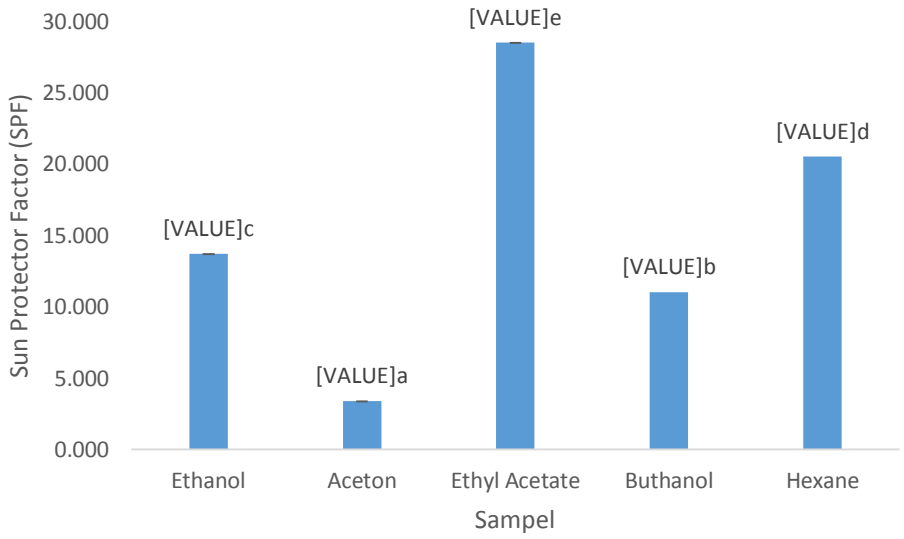

Fig. 3: Sun Protector Factor of brown seaweed extract. Data are expressed as mean of triplicates with standard deviation. Values with the same superscript letter are not statistically significant at the 5% level

Bioactive component such as phenol hydroquinone, flavonoids, and triterpenoids expected to have photo-protective activity[35]. Based on data above, SPF from extract with different solvent exhibit different SPF characteristic. Saewan and Jimtaisong[36] state that flavonoid which found in seaweed can act as ultraviolet protector. Phenolic compound especially flavonoid classes consist of chromophore groups that can absorb ultraviolet light so it can reduce the damage of skin[37]. Steinberg[38] associated the quantity of photoprotective compounds of brown seaweed to the specific area in which the seaweed was collected. For example, tropical algal species tend to use their phenolic as photoprotectors, while those in temperate areas (e.g., kelp species) synthesize secondary metabolites as a defense against herbivores.

Among brown algae, several secondary metabolites might be used for photoprotective purposes, such as phenolic compounds[39], carotenoids[40,41], and mycosporine-like amino acids (MAAs). The synthesis of photoprotective compounds in brown algae may be regulated by extrinsic factors such as UV radiation and PAR. MAA synthesis is rarely reported in brown macroalgae, and is most commonly studied in red macroalgae[42,43]. Phenolic compounds[44,45] and carotenoids[46] are known to be the most abundant compounds in brown macroalgae and their syntheses have been directly related to solar radiation levels. In addition to their role in photoprotection, these compounds have shown high antioxidant activity[47-49] and antitumoral activities[50-52].

Characterization

Ultraviolet Visible Spectrum

The species of brown seaweeds showed similar absorption profiles for the taxonomic group with the detection of ten main regions of UV–Vis absorbances. Considering the UV spectral ranges and putative absorption substances, four bands were selected and designated as Band A (200-260 nm), Band B (265-300 nm), Band C (305–347 nm), and Band D (358–377 nm). In the case of Photosynthetically Active Radiation (PAR) spectra, six main bands were also considered as follows: Band E (400–425 nm), Band F (440–455 nm), Band G (490-520 nm), Band H (530-545 nm), Band I (580–640 nm), and Band J (650–670 nm).

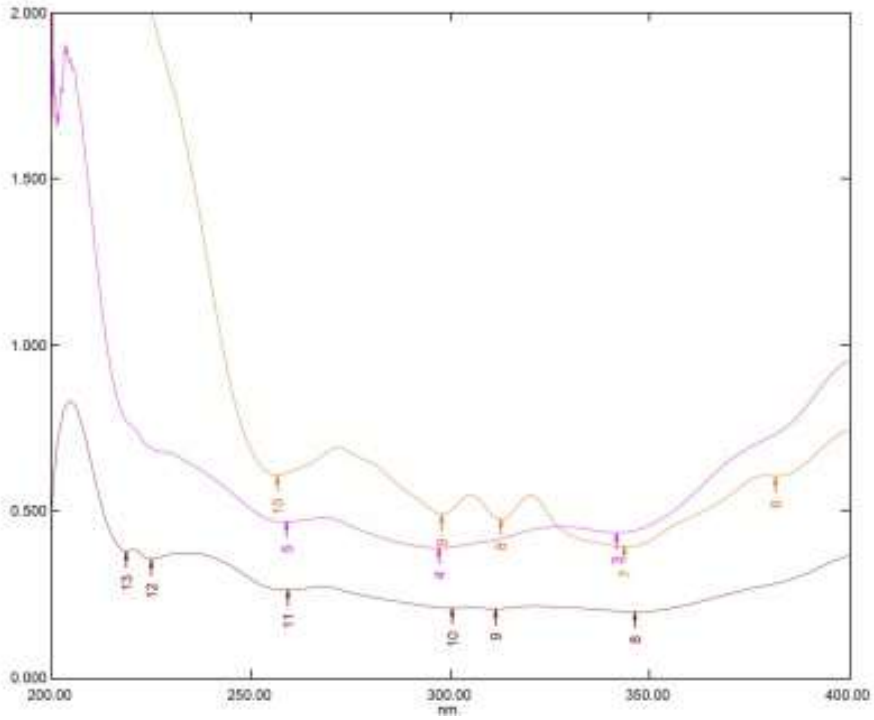

Fig. 4: Ultraviolet Radiation spectrum from brown seaweed extracts

Figure 4 showed the absorption spectra of brown seaweed extracts measured in ethanol at wavelength regions of 200-400 nm for phenolic component and 400-600 nm for carotenoid and chlorophyll presented by Figure 5. These results indicate that *Sargassum* sp, *Turbinaria* sp, and *Padina* sp extract contain phytochemicals such as phenolic, carotenoid (fucoxanthin) and chlorophyll which potential as antioxidant active component.

Slovehenko and Merlzlyak[53] considered that phenolic compounds-phlorotannins absorbance spectrum showed two bands, one being located in the range between 240 and 280 nm, which covers Band A and Bof brown seaweed spectra. In other seaweed groups, the wavelength absorption range of Band C is characterized by absorption due to mycosporine-like amino acids[54]. However, these compounds have not been recognized as abundant in brown seaweed[43,55]. Band D (358–377 nm) is characterized in macro seaweed by the presence of coumarins[56], another family of phenolic compounds. In brown seaweed, the main phenolic compounds recognized as photoprotective agents against UV radiation are phlorotannins[44,57].

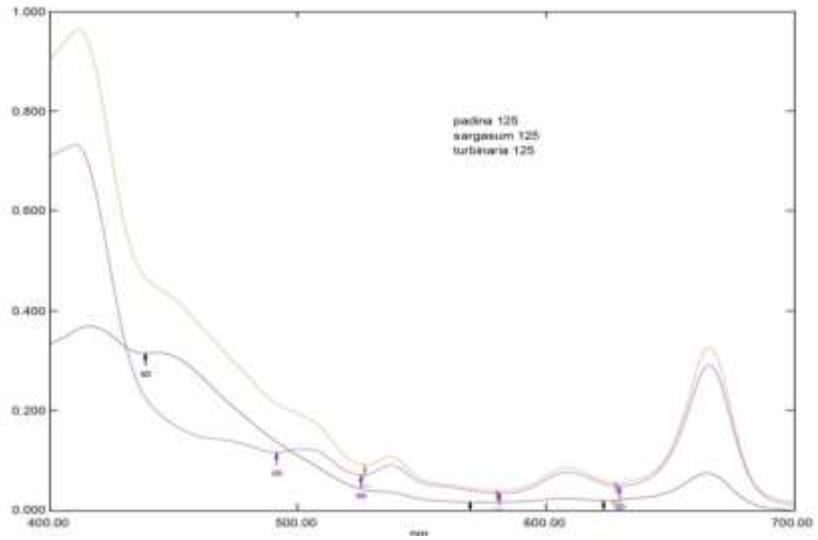

Fig. 5: Photosynthetically active radiation spectrum from brown seaweed extracts

The second area (400–700 nm) with a predominant and evident peak group was separated in Bands E, F, G, H, I, and J. This absorption section may be characterized by a consortium of the blue absorbance peak of chlorophylls and carotenoids. Carotenoid (fucoxanthin) in the sample can be evaluated by it peak absorbance at 420 nm. Ethyl acetate extract of *Sargassum* sp, *Padina* sp, and *Turbinaria* sp contains fucoxanthin. The highest fucoxanthin content is in *Padina* sp, followed with *Sargassum* sp and the lowest is *Turbinaria* sp. Beside carotenoid (fucoxanthin) seaweeds extract contains chlorophyll which exhibits maximum absorbance at 672 nm. *Padina* sp shows the highest chlorophyll, followed with *Sargassum* sp and the lowest *Turbinaria* sp.

Infra Red Spectrum

Firstly, the FT-IR spectral analysis of *Sargassum* sp extract revealed that the spectral range of obtained functional group ranged between 400 and 4000 cm^{-1} shown in Fig. 6. From the results, it was observed that the peaks 378,05 cm^{-1} may be due to C-halide bond. Followed by peak signals recorded in 925.83 cm^{-1} may be due to nitrogen grouping (N-O). A peak at 1712.79 cm^{-1} may be due to the presence of carbon, oxygen (-C=O). A sharp peak observed in 2360.87 cm^{-1} represents the possible presence of nitrogen, hydrogen atoms (N-H) bond. The vibration stretch recorded at 2854.65 and 2924.09 cm^{-1} represents the presence of (C-H) bond. Finally, a broad band at 3363.86 cm^{-1} may be phenol hydroxyl group (OH-).

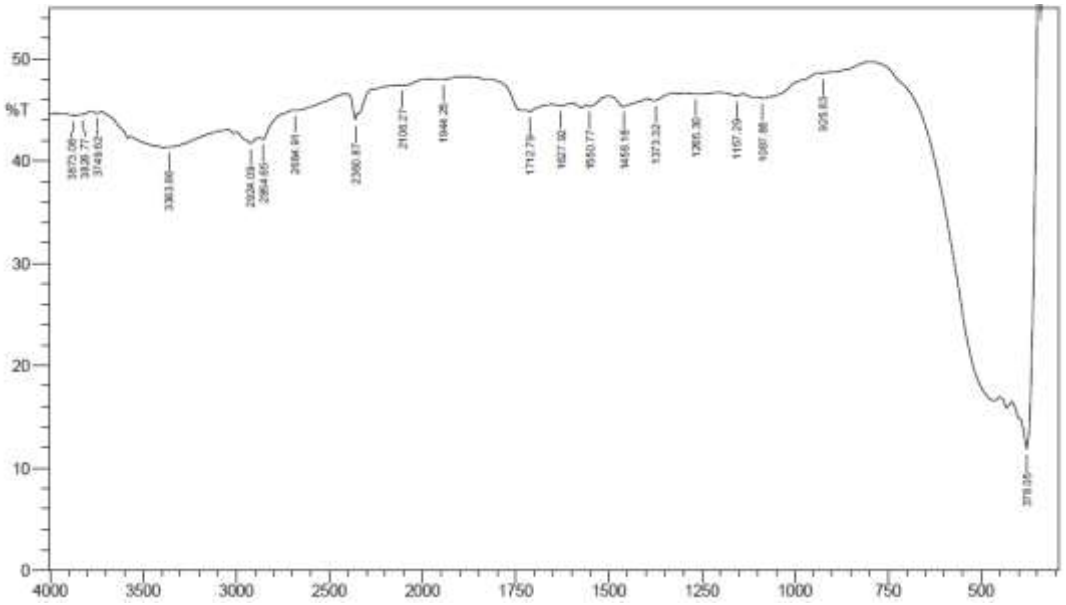

Fig. 6: IR spectrum of *Sargassum* sp extract

Moubayed et al.[58] analyze chemical constituents of *Sargassum* sp cells using FTIR Spectroscopy. High O–H absorption ranging between 3400 cm^{-1} and 3300 cm^{-1} which related to the main chemical groups phenols namely hydroxyl amide I, amide II and primary amine groups present on the cell walls. FTIR Chemical analysis revealed that the major constituents particularly in *Sargassum* sp were phenolic nature[59,60] to which both potent antimicrobial and antioxidant activities are associated.

Secondly, The *Padina* sp extract FT-IR spectral analysis is shown in Fig. 7. It was observed that the peaks 376.05, 432.05 and 470.63 cm^{-1} may be due to C-halide bond. The peak 1242.16 to 2237.50 cm^{-1} may possible presence of carbon (C=C) double bond. The sharp peak observed in 2360,67 cm^{-1} represents the possible presence of nitrogen, hydrogen atoms (N-H) bond. The vibration stretch recorded at 2854.65 and 2924.09 cm^{-1} represents the presence of (C-H) bond. Finally, a broad band at 3371.57 cm^{-1} may be phenol hydroxyl group (OH-).

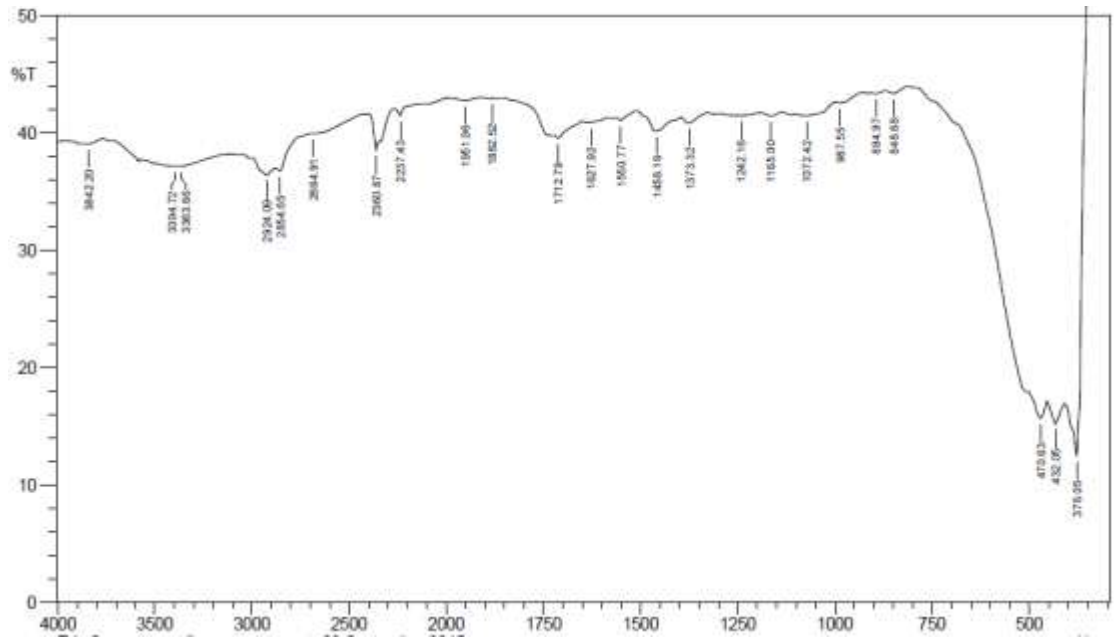

Fig. 7: IR spectrum of *Padina* sp extract

Padina australis shows absorbance at 3462.20 cm^{-1} that represent of hydroxyl (OH-) group, and 2923.33 cm^{-1} and 2852.43 cm^{-1} represent of alkane (C-H) group[61]. It also shows absorbance at 1242.16 to 2237.50 cm^{-1} which indicated the presence of carbon (C=C) double bond. Furthermore, absorbance at 1737.74 cm^{-1} that represent of (C=O) group and 1031 cm^{-1} that represent of symmetric C-O-C group was found in *Padina australis* extract.

The last, FT-IR spectral analysis of *Turbinaria* sp extract revealed that the spectral range of obtained functional group ranged between 400 and 4000 cm^{-1} shown in Fig. 8. From the results, it was observed that the peaks 339.47 cm^{-1} may be due to C-halide bond. A sharp peak at 1627.92 cm^{-1} represents of carbon, carbon (C=C) bond. The sharp peak observed in 2360.87 cm^{-1} represents the possible presence of nitrogen, hydrogen atoms (N-H) bond. The vibration stretch recorded at 2854.65 and 2924.09 cm^{-1} represents the presence of (C-H) bond. Finally, a broad band at 3394.72 cm^{-1} may be phenol hydroxyl group (OH-).

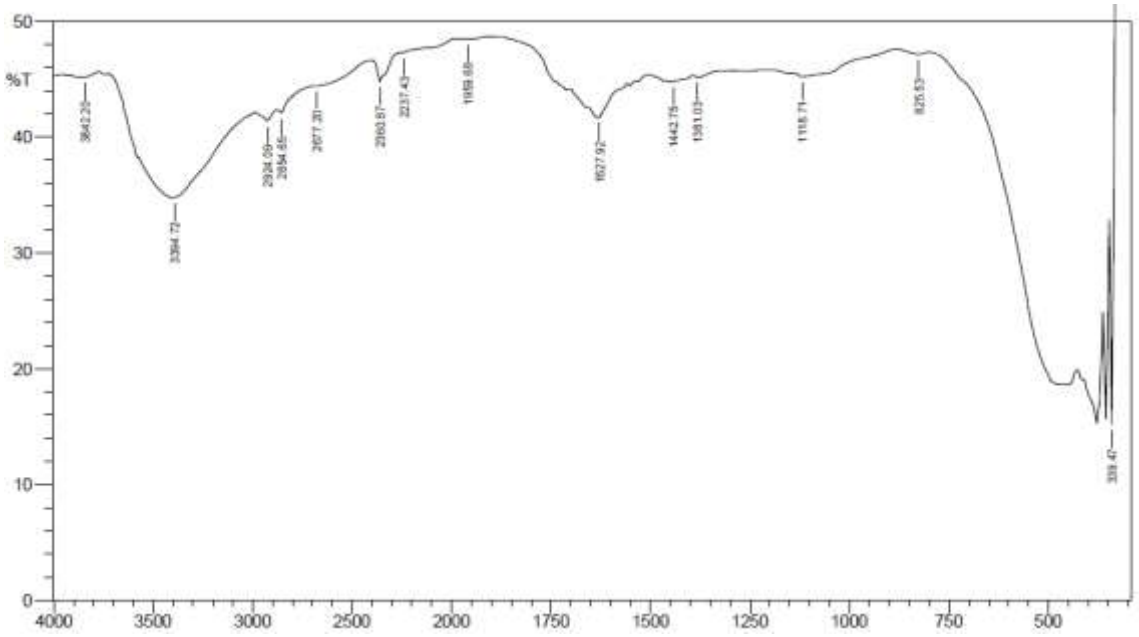

Fig. 8: IR spectrum of *Turbinaria* sp extract

Hemalatha et al.[62] analysis revealed that the spectral range of obtained functional group from *T. ornata* ranged between 400 and 4000 cm^{-1}. It contains C-Br bond, nitrogen grouping (N-O), carbon, oxygen (C-O) bond, carbon (C=C) double bond, nitrogen, hydrogen atoms (N-H) bond, (C-H) bond, and phenol hydroxyl group (OH-). *T. ornate* Fraction contains phenolic (phlorotannins), based on the UV-Vis and FT-IR spectral analysis[62]. Phenolic compounds could elevate the value of seaweeds as functional ingredients in pharmaceuticals or functional foods.

Conclusion

Total phenolic content of *Sargassum* sp 5,20 mg GAE/g, *Padina* sp 27,45 GAE/g and *Turbinaria* sp 1,47 GAE/g. Total antioxidant of *Sargassum* sp 0,496 mg AAE/g, *Padina* sp 0,295 mg AAE/g, and *Turbinaria* sp mg AAE/g, and. SPF value of *Padina* extract was ethyl acetate followed with hexane, ethanol, butanol and acetone. The SPF value were 28.505; 20.530; 13.705; 11.040; 3.403, respectively. Brown seaweed extract consists of phenolic compound, carotenoid and chlorophyll using spectrophotometer UV-vis. FTIR spectra of brown seaweed extract show *Sargassum* sp contain (C-halide), (N-O), (-C=O), (N-H), (C-H) and (OH-) groups, *Padina* sp (C-halide), (C=C), (N-H), (C-H) and (OH-) groups, and *Turbinaria* sp (C-halide), (C=C), (N-H), (C-H) and (OH) groups.

Conflict of interest statement

We declare that we have no conflict of interest.

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