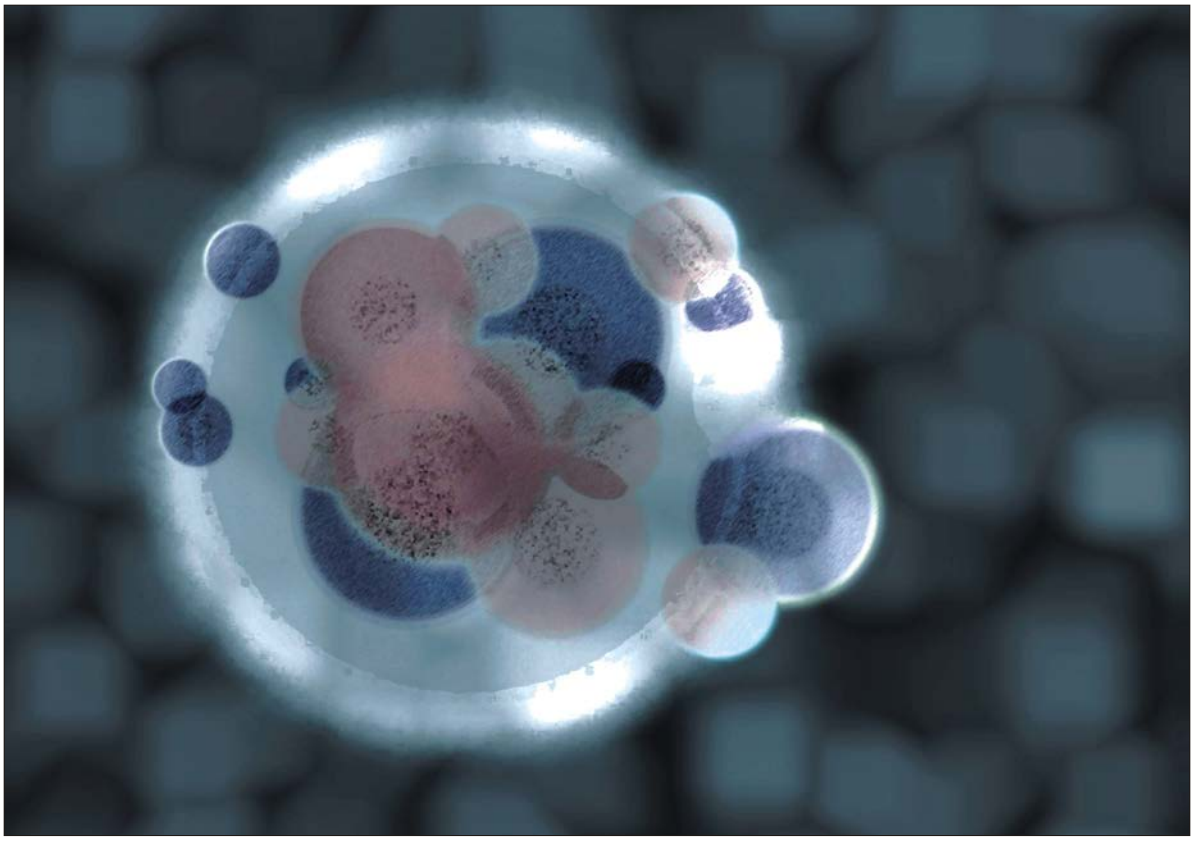

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Mini Review
Thalassia hemprichii Seagrass Extract as Antimicrobial and Antioxidant Potential on Human: A Mini Review of the Benefits of Seagrass

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Abstract

Seagrass is a flowering plant (*Angiospermae*) which can adapt itself to survive in the seawater. *Thalassia hemprichii* is one type of seagrass that grows in Indonesian water areas. Seagrass is often found in the water areas of Lampung, Bali, Kepulauan Seribu, Manado and Wakatobi. *Thalassia hemprichii* is not used only to maintain the sea ecosystem, especially the shallow sea, but also to be processed as medicines and cosmetics. *Thalassia hemprichii* can be used as medicines because it contains secondary metabolite compound which has the potential to be an antimicrobial that fights against agents of pathogenic diseases on human. As the basic material of cosmetics, *Thalassia hemprichii* contains high antioxidants which can counteract free radicals. It is an added values that *Thalassia hemprichii* has to be the basic material for cosmetic products. Besides those some functions, *Thalassia hemprichii* extract has the potential as antifungal, antiviral, antifertility, anticancer and antidiabetes. In this review, the authors summarize recent study focused on the biological effects *Thalassia hemprichii* seagrass extract as antimicrobial and antioxidant.

Key words: Seagrass, extract, *Thalassia hemprichii*, antimicrobial, antioxidant

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INTRODUCTION

Indonesia, a country in Southeast Asia has a diverse flora that potential as herbal medicines. Herbal medicines are known to have anti-inflammatory, antimicrobial and antioxidant potential. Herbal medicines are known to have various potential health benefits such as antifungal, antiviral, antifertility, anti-inflammatory, anti-cancer, anti-diabetes, antimicrobial and antioxidants. Currently, according to the literature, many researchers are focused on finding plant derived medicines[1-3] because medicinal plants had been reported to be safe and without side effect[4]. Many herbal medicines can be used for the treatment of several diseases and have been studied in the pathomechanism of these herbal medicines in infectious diseases, metabolic and degeneration both *in vivo* on human and animal experimental such as *Curcuma xanthorrhiza*, *Syzygium aromaticum*[5], red fruit (*Pandanus conoideus*)[6,7], *Curcuma longa*[8-10], Miana leaves (*Coleus scutellariodes* (L) Benth)[11,12], *Musa paradisiaca* L. (MPL) fruit[13], *Plectranthus scutellarioides* (L.) R.Br. (ELEP)[14] andaliman fruit (*Zanthoxylum acanthopodium* DC)[15]. Other medicinal plants found in Indonesia are seagrass which have the potential for antimicrobial and antioxidant.

Seagrass is water plant flowering and has adaptability to life and grow in the marine environment[16]. Seagrass is water plant flowering and has adaptability to life and grow in the marine environment that have economic and health benefits[16]. Seagrass beds occur in shallow coastal areas around the world[17]. The diversity of marine habitats in Indonesia is among the highest in the world and Indonesian seagrass diversity is comparable to other countries in the region. Indonesian seagrasses either form dense monospecific meadows or mixed stands of up to eight species. *Thalassia hemprichii*, *Enhalus acoroides*, *Halophila ovalis*, *Halodule uninervis*, *Cymodocea serrulata* and *Thalassodendron ciliatum* usually grow in monospecific beds and muddy substrates on the seaward edges of mangroves often have meadows of high biomass[18].

There are many varieties of seagrass in Indonesia, 15 of 52 varieties of seagrass that has been found in the world until now are found in Indonesia. They consist of 7 genera: *Enhalus*, *Halophila*, *Thalassia hemprichi*, *Cymodocea*, *Halodule*, *Syringodium* and *Thalassodendron*[19,20]. *Thalassia hemprichii* are the common seagrass that can be found in the water area of Indonesia. It can be found in South Sulawesi[21], Kuta and Gerupuk Bays, Lombok Island[22], Ambon, Maluku[23], Alor, Bali, Banggai, Bangka, Belitung, Derawan, Halmahera, Karimunjawa, Luwuk, Pulau Bira, Pulau Pari, S. Nasik, Tual and Wakatobi[24]. Monospecific beds of *Thalassia hemprichii* are the most widespread throughout Indonesia and occur over a large vertical range from the intertidal zone down to the lower subtidal zone[18].

Therefore, the aim of this review is to provide an overview of the effects of *Thalassia hemprichii* seagrass extract as antimicrobial and antioxidant on human. For this purpose, electronic databases including Scopus, Pubmed and Google Scholar were searched for *Thalassia hemprichii* seagrass and it was examined by *in vitro*, *in vivo*, or clinical trials to determine the effects of *Thalassia hemprichii* as antimicrobial and antioxidant.

The vegetation of *Thalassia hemprichii* seagrass is the area of food hunting, breeding and habitat of several plants and animals[25]. Anthropogenic activities in coastal zones such as construction on the coastline, tourism, near-shore shing and aquatic activities can have long-lasting negative effects on *Thalassia hemprichii* seagrass beds and coastal ecosystems[26,27]. *Thalassia hemprichii* has secondary metabolite mechanism which is used to counteract predator attack and to survive in the environment[28]. Many secondary metabolite compounds have been used as medicines or models to make new medicines. Some of them are antimicrobial and antioxidant.

Seagrass is a commodity that has been widely used by community both traditionally and modern. Traditionally, seagrass has been utilized, among others, for making basket, burned to get the salt, soda or warmers, for filling mattresses, as thatched roofs, for compost and fertilizer, used for sound insulation and temperature, can in lieu of yarn in making nitrocellulose and so on. While modern use is as waste filters, beach stabilizers, materials for paper, fertilizer and fodder, as well as medicinal ingredients[16].

Seagrasses are also known to have the potential to be used as pharmaceutical raw materials. Kannan *et al*.[29] explained that *Thalassia hemprichii* has bioactive potential as antioxidants and contain compounds phenolic group. *Thalassia hemprichii* collected from Pamban, Tamil Honey, India is known to contain potential bioactive compounds as antibacterial, antifungal, anti protozoa, antiviral, antifertility and ingredients drugs that affect cardiovascular system[30]. The seagrass[20] is shown on Fig. 1.

ANTIMICROBIAL POTENTIAL OF *THALASSIA HEMPRICHII* SEAGRASS

Seagrasses produce antimicrobial/antibacterial compounds to reduce or control the microbial growth[31,32]. The secondary metabolite that is produced by many organisms

Fig. 1: *Thalassia hemprichii*
Source: Waycott *et al.*[20]

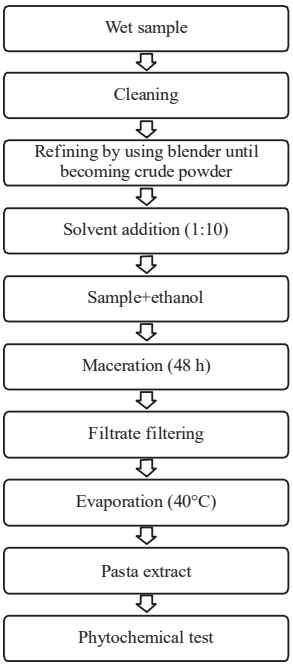

Fig. 2: Flow chart of making the extract of *Thalassia hemprichii*
Source: Dewi *et al.*[36]

and it is known to have many biological activities that can be utilized by human being. Some of the biological activities of the secondary metabolite are anticancer, antibacterial, antioxidant and antifungi[30,33,34]. The technique to isolate the secondary metabolite compound of a particular natural material is known as extraction. Extraction is one of substance separation processes that are needed from a plant material[35].

The method of extraction relies on the solubility of the compound to be extracted in the solvent used[35]. The success of the extraction process is also influenced by some factors so that to need to carefully choose the method of extraction used to extract the secondary metabolite compound (Fig. 2)[36].

The extraction of *Thalassia hemprichii* which is continued by doing phytochemical test shows that *Thalassia hemprichii* has some bioactive compounds which can become anticancer, antibacterial and antifungal substances[37]. Some research shows the advantage of *Thalassia hemprichii* extract as antibacterial. The test on *P. aeruginosa*, *S. epidermidis* and *M. luteus* shows that the highest inhibitory zone is at a concentration of 200 µg/disk in the ethyl acetate extract (Table 1)[38].

The result of bioassay test shows that the extract and fraction of *Thalassia hemprichii* has the potential of *S. aureus* (gram-positive) antibacterial while on *E. coli* bacteria (gram-negative), it does not have inhibitory power (Table 2)[39].

Other research shows that phytochemical test done shows the extracts of *Enhalus acoroides* and *Thalassia hemprichii* contain bioactive compounds of the

Table 1: Qualitative test from fresh seagrass samples

	Seagrass species			
Bacteria	*C. racemose*	*E. cottonii*	*G. verrucosa*	*T. hemprichii*
P. aeruginosa	-	-	+	+
S. epidermidis	+	+	+	+
M. luteus	-	+	-	+

Source: Siregar *et al.*[38], -: Absent, +: Present

Table 2: Diameter of inhibitory zone of some antibacterial concentrations on the tested bacteria

	Inhibitory zone diameter (%)		
Tested bacteria	5	10	15
S. aureus	2.76±0.31	4.74±0.30	6.13±0.39
E. coli	0.73±0.09	2.02±0.25	3.08±0.29

Source: Rahmawati *et al.*[39]

types of flavonoid, alkaloid and steroid. The toxicity test with BSLT method administered shows that the methanol extract of *Thalassia hemprichii* is very toxic with the LC_{50} value of 5.74 ppm. Meanwhile, the n-hexane extract of *Enhalus acoroides* is not toxic with the LC_{50} value of 1309.42 ppm (Table 3)[40].

Thalassia hemprichii seagrass is a great pool of action bacteria resource[41]. *Thalassia hemprichii* has ability to produce antimicrobial metabolites against human pathogens. Besides it, the research also proves that *Thalassia hemprichii* has a good inhibitory power against pathogenic bacteria with the inhibitory concentration minimum of less than 10 µg mL^{-1}. Extracts at 4-fold concentrations can destroy target cells

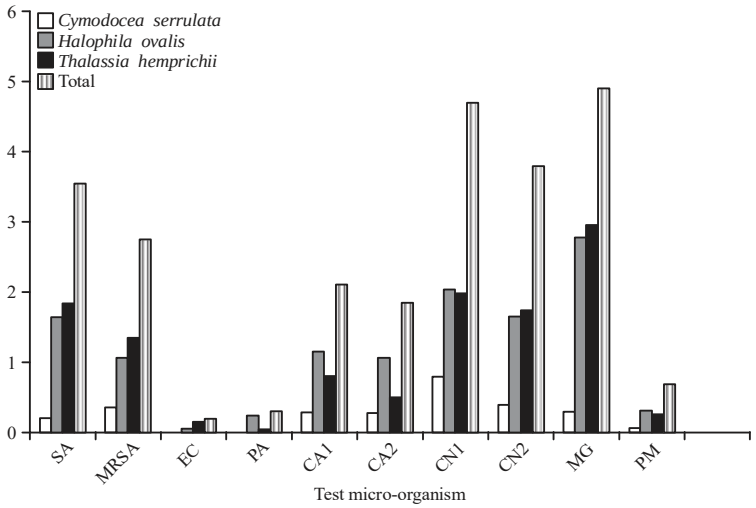

Fig. 3: Antimicrobial activity of endophytic fungal crude extracts against each test microorganism
Source: Supaphon *et al.*[42], SA: *Staphylococcus aureus* ATCC 25923, MRSA: Methicillin-resistant *S. aureus*, EC: *Escherichia coli* ATCC 25922, PA: *Pseudomonas aeruginosa* ATCC 27853, CA1: *Candida albicans* ATCC 90028, CA2: *C. albicans* NCPF 3153, CN1: *Cryptococcus neoformans* ATCC 90112 (flucytosine-sensitive), CN2: *C. neoformans* ATCC 90113 (flucytosine-resistant), MG: *Microsporum gypseum* clinical isolate, PM: *Penicillium marneffei* clinical isolate

Table 3: Minimum inhibitory concentration (MIC) of *Thalassia hemprichii* and *Enhalus acoroides*

	Minimum inhibitory concentration (μg mL^{-1})		
Sample	*E. coli*	*S. aureus*	*B. subtilis*
Ethanol extract of *T. hemprichii*	500.00	125.00	500.0
Ethanol extract of *E. acoroides*	250.00	62.50	250.0
Ethyl acetate extract of *T. hemprichii*	125.00	250.00	125.0
Ethyl acetate extract of *E. acoroides*	31.25	31.25	62.5
N-hexane extract of *T. hemprichii*	62.50	62.50	125.0
N-hexane extract of *E. acoroides*	31.25	15.62	250.0

Source: Purnama and Brahmana[40]

Table 4: Result of phytochemical test of *Thalassia hemprichii* with different methanol concentration (mg g^{-1})

	Methanol concentration (%)		
Parameters	70	80	90
Flavonoid	0.51±0.006	0.71±0.01	0.97±0.006
Alkaloid	7.26±0.03	7.83±0.02	8.42±0.02
Tannin	4.47±0.02	5.14±0.09	5.67±0.05
Phenol	3.13±0.01	3.53±0.01	4.09±0.06

observed using an electron microscope (Fig. 3)[42] and the result of phytochemical test of *T. hemprichii* with different methanol concentration (mg g^{-1}) (Table 4).

Thalassia hemprichii showed the highest antibacterial activity against both strain of *V. harveyi* with same inhibition zones of 11.50±1.29 mm[43]. This is in accordance with the report by Premanathan *et al.*[44] where *Thalassia hemprichii* showed antibacterial activity against 4 pathogens; (*Bacillus subtilis*, *Staphylococus aureus*, *Agrobacterium tumefaciens* and *Escherichia coli*) tested.

From the seagrass gathered from Tamil Nadu, India, *Cymodocea serrulata*, *Enhalus acoroides*, *Halophila ovalis*, *Halodule pinifolia* and *Thalassia hemprichii*, it is shown that *Thalassia hemprichii* has the biggest inhibitory zone at 100 mg mL^{-1} concentration[45]. The ability of *Thalassia hemprichii* as an antibacterial is because of the content of bioactive compounds. Some of the bioactive compounds are:

Flavonoid: Flavonoid, a class of polyphenolic compounds, occurs in any of the five chemical structures like flavones, flavonols, flavanons, flavanols and anthocyanidins. The presence of flavonoids was indicated in *Thalassia hemprichi* seagrass[46]. Flavonoid, especially a compound which solve in water can be extracted by using[47] ethanol 70%. Flavonoid is a phenol compound. Therefore, its color changes if alkaline solution or ammonia is added to it so that it is easily detected on chromatogram or in the solution. This compound contains aromatic system which conjugates and it shows strong absorption band on ultraviolet spectrum area (V) and visible spectrum. The occurrence of red, yellow or orange colors in the amyl alcohol layer in the phytochemical test shows the presence of flavonoid.

Thalassiolin D, a new O-glucoside sulfate flavone along with 3 types of flavonoids, two steroids, p-hydroxybenzoic acid, 4,4'-dihydroxybenzophenone and nitrogen compounds, octopamine has been isolated from *Thalassia hemprichi* seagrass, collected from the Red Sea of Arabia[48]. Flavonoid group compounds reportedly play an active role as antifouling and act as isolates against the attachment of organisms[49]. Other studies have shown that *Thalassia hemprichii* has the potential to inhibit pathogenic bacteria and fungal strains[50].

Siregar *et al.*[38] explain that flavonoid compounds has the ability to inhibit bacterial growth with several different mechanisms, among others flavonoids cause the damage of bacterial wall permeability, microsomes and lysosomes as a result interaction between flavonoids and bacterial DNA.

Alkaloid: Alkaloid is derived from plant sources, they are basic, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or other animals. Alkaloid is often used in the field of pharmacology[51]. Research shows the presence of antibacterial alkaloid bioactivity[52]. Decrease the diameter of the inhibition zone in *Thalassia hemprichii* extract shows that antibacterial active ingredients are bacteriostatic.

The physical properties of isolated alkaloids are crystalline solids with a particular melting point or have a range of decomposition. The chemical characteristic of alkaloid is alkaline. The alkaline characteristic of alkaloids causes these compounds very easy to decompose, especially by heat and rays in the presence of oxygen. Although the mode of action of these alkaloids is not known in detail, some studies have shown that bisindole monoterpenoid alkaloids, with similar structures, act as DNA-intercalating agents or like topoisomerase inhibitors[52].

Quantitative phytochemical testing of *Thalassia hemprichii* showed that at 90% methyl concentration, alkaloids levels of 8.42 mg g^{-1} is obtained. Alkaloid has the function as antibacterial by disrupting the peptidoglycan component of the bacterial cell so that the cell wall layer is not completely formed and causes the cell's death. Another mechanism of alkaloid antibacterial is that alkaloid component is known as the DNA intercalators and inhibitor of bacterial cell topoisomerase enzymes[53].

Biological activity of alkaloid compounds caused the presence of base groups nitrogenous. There is a base group when experiencing contact to bacteria will react with the amino acid compounds that make up bacterial cell walls and also bacterial DNA which is the main constituent of the cell nucleus; it is the center of everything cell activities. This reaction occurs because of chemically a compound that is of a nature bases will react with acidic compounds i.e., amino acids because of the most amino acids reacted with alkaline groups of alkaloid compounds. This change in the arrangement of amino acids is clear will change the genetic balance in acid DNA so that bacterial DNA will having damaged. DNA damage in the nucleus of the bacterial cell will push lysis of the cell nucleus, so the cell will get damage. Cell damage resulting in bacterial cells not being able to do metabolism so it will experiencing lysis (destroyed)[54].

Tannin: Tannins are polyphenolic secondary metabolites of higher plants and are either galloyl esters and their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallo-tannins, ellagitannins and complex tannins), or they are oligomeric and polymeric proanthocyanidins that can possess different inter flavanyl coupling and substitution patterns (condensed tannins)[55]. The tannins appear as light yellow or white amorphous powders or shiny, nearly colourless, loose masses, with a characteristic strange smell and astringent taste.

Antibacterial activity of *Thalassia hemprichii* seagrass extract can be made possible by the presence of compounds dissolved in water that have antibacterial activity. According to Abd El-Hady *et al.*[56], some active compounds found in seagrass antimicrobial, including tannin, saponins, terpene, alkaloids and glycosides. Frequently an increased tannin production can be associated with some sickness of the plant. Therefore, it is assumed that the biological role in the plant of many tannins is related to protection against infection, insects, or animal herbivory. In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic and haemostatic pharmaceuticals[55].

Significant positive correlation was found between phenol and tannin contents of seagrass leaves and rhizomes[57]. The mechanism of action of tannin antibacterial is by reproducing protein. Tannin antibacterial has its effect through the reaction on the cell membrane, the inactivation of enzyme and genetically material function. The mechanism of tannin as an antibacterial is by inhibiting the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed. Tannin has the activity of antibacterial which is related with its ability to inactivate microbial cell adhesin, enzyme and disrupting the transport of protein on the layer within the cell[58].

Tannin has a phenol compound which has a hydroxyl group in it, then the mechanism on deactivates bacteria by utilizing differences the polarity between lipids and clusters hydroxyl. If bacterial cells are getting more contains a lot of lipids, it is needed high concentration to make the bacteria lysis[38].

ANTIOXIDANT POTENTIAL OF *THALASSIA HEMPRICHII* SEAGRASS

Free radical: *Thalassia hemprichii* having the function as anti-bacterial, it also serves as the antioxidant. There is some evidence that free radical damage contributes to the etiology of many chronic health problems such as emphysema, cardiovascular and inflammatory diseases, cataracts and cancer[59]. A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants[60].

Antioxidant: Antioxidants in biological systems have multiple functions such as to protect from oxidative damage[61]. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property[60]. Antioxidant is a molecule that is able to slow or prevent the process of another molecular oxidation. Oxidation is a chemical reaction which can produce free radical and trigger chain reaction that can break cells[62]. Antioxidants in biological systems have multiple functions, including depending against oxidative damage and in the major signaling pathways of cells.[63]The function of antioxidant is to prevent free radical.

Seagrasses have strong antioxidant potential[63]. Some research has proved that *Thalassia hemprichii* has high antioxidant level. The extraction of *Thalassia hemprichii* by using maceration and n-hexane, ethyl acetate and methanol solvent for 1×24 h results in the IC_{50} value of 25.98344501 after antioxidant activity test is done on *Thalassia hemprichii* seagrass leaf. This value shows that the tested sample can inhibit free radicals very strongly. The sample contains big potential to be used as natural antioxidant source, as shown[64] by Table 5.

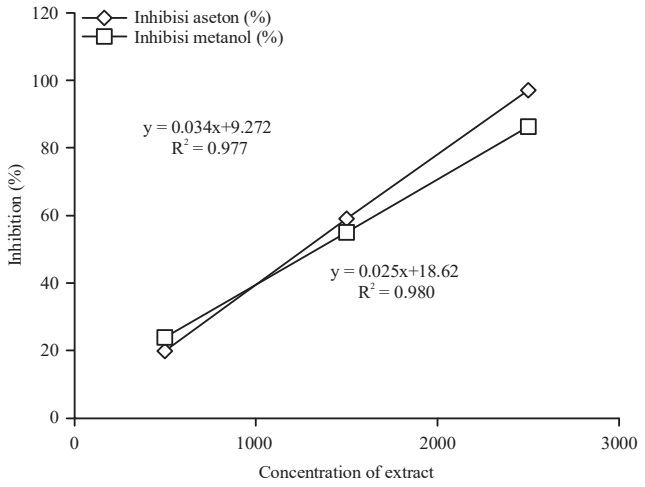

Fig. 4: *Thalassia hemprichii* antioxidant activity curves
Source: Ulfa *et al.*[65]

Table 5: IC_{50} value of *Thalassia hemprichii* seagrass leaf extracts

Extract sample	Concentration (ppm)	Graph of an equation	IC_{50} value
n-hexane	75	y = -0.51x+121.83	139.50
	125		
	175		
Ethyl acetate	75	y = -0.25x+56.59	25.98
	125		
	175		
Methanol	75	-	-
	125		
	175		

Source: Tristanto *et al.*[64]

The test on *Thalassia hemprichii* extract from Jepara sea, the value of IC is 50% which means that *Thalassia hemprichii* leaf extract can demonstrate antioxidant activity of 563.88-2039.8 mg L^{-1}. The bioactive components found are alkaloid, flavonoid, phenol, hydroquinone, steroid, saponin and tannin which means that *Thalassia hemprichii* can functions as natural antioxidant source that is equal to other antioxidant sources likes fruits (Fig. 4)[65].

Besides being consumed to get the benefit as an antioxidant, *Thalassia hemprichii* also has the ability to break down free radicals. From 7 species of seagrass, *Thalassia hemprichii* and *Cymodocea serrulate* showed high free radical flushing activity in their habitat. *Thalassia hemprichii* also has a unique secondary metabolite that can be used not only as an antioxidant, but also as an anticancer. *In vitro* analysis using HeLa cells shows that semi polar and polar extracts show potential anti-cancer properties. The level of cell lethality with semi polar extract is higher than that of polar extract, but it is not different from doxorubicin cancer drug.

Another study demonstrates the potential of *Thalassia hemprichii* extract for decrease glucose serum level. Experiments on animals given *Thalassia hemprichii* ethanol extract shows decreased glucose serum ($p<0.01$). Post-treatment using *Thalassia hemprichii* also decreases cholesterol, triglycerides and LDL in animals. There is an increase in HDL in post-treatment along with a decrease in creatinine, urea level and weight[66].

Phenolic acids were detected in the seagrasses also. Seagrasses collected at Diani Beach, Kenya, yielded chromatograms that were nearly indistinguishable. The phenolic acids were in the leaves of *Thalassia hemprichii*[67]. There was a positive relationship between total phenol content and antioxidant activity. The research conducted by Kannan *et al*.[63] that there was a significant correlation between total phenolic content and total antioxidant activities.

This study discovered the *Thalassia hemprichii* that can be beneficial for human health. This study will help the researchers to uncover the critical areas of *Thalassia hemprichii* seagrass extract that many researchers were not able to explore. Thus a new theory on antimicrobial and antioxidant potential of *Thalassia hemprichii* seagrass extract may be arrived at.

CONCLUSION

Thalassia hemprichii extract can be used as an anti-bacterial agent against several agents of pathogen in humans. *Thalassia hemprichii* extract can be used as a natural antioxidant. Besides, it has the potential as an anticancer and antidiabetic substance.

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