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A mini review on biological activities of genus *Thalassia*: A marine seagrass

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Abstract
Marine organisms produce a wide range of secondary metabolites that show the potential for the development of new moieties for the treatment of various diseases. Recently, several studies on secondary metabolites obtained from marine seagrasses resulted in the identification of pharmacologically active constituents. *Thalassia*, a marine seagrass genus belonging to the Hydrocharitaceae family, consists of two therapeutically useful species, namely, *Thalassia testudinum* and *Thalassia hemprichii*. The phytoconstituents present in the *Thalassia* species showed a variety of chemical compounds with proven therapeutic applications, such as alkaloids (antioxidant, larvicidal activity), glycosides (anticancer, antioxidant, larvicidal activities), fatty acids (anticancer activity), flavone glycosides (Anticancer activity), tannins, phenols (antioxidant, anticancer activities), ketones (antiviral activity), sterols (antioxidant, antiviral activities), flavonoids (anticancer activity), sulphates (antibacterial activity). This review aims to assemble the pharmacological activities of *T. testudinum* and *T. hemprichii* of the *Thalassia* genus, their phytoconstituents, methods for extraction, pharmacological actions, and their mechanisms and IC_{50} values. Hence, the results highlight the potency of these two species as natural therapeutic molecules for the treatment of diseases.

Keywords: *Thalassia*, seagrasses, phytoconstituents, therapeutic activities, anticancer

Introduction
Over the past 50 years, marine natural compounds have attracted the interest of researchers worldwide. In comparison to terrestrial natural compounds, marine organisms are extraordinary sources of novel therapeutic compounds that have a wider range of structural and chemical features [1]. Various marine species have produced a number of chemically different constituents, some of which are being studied for their potential use in the development of novel medications for therapeutic use [2]. Additionally, the European Medicines Agency (EMA) and Food and Drug Administration (FDA) have approved several medications developed from marine sources [3]. In recent years, various marine organisms and animals, including sea slugs, tunicates, soft corals, sponges, sea hares, bryozoans, nudibranchs, and marine species, have produced a variety of bioactive compounds [4].
Seagrasses are important sources of biologically active components and are eukaryotic organisms that can establish themselves in the shallow water sections of the tropical, subtropical, and temperate oceans, with the exception of Antarctica [5, 6].
These are the only flowering species that can grow in a marine environment. There are around 60 species of marine seagrass that are divided into four marine families: Zosteraceae, Posidoniaceae, Cymodoceaceae, and Hydrocharitaceae [7].
Phytochemicals or active constituents such as terpenoids, polyphenols, glycosides, alkaloids, flavonoids, and the halogenated substances produced by various species of seagrass showed antioxidant, anticancer (antitumor), anti-inflammatory, antifungal, antimicrobial, antimalarial, antidiabetic, antiviral, antiprotozoal, and cytotoxic properties that are effective in the prevention and treatment of various diseases [8].

Thalassia
Thalassia is marine seagrass genus [9] in the Hydrocharitaceae family and is found in the tropics and subtropics. *Thalassia* contains two morphologically similar but geographically distinct species in tropical waters, namely *T. testudinum Banks ex Konig*, also called turtle grass, and *T. hemprichii* (Ehrenberg). They are widely spread in shallow coastal regions of the Western Atlantic and Indo-Pacific, *Thalassia* beds can be found in marine coastal or lagoons estuaries, which are frequently surrounded by coral reefs or mangroves [10, 11]. They are considered as "twin species" [12].

The active constituents, such as glycosides, fatty acids, saponins, sulphates, polyphenols, sterols, terpenes, tannins, alkaloids, and proanthocyanins are present in *Thalassia* species are responsible for various therapeutic activities (Table 1.) [13].

Table 1: Phytoconstituents of *Thalassia* species

Phytoconstituents	Examples	Source	Activities
Glycosides	Glucopyranoside, Dihydroxy-3',4'-dimethoxyflavone, kaempferol 3-O-(6″-O-p-coumaroyl)-β-glucoside	*T. testudinum* leaves *T. testudinum* leaves *T. hemprichii* Extract	Anticancer activity Antioxidant activity Antiviral activity
Flavonoids	Apigenin, Dihydroxy-3, 4-dimethoxyflavone 7-O-d-glucopyranoside, Chrysoeriol, Trimethoxyflavone Thalassiolins A-B,	*T. hemprichii* Leaves	Anticancer activity
Alkaloids	-	*T. hemprichii* Leaves	Antioxidant activity, Larvicidal
Flavone Glycosides	Malonylated flavone glucosides, Flavone glucuronides, Sulphated Flavones, Methoxy Flavone glycosides	*T. hemprichii* Extract	Anticancer activity
Fatty Acids	Palmitic acid, Stearic acids, Linolenic acid, Oleic acid, Linoleic acid, Stearic acids	*T. testudinum* Extract	Anticancer activity
Phenol	Sulphate ester Chicoric acid, Chrysoeriol 7-O-D-glucopyranoside,	*T. testudinum* Leaves *T. hemprichii* Leaves *T. testudinum* Extract	Antioxidant activity Anticancer activity Anticancer activity
Sulphates	Isoscutellarein 7-O-xylopyranoside-2-O-sulfate, luteolin 7-b-d-glucopyranosyl-200-sulfate	*T. hemprichii* Extract *T. testudinum* Extract	Antibacterial activity Antiviral activity
Sterols	Triterpene-steroids, β-stigmasterol	*T. hemprichii* Extract	Antioxidant activity Antiviral activity
Ketone	4,4′-dihydroxybenzophenone	*T. hemprichii* leaves	Antiviral activity

Therapeutic applications

Anticancer activity

Researchers evaluated the cytotoxic potential of the choroform (nonpolar components) fraction present in the hydroethanolic extract of *T. testudinum* collected from Guanabo Beach, Cuba. The extract was obtained by maceration and analysed by gas chromatography-mass spectrometry. A total of 69 compounds were found in the chloroform fraction, including unsaturated and saturated fatty acids (ω -6 and ω -3), sterols, phenolic acids, and glycerides. Amongst fatty acids, palmitic acid was the most potent, at 48.4%, followed by linolenic, oleic, linoleic, and stearic acids. Cell viability was evaluated at various concentrations of chloroform fraction from 0.01-1000 µg/mL by the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay on human immortalised endothelial EA.hy926 and human lung carcinoma A549 cell lines. DMSO-1%-treated and untreated cells were used as controls for the assay. The result showed that the chloroform fraction of the hydroethanolic extract from seagrass, *T. testudinum,* exhibited concentration-dependent cytotoxicity, and it was most toxic to the A549 cell line, with an IC_{50} of 20.4 µg/mL, followed by the EA. hy926 cell line, which had an IC_{50} of 248.4 µg/mL [14].

Another study was carried out to prove the chemopreventive and antimutagenic effects of *T. testudinum*. The hydroethnolic extract was prepared by maceration, and non-polar constituents were removed by chloroform extraction. The Electrospray Ionisation Mass Spectrometry (ESIMS) technique was used to isolate Thalassiolin B (1-chrysoeriol 7-d-glucopyranosyl-2"-sulfate), and its structure was established by 1H and 13C NMR. The inhibitory activity of the hydroethanolic extract, its fraction, and Thalassiolin B was tested spectrophotometrically against phase I metabolising enzymes CYP1A and CYP2B obtained from rat and human cells. The *in vitro* antimutagenicity studies were performed against benzopyrene-induced mutagenesis by the Ames test in *Salmonella typhimurium* strain TA98. *In vivo*, antimutagenic effects were tested on Balb/c male mice. The results showed that CYP1A1 (the phase I metabolising enzyme) was more sensitive to the tested compounds in comparison to the other enzymes. Mixed type of inhibition were also observed with Ki values of 54.16±9.09 µg/mL, 5.96±1.55 µg/mL and 3.05±0.89 µg/mL for hydroethanolic extract, its fraction, and thalassiolin B, respectively. Additionally, the compounds significantly reduced the *in vitro* mutagenicity caused by BP in the range of 27% to 34%. Furthermore, at an oral dose of 100 mg/kg, *T. testudinum* treated mice significantly reduced the BP-induced micronuclei and oxidative damage in mice [15].

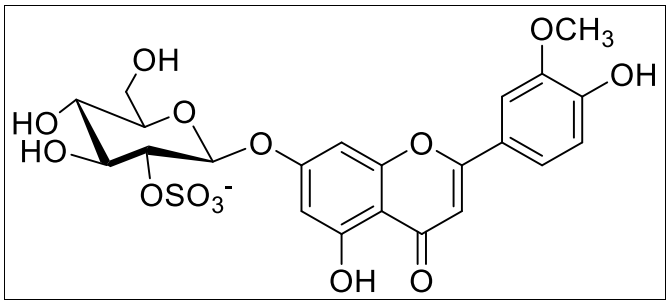

Fig 1: Thalassiolin B obtained from hydroethanolic extract of *T. testudinum*

The cytotoxic and anticancer potential of an aqueous ethanolic extract from *T. testudinum* was evaluated using PC12, 4T1, Caco-2, and HepG2 tumour cell lines and MCDK, BHK2, CHO, 3T3, and VERO non-tumor cell lines. The extract was prepared by maceration and evaluated by chromatographic and spectroscopic techniques. It showed the presence of dihydroxy-30, 40-dimethoxyflavone 7-O-b-D-glucopyranoside, apigenin 7-O-b-D-glucopyranosyl-2″-sulfate (thalassiolin C), chrysoeriol 7-O-b-D-glucopyranoside, chrysoeriol-7-Ob-D-glucopyranosyl-2″-sulphate (Thalassiolin B), luteolin-30-sulfate, apigenin, and chrysoeriol compounds. Cell viability and DNA damage were determined using the MTT assay, trypan blue dye exclusion, neutral red assay and comet assay (single-cell gel electrophoresis). The tumour cells were more sensitive to aqueous ethanolic extract of *T. testudinum* than non-tumour cells, and the tumour cell line HepG2 cells showed greater sensitivity with an IC_{50} of 102 µg/ml for the MTT assay and 106 µg/ml for the Neutral Red Assay. The cytotoxic action showed by other tumour cell

lines, Caco-2, 4T1 cells, and PC12, with an IC_{50} of 129 µg/ml-165 µg/ml. The normal cells exhibited no cytotoxicity with an IC_{50}>1000 µg/ml. The study of cytosolic Ca^{2+} accumulation, production of ROS, cell viability, and nuclear damage was done by a high-content screening multiparametric assay (HCS) conducted on HepG2 cells using a flow cytometer. Treatment of HepG2 cells with extract showed a reduction in cell viability, increased the amount of cytosolic Ca^{2+} and intracellular ROS, induced DNA fragmentation and nuclear damage in a concentration-dependent manner [16].

The polyphenolic fraction from *T. testudinum* extract showed, concentration dependent cytotoxicity in human colorectal cancer cells. *T. testudinum* was extracted by maceration and non-polar fraction were removed using chloroform extraction. Cell viability assay, Crystal violet assay were performed on cancer cells HCT116, HCT15, SW620, and HT29 and normal cells lines (FHC, HEL299 and MCF10A). The potential proapoptotic effect of the extract was assessed by flow cytometry in cancer cell lines using Annexin V-FITC/propidium iodide. Additionally, reactive oxygen species (ROS) generation was measured by fluorescence using DCFH DA staining, and concentration of sulfhydryl by spectrophotometry. *In-vivo* antitumor activity of polyphenolic fraction was evaluated in xenograft model in nu/nu mice. PBS (Phosphate buffer saline) was used as a negative control, while cisplatin was used as a positive control. *In vivo* proapoptotic effect was evaluated by immunohistochemistry using anti-Bcl-2 and anti-caspase 3 antibodies. The result showed, polyphenolic fraction exerts cytotoxicity in colorectal cancer cells. Also, extract showed a significant, depletion of sulfhydryl concentration and increase in the intracellular ROS generation in HCT15 cells. The polyphenolic fraction prevented growth of tumors and induced apoptosis in the xenograft mice model. During the toxicological evaluation in mice, no signs of toxicity were observed in extract treated group in comparison to cisplatin. Thalassiolin B and polyphenolic fraction showed a decrease in survival percentage of cancer cells with an IC_{50} of 38.75±3.57 µg/ml and 22.47±1.30 µg/ml. These studies showed *T. testudinum* as a potential agent for anticancer activity [17].

In vivo and *in-vitro* cytotoxic efficiency of the polyphenolic extract of *T. testudinum* was assessed for colorectal cancer in colon tumour cell lines (SW480, CT26 and RKO) and in a syngeneic allograft murine colorectal cancer model. The polyphenolic fraction was obtained by maceration of *T. testudinum* leaves using ethanol. Various flavonoids such as, luteolin, apigenin, chrysoeriol-7-O-β-D-glucopyranoside and thalassiolin B were found in the extract. The cytotoxic effects of extract in concentration range of 1 to 1000µg/mL was evaluated by MTT assay. The colon cancer cell line, SW480 showed the maximum sensitivity to the polyphenolic extract of *T. testudinum* treatment, with an IC_{50} values of 58.9, 174.9 and 115.3 µg/mL for SW480, RKO, and CT26 cell line, respectively. Chorioallantoic Membrane (CAM) assay and wound healing migration assay was performed for the inhibition of angiogenesis and the migration ability of EAhy926 cells. In CAM assay, *T. testudinum* extract (50 g/disc) completely reversed bFGF-induced blood vessel formation, followed by angiogenesis suppression. Wound healing migration assay showed inhibition of the invasion and migration of EA. hy926 cells. The *In-vivo* anticancer effect was assessed using Balb/c mice. Cisplatin was used as a positive control. *T. testudinum* extract activated an ATF4-P53-NFB-specific gene which promote the anticancer immune cell death in the *In-vivo* synthetic allograft model of the murine CT26 colon cancer. The polyphenolic fraction reduced growth of the tumour cells and improved overall organism survival rate [18].

Anti-oxidant activity

Antioxidant activity of aqueous ethanolic extract of *T. testudinum* (BM-21) was evaluated *in-vitro* using four different free radical scavenging assays (RO_2•, HO•, DPPH• and O_2^{-}•). Fractionation of the BM-21 by RP-C18 flash chromatography and evaluation by spectoroscopy reported the presence of 11 compounds in the fractions, including one novel sulphate ester compound, ethyl 4-(sulfoxy) benzoate, as well as previously described 10 phenolic compounds such as, 4-hydroxybenzaldehyde, apigenin 7-O-β-D-glucopyranosyl-2″-sulfate,4-hydroxybenzoic acid, chrysoeriol-7-O-β-D-glucopyranosyl-2″-sulfate (thalassiolin B), apigenin 7-O-β-D-glucopyranoside, chrysoeriol 7-O-β-D-glucopyranoside, 5,7 dihydroxy-3',4'-dimethoxyflavone, chrysoeriol, 7-O-β-D-glucopyranoside, luteolin-3'-sulfate and apigenin. DMSO was used as a control, while, ascorbic acid and quercetin were used as standards for free radical scavenging assays (RO_2•, HO•, DPPH• and O_2^{-}•). Extract exhibited dose dependent antioxidant activity with hightest % inhibitory activity against RO2• radical 99.4±0.4% with an IC_{50} of 131.0±3.2 µg/ml. Other radical scavenging assays showed, IC_{50} between 171 to 131 µg/mL and % inhibition between 99 to 75% [19].

Similarly, another study investigated the antioxidant effect of BM-21, at concentrations of 4 mg/kg, 40 mg/kg, and 400 mg/kg on the acrylamide (ACR) induced distal axonopathy in male OF-1 mice. The BM-21 extract from the leaves of *T. testudinum* was obtained by maceration and extract showed the presence of thalassiolin B (about 5.8±0.9%) as a lead compound, along with a large concentration of polyphenols including thalassiolin A and flavones. Animals were given acrylamide at the dose of about 70 mg/kg, followed by BM-21 at doses of about 4, 40, and 400 mg/kg. The neurobehavioral indices (rotarod test), electrophysiological indices (CMAP), as well as oxidative test parameters such as glutathione, total hydroperoxides, superoxide dismutase and glutathione peroxidase were evaluated. The result of rotarod test showed that treatment with BM-21 improved abnormal motor coordination to near normal levels. Electrophysiological indices demonstrated that, at the dose of 4 mg/kg, CAMP of the mice was unchanged but at 40 mg/kg, the treated group indicated a mild to modest degree of recuperation of duration and amplitude, while BM-21 at 400 mg/kg showed restoration of both parameters towards its normal level. Studies in the homogenates of brain, sciatic nerve and cerebellum, showed that at 400 mg/kg of BM-21, restored glutathione (GSH) and total hydroperoxides (THP) in the sciatic nerve along with superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities [20].

Studies were conducted to evaluate the efficacy of a cream containing aqueous ethanolic extract of *T. testudinum* on UVB-damaged skin of mice. Mice skin was exposed to UVB radiation and the skin damage was assessed using macroscopic and histopathological studies. The results showed that the application of cream resulted in a dose dependent decrease in macroscopic skin alterations such as, scabs, erythema, wrinkling and roughness of the skin and maximal beneficial effects occurred at 240 µg/cm^2. The cream treatment also showed decrease in hyperqueratosis, acanthosis, collagen, infiltrating inflammatory cells, and

elastic fibres degradation and vascular damage in histopathological studies. Fractionation of the plant extract was done using chloroform (fraction C), water-saturated n-butanol (fraction B) and n-hexane (fraction H). These fractions also showed beneficial effects on skin in the macroscopic and histopathological studies. The DPPH scavenging property and total phenolic contents of the fractions were conducted and results indicated that fraction B showed largest free radical scavenging effect with an EC_{50} value of 97 µg/mL and total phenolic content of 39 ± 1.5%. Further fractionation of the fraction B using size exclusion column chromatography followed by C18 reversed phase HPLC purification resulted in isolation of 4-hydroxybenzoic acid along with thalassiolin B. The mice skin regeneration studies and DPPH scavenging activity conducted and showed that serial administration of thalassiolin B markedly restored 99% of skin damage. 4-hydroxybenzoic acid showed no skin regenerating activity. The radical trapping assay showed that, Thalassiolin B was found to scavenge DPPH radicals with an EC_{50} of 100.0 µg/mL. whereas, 4-hydroxybenzoic acid did not show any activity [21].

Antioxidant and total phenolic content of ethanolic extracts 100%, 75% and ethanol 50% of *T. hemprichii* was evaluated using Molyneux method and modified Folin-Ciocalteu method respectively. Positive control was ascorbic assay. Result stated that ascorbic acid exhibited hightest % inhibition of 78.055±0.756% and the ethanolic extracts in concentrations of 100%, 75% and 50% exhibited inhibition of 38.035±0.252%, 51.809±0.164% and 52.212±0.735% respectively. The total phenolic content present in the extracts were in the range of 0.52-0.55 GAE/100 g. Further, the antioxidant activity of the extract was assessed against *C. histolyticum* collagenase spectroscopically using Wittanauer method. The extract showed 50% inhibitory activity at 20 µl showing that *T. hemprichii* could be used as anti-wrinkle agent in cosmetics [22].

Researchers conducted study for optimization of process of extraction for the seagrass *T. hemprichii* in order to maximize its antioxidant effect and total phenolic content. Three different extraction conditions were studied by varying the ethanol concentration, temperature of extraction and by addition or absence HCl-1N during extraction. The antioxidant activity was evaluated with the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, for that, ascorbic acid was used as a positive control, and the total phenolic content was determined by the Folin-Ciocalteu method. An ANOVA analysis was performed, to demonstrate the significance of varying conditions on the total antioxidant and total phenolic condition. The results showed, that extraction conducted using 50% ethanol with addition of HCl-1 N at 60 °C yielded extract with antioxidant activity (IC_{50} 83.48 µg/ml) and total phenolic content of 41.09 mg GAE/gm of extract [23].

Another study, reported the antioxidant potential of a methanolic extract from *T. hemprichii* in comparisonsto other seagrasses. The extract was made using the maceration technique using methanol and concentrated using a rotary evaporator. The antioxidant activity of the methanolic extract was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay on TLC plates. TLC plates were established using 10% chloroform in methanol as a mobile phase. The active antioxidant seagrass constituents of *T. hemprichii* were detected as yellowish white spots against a purple background, produced by bleaching the DPPH reagent. A total of 8 spots were detected on the TLC plate, indicating strong antioxidant activity. A solution of gallic acid was positive control, while ascorbic acid and blank TLC plate were used as negative controls. The total phenolic contents of the methanolic extract from *T. hemprichii* were determined by the modified Folin-Ciocalteu method and was found to be 0.4187±0.007mg [24].

Anti-viral activity

Researchers examined the therapeutic effectiveness of *T. testudinum* and *T. hemprichii* for their antiviral activity. These studies evaluated the HIV integrase inhibitory property of seagrass *T. testudinum* and Thalassiolins A-C, a series of water-soluble flavones, isolated from *T. testudinum.* The extract was obtained by blending seagrass with deionized water and maceration technique. The lyophilized section of the extract was further purified by C18 reversed phase high performance liquid chromatography and size exclusion chromatography. The compound luteolin 7-b-d-glucopyranosyl-200-sulfate was identified as Thalassiolin A. Thalassiolin B and Thalassiolin C were also identified in the extract (fig.3). The antiviral activity of Thalassiolin A-C was evaluated using invitro by strand transfer inhibitory and integrase terminal cleavage activities in the presence of metal cofactor Mn^{+2}. The result showed, Thalassiolin A showed IC_{50} of 2.1 µg/ml for integrase terminal cleavage and IC50 of 0.4 for strand transfer activity and Thalassiolins B and C showed IC_{50} of 30-100mM. Further, the HIV replication inhibitory activity was established by MAGI cell indicator assay with an IC_{50} of 30 µM [25].

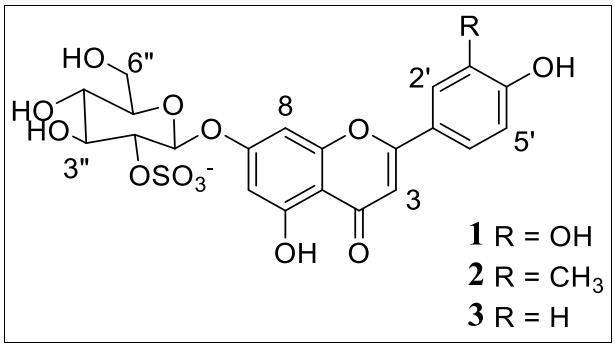

Fig 3: Structures of the thalassiolins isolated from *T. testudinum*: Thalassiolin A (1), Thalassiolin B (2), and Thalassiolin C (3).

Study reported anti-viral activity from a novel compound, Thalassiolin D, it is a flavone O-glucoside sulphate obtained from, methanolic extract of seagrass *T. hemprichii*. Fresh material of *T. hemprichii* were prepared by maceration. Then filtrates were dried in rotatory evaporator under the reduced pressure at 40 °C. Then the total extract was subjected to column chromatography to obtain the fractions. The extract contained the new compound, Thalassiolin D with three other flavonoids, two steroids, 4, 4′-dihydroxybenzophenone and p-hydroxybenzoic acid, and the nitrogen molecule octopamine. The structure of the thalassiolin D was identified by a spectroscopic study using MS and 1D and 2D NMR data as diosmetin 7-O-glucosyl-2″-sulphate. The extract of *T. hemprichii* and the other isolated flavonoid compounds were exposed to an *in vitro* antiviral HCV protease assay, and the result showed that extract and Thalassiolin D showed antiviral activity with IC_{50} of 23 µg/ml and 16 µM, whereas diosmetin 7-O-β-glucoside exhibited weak antioxidant activity, with an IC_{50} of 37µgM [26].

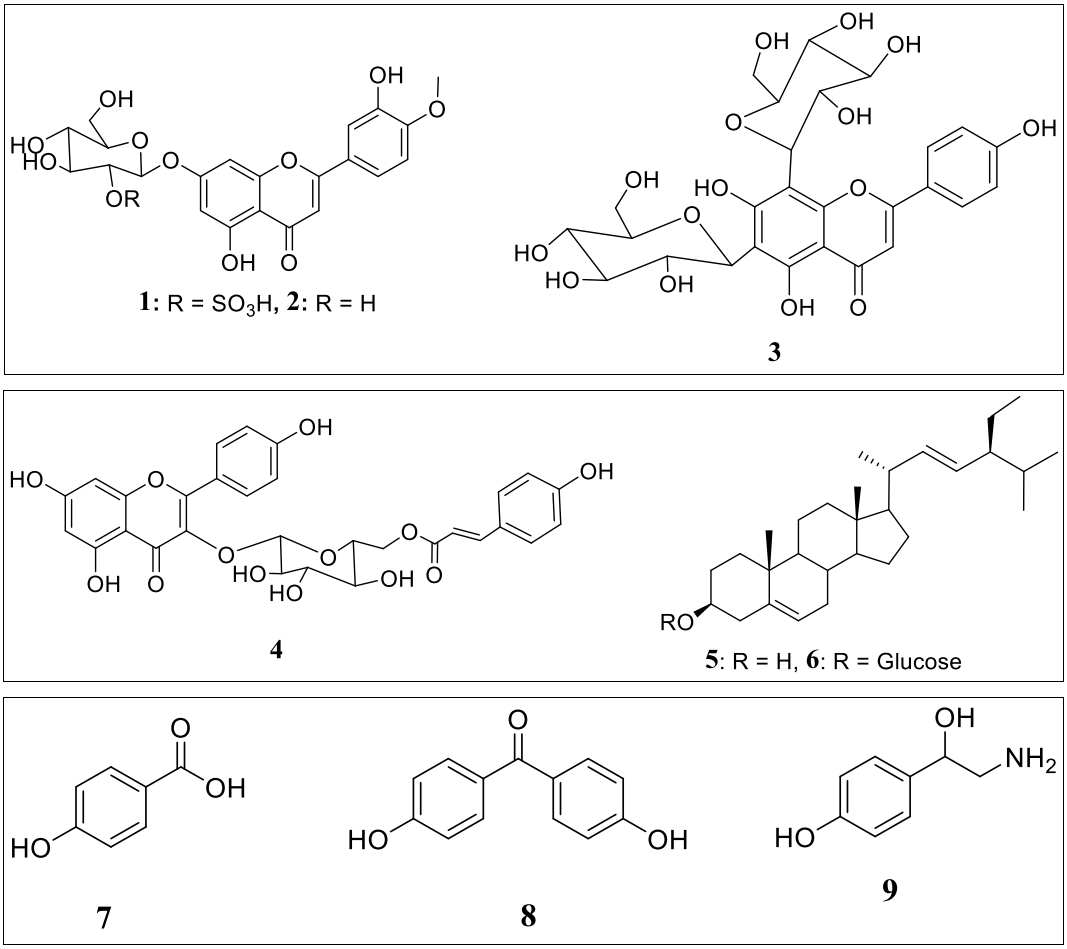

Fig 4: Isolated compounds diosmetin 7-O-β-glucoside-2″-sulphate (1), diosmetin 7-O-β-glucoside (2), apigenin 6,8-C-β-diglucoside (3), kaempferol 3-O-(6″-O-p-coumaroyl)-β-glucoside (4), β-stigmasterol (5), β-stigmas- terol 3-O-β-glucoside (6), p-hydroxy-benzoic acid (7), 4, 4′-dihydroxybenzophenone (8) and octopamine (9) from *T. hemprichii*.

Anti-diabetic activity

Researchers worked on the evaluation of antidiabetic potential from methanolic extract of *T. hemprichii* using alpha-amylase inhibitory assay. The methanolic extract of *T. hemprichii* was extracted and its activity was compared with acarbose as standard. The methanolic extract of *T. hemprichii* was ineffective at inhibiting the activity of the enzyme alpha-amylase at doses of 500 mg/ml and 250 mg/ml and with percent inhibition of 0.66% and 13.77% respectively in comparison to acarbose with an inhibition of 18.88% and 0.90% [27].

The ethanolic extract of root, stem and leaves from seagrass *T. hemprichii* was prepared by maceration using 80% ethanol and evaluated for its anti-diabetes activity using α-glucosidase inhibition assay. The α-glucosidase inhibitory activity was conducted using microplate reader at 400 nm and acarbose was used as a positive control. The research indicated that ethanolic extract of *T. hemprichii* has a moderate inhibitory action against α-glucosidase and with an IC_{50} of 425.86±5.15 µg/mL [28].

Larvicidal activity

The study investigated the larvicidal potential of *T. hemprichii* from two different locations and from the various parts of the plant against Ae. aegypti larvae. The seagrass were collected from Menjangan Island and Prapat Agung Beach**,** West Bali National Park, Indonesia. The samples of extract were divided according to their organs, such as leaves, rhizomes, and roots, and extracted by maceration with methanol. High performance liquid chromatography (HPLC) used to analysed the extract and it showed, presence of alkaloids, flavonoids, and saponins. The larvicidal activity was evaluated by a larvicidal screening test. A control group involved both positive and negative control. The negative control was distilled water (100 ml), whereas the abate solution about the 0.01% concentration was the positive control. The result showed that, *T. hemprichii* leaves contained higher larvicidal potential than rhizomes and roots, and its mortality rate was 100%. The HPLC chromatogram showed the *T. hemprichii* leaves, roots, and rhizomes from different places had a relatively identical form of active constituents. From all the extracts, the methanolic extract of *T. hemprichii* from Menjangan Island demonstrates maximum activity [29].

Studies were conducted to evaluate the efficacy of *T. hemprichii* from the Saudi Arabian Red Sea against Ae. aegypti mosquito. The ethanolic extract from *T. hemprichii* was prepared by maceration using ethanol. The assessment protocol of the World Health Organization (WHO) was used to check the sensitivity of early 4th instar larvae or late 3rd instar larvae Ae. aegypti to the ethanolic crude extract. Bug slug was used as a positive control. After treatment with the extract, a positive correspondence was observed between the applied concentrations and the percentage mortality of the 4th larval stage of an Ae. aegypti mosquito. However, the positive control Bug Slug had the high efficacy and potency for the mosquito vector larval, with a LC_{50} values of 0.0287 ppm, upper confident limit (UCL) of 0.04 ppm and the lower confident limit (LCL) of 0.02 ppm, compared to the *T. hemprichii* extract, which showed an LC_{50} of 201.7ppm, the

lower confident limit of 181.9 ppm, and the UCL of 194.9 ppm [30].

Larvicidal activity of ethanolic extract of *T. hemprichii* collected from Mandapam, India's south-east coast was studied. Ethanolic extract was prepared by percolation and concentrated by rotatory evaporator. Saponin, steroids, phenols, sugars, alkaloids, tannins, terpenoids, and proteins were detected in the extract. Batches of 4th instar larvae of an Ae. aegypti were transmitted in the enamel bowl enclosing 1 ml of plant extract (dissolved in DMSO) and 199 ml of distilled water. Each experiment was carried out in three replicates along with a control group. The control group contained 199 mL of distilled water and 1 mL of DMSO. After the treatment, the number of dead larvae was count and mortality was calculated by abbott's formula. The result showed, an ethanolic extract from the root of *T. hemprichii* demonstrated larvicidal activity with a LC_{50} of 0.096±0.002 µg.ml^{-1} and a LC_{90} of 0.121±0.069 µg.ml^{-1} [31].

Researchers evaluated larvicidal activity of *T. hemprichii*, collected from southern corniche region of Jeddah governorate, Saudi Arabia, against 4th instar mosquito larvae of Anopheles d'Thali. The extraction was carried out on a Soxhlet apparatus by absolute acetone and concentrated by a rotatory evaporator. Phytochemicals such as saponins, flavonoids, alkaloids, terpenoids, and steroids were found in the extract. Larvicidal treatments were done by exposing the early 4th instar larvae of An. d. thali to 150-300 ppm concentrations of the extract. Three replicates of five samples per concentration were prepared. Then the mortality of the larvae in each sample was recorded. *T. hemprichii* showed an IC_{50} and IC_{90} of 197.3 ppm and 293.8 ppm [32].

Anti-inflammatory effect

Chloroform and butanolic fractions of *T. testudinum* collected from Cuban coast was evaluated for its anti-inflammatory and analgesic properties. The *in vivo* anti-inflammatory and analgesic activities of the extract in mouse were evaluated by writhing tests, cyclo-oxygenase (COX) inhibition assay, phospholipase A2 (PLA2) inhibition assay, croton oil mouse ear oedema, and carrageenan paw oedema assay. Result showed that chloroform and butanolic fractions inhibit COX enzyme above 80%. The *T. testudinum* extract, its butanolic and chloroform fraction inhibited the phospholipase A2 human recombinant enzyme by 70%, 50% and 90%. The percentage inhibition of *T. testudinum* extract in croton oil induced mouse ear oedema and writhing tests was 60-70 and 70-90% respectively. Further, *T. testudinum* extract at 25mg/kg inhibited the oedema induced by carrageenan in rat paw by 90%. Researchers concluded, that *T. testudinum* extract with butanolic and chloroform fractions inhibited COX enzyme and showed anti-inflammatory as well as analgesic effect [33].

Anti-bacterial activity

Researchers reported the antibacterial effect of a crude methanolic extract from seagrass *T. hemprichii*, collected from Barrang Lompo Island, Makassa. Phytochemical analysis showed the presence of flavonoids, alkaloids, and steroids, along with reducing sugars in the extract. The antibacterial potential of the extract was evaluated by biofilm bacterial inhibition assay using an Agar diffusion method. The effectiveness of *T. hemprichii* extract against the biofilm-forming bacteria was compared with chloramphenicol solution, which was used as a positive control, and methanol, used as a negative control. The growth inhibitory properties of *T. hemprichii* extract were also examined for each bacterial isolate of biofilm forming bacteria. The results stated that, the crude methanolic extract of the seagrass *T. hemprichii* demonstrated better antibacterial activity for biofilm forming bacteria. The diameter of the inhibitory zone for the methanolic extract of *T. hemprichii* was 26 mm, similar to the inhibitory zone of chloramphenicol which formed a 28 mm inhibition zone, while the negative control, methanol showed no inhibitory zone [34].

The antibacterial effect of a methanolic extract from *T. hemprichii* seagrass collected from the Egyptian Red Sea coast, was studied. At room temperature, *T. hemprichii* was continuously extracted with methanol by the maceration technique. Column chromatography was used for the isolation of compounds and Spectral analysis techniques such as 13C NMR and 1H NMR were used to establish the structures of the isolated compounds. The extract showed presence of a novel compound, namely, 8-hydroxyapigenin 7-O-xyloside-2-O-sulfate, with some already reported compounds, rosmarinic acid, caffeic acid, isoscutellarein 7-O-xylopyranoside-2-O-sulfate and isoscutellarein 7-O-xylopyranoside. The antibacterial activity of *T. hemprichii* extract was investigated using an agar plate diffusion assay. *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus aureus* were used as bacterial strains, while *Candida albicans* and *Aspergillus niger* were used as fungal strains for the assay. The result showed, minimum inhibitory concentrations (MIC) of extract was 0.1-10 µg/mL. The potent antibacterial activity of the extract could be attributed to the compounds isolated [35].

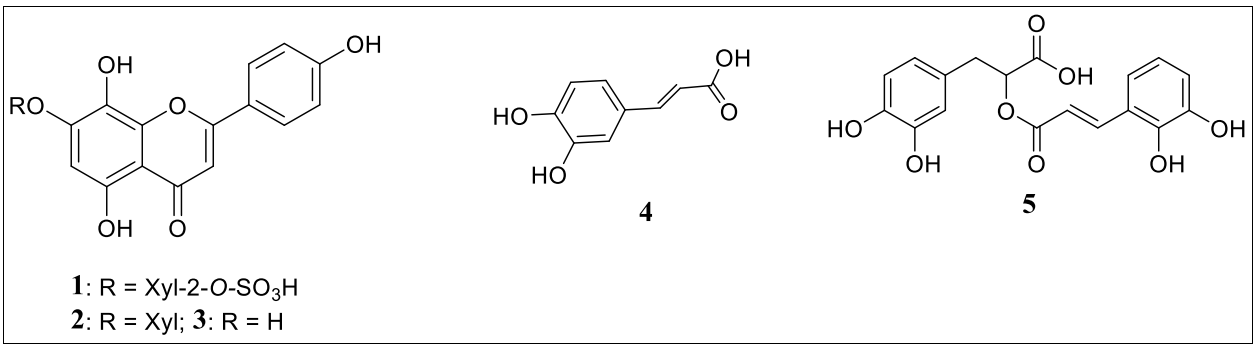

Fig 5: Isolated compounds 7-O-c-xylopyranoside-2"-O-sulfate (1), isoscutellarein 7-O-c-xylopyranoside (2), isoscutellarein (3), caffeic acid (4), and Rosmarinus acid (5) from *T. hemprichii*.

Another study compared the antimicrobial and antibacterial properties obtained from various solvent extracts of *T. hemprichii*. Dried powder of *T. hemprichii* was extracted with organic solvents like, acetone, diethyl ether, ethyl acetate,

methanol, ethanol, and water. The antibacterial potential of various solvents was assessed by the disc diffusion method. The result showed, ethanol and acetone crude extract from the seagrass *T. hemprichii*, demonstrated a large range of antibacterial activity as compared with other extracts. So, acetone and ethanol crude extracts were further fractionated and purified by column chromatographic technique. The antimicrobial activity of purified bioactive fractions tested against *Klebsiella pneumoniae, Salmonella typhi, Bacillus subtilis, Vibrio cholera, Enterobacter aerogenes, Escherichia coli, Staphylococcus aureus, Proteus mirahbilis* bacterial strains. These bioactive fractions showed significant antibacterial and antimicrobial action. Acetone extract showed antimicrobial activity against all the tested organisms, whereas diethyl ether crude extract inhibited *E. aerogenes, E. coli, S. aureus,* and *P. mirahbilis* bacterial strains more effectively [36].

Conclusion

The summary of this review was to compile the therapeutic activities of various phytoconstituents obtained from the seagrass genus *Thalassia* for better human health. The reported compounds were categorized according to their therapeutic activities. Numerous phytoconstituents that were discussed are Glycosides, Flavonoids, Alkaloids, Fatty Acids, Phenols, Sulphates, Sterols, Ketones, etc. In order to highlight the bioactive properties of the phytoconstituents, such as anticancer, antioxidant, anti-inflammatory, antimicrobial, antifungal, antiviral, anti-dengue, with mild anti-diabetic potential, various novel compounds such as, BM-21, ethyl 4-(sulfoxy) benzoate, 8-hydroxyapigenin 7-O-xyloside-2-O-sulfate, Thalassiolin D, Thalassiolin A-C are potentiate the importance of seagrasses, we compiled an extensive collection of therapeutically efficient extracts from *Thalassia* species that have been reported in the last decades.

The development of new analytical methods, including high-performance liquid chromatography, gas chromatography combined with mass spectroscopy, and column chromatography, can speed up comparative studies for the presence or absence of known compounds and increase the effectiveness of the novel bioactive compounds. Constituents extracted from the different extracts should be assessed for their safety and efficacy in numerous cell lines and different animal models, before being used to develop medications from them and tested in clinical trials. The unavailability of research on the pharmacokinetics and bioavailability of the identified phytoconstituents from the *Thalassia* genus limits their use.

Additionally, no phytoconstituents identified from the *Thalassia* genus are offered for sale commercially. As a result, it is clear that natural products obtained from marine angiosperms specially seagrass still have tremendous potential for various diseases and lifelong disorders.

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