Original Article

Antibacterial activity against *Salmonella typhi* and phytochemical screening of seven seagrass species from the coast of Tanzania

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Abstract

Seagrasses in Tanzania have traditionally been used as a remedy against various diseases including stomach problems. This study determined antibacterial activities and phytochemical composition of seven seagrass species against *Salmonella typhi*, a causative agent of typhoid fever. Crude extracts were obtained using methanol, dichloromethane and hexane solvents. All extracts showed antimicrobial activities against *S. typhi*. Hexane extracts showed highest activities with maximum inhibition zone and lowest Minimum Inhibition Concentrations (MIC). The seagrasses *Halodule uninervis* and *Cymodocea serrulata* exhibit strong antimicrobial activity against *S. typhi*, by having the lowest MIC of 0.39 mg/ml. Most extracts were non-toxic, with *Cymodocea rotundata* having the lowest toxicity level (LC_{50} = 2521.31 mg/ml) and *Thalassia hemprichii* the highest (LC_{50} = 0.038 mg/ml). Seven phytochemical groups, namely alkanoids, saponins, tannis, diterpenes, flavonoids, phenolic and cardiac glycosides were detected. There was no significant difference in antimicrobial activity and phytochemical content between leaves and root extracts. This study established for the first time that seagrasses of Tanzania contain promising antibacterial bioactive compounds against *S. typhi*. The results corroborate indigenous knowledge and may be useful in the development of novel antibacterial drugs that will help to solve problems of antibiotic resistance among pathogenic bacteria like *S. typhi*.

Keywords: minimum inhibition concentration, cytotoxicity, typhoid fever, Tanzania

Introduction

Seagrasses are amongst the marine organisms that are rich in secondary metabolites; some of which have important pharmacological properties used as treatments against bacteria diseases, fungal diseases, cancer, arthritis, inflammatory conditions, and viral diseases (Athiperumalsamy *et al.*, 2008; Kumar *et al.*, 2008; Nazar *et al.*, 2009; Yuvaraj *et al.*, 2012; Rengasamy *et al.*, 2013; Goda *et al.*, 2020; Kim *et al.*, 2021). Traditionally, roots and/or leaves of different seagrasses have been used as remedies against various diseases in different parts of the world including in coastal regions of Tanzania (De La Torre-Castro and Rönnbäck, 2004). In Tanzania, *Cymodocea* spp. are used as a remedy for skin diseases, fever, cough and is believed to help during pregnancy as a tranquilizer for babies while *Halophila* spp. are known to have potential against malaria, skin diseases as well as effective in early stages of leprosy (De La Torre-Castro and Rönnbäck, 2004). Additionally, the seagrass *Thalassia ciliatum* is popular for the relief of small pox and fever while *Enhalus acoroides* roots are used as a remedy against stings from different kinds of rays, muscle pain, fever, wounds and stomach problems (De La Torre-Castro and Rönnbäck, 2004), including typhoid.

Typhoid fever is a potentially severe and occasionally life-threatening bacterial illness caused by the bacterium *Salmonella enterica* serovar *typhi* (commonly known as *Salmonella typhi*). The illness is often characterized by the insidious onset of sustained fever, headache, malaise, anorexia, relative bradycardia, constipation or diarrhea, and non-productive cough. The disease is endemic to areas that are characterized by rapid population growth, increased urbanization, limited safe water and health systems, such as Africa, India, South and Central America (Uneke, 2008). The disease is known to be transmitted through the fecal-oral route via contaminated water and food, especially by food-handling carriers, and human beings are the only known reservoir and host for typhoid fever (Butter, 1992). Typhoid fever is of important socioeconomic impact because it may take several months for a patient to recover and be able to work normally again. According to the World Health Organization (WHO), the morbidity and mortality rate caused by typhoid fever worldwide was estimated to be 11 - 20 million cases leading to 128,000-161,000 deaths every year, and poor communities and vulnerable groups including children are most affected (World Health Organization, 2018). In addition, climatic variables such as rainfall, vapour pressure and temperature have an important effect on the transmission and distribution of typhoid infections in human populations (Kelly-Hope *et al.*, 2007). A study conducted in Vietnam showed that typhoid incidence was seen to increase with temperature, rainfall and river level at time lags ranging from three to five weeks. For example, it was shown that for a 0.1 meter rise in river level, the number of typhoid cases increased by 4.6 % above the threshold of 4.0 meters. On the other hand, with a 1 °C rise in temperature, the number of typhoid cases could increase by 14.2 % (Dewan *et al.*, 2013). Moreover, it was also reported that typhoid incidences varied with geographical and other environmental conditions. For instance, in Zhejiang Province, China, auto-correlation analysis and spatio-temporal scan statistics revealed that high-risk clusters of typhoid fever were located mainly in the coastal regions but scattered across the province (Hua *et al.*, 2017). Along the coast of Tanzania on Pemba Island a rate for typhoid fever of 110 cases/100,000 population/year was reported (Thriemer *et al.*, 2012) while in Singida region (inland), the incidences of 580 - 1,400/100,000 persons were observed (Malisa and Nyaki, 2010). As for other diseases, the biggest challenge for typhoid is the emergence and spread of multidrug-resistant strains of bacteria causing typhoid fever, leading to significant morbidity and mortality (Gupta, 1994). In Tanzania, Msemo *et al.* (2019) reported that 89.9 % of patients diagnosed with *S. typhi* were resistant to amoxicillin, 81.0 % to chloramphenical and 92.1 % to trimethoprim sulfamenthoxazole. Hence, there is a great need to explore and develop new drugs from locally used medicinal plants.

Worldwide, plants have been found to be interesting sources of new drugs to overcome the antimicrobial resistace challenge. It is reported that about 80,000 flowering plants are used in medicine throughout the world (Leamann, 2011). Likewise, various seagrasses have been reported to have phytochemical compounds such as tannins, sterols, terpenoids, steroids, catachols and flavanoids with potential for pharmacological development (Yuvaraj *et al.*, 2012; Regalado *et al.*, 2012; Rengasamy *et al.*, 2013; Goda *et al.*, 2020; Kim *et al.*, 2021). In Tanzania most of the population depend on medicinal plants for their primary health care and about 1200 species of higher plants have been used as medicine (Mahunnah *et al.*, 2012). However, little is known on the potential natural products from the marine environment, in particular seagrasses. In this study the antibacterial activities of the extracts of seven seagrass species were explored against pathogenic bacteria *S. typhi*. These species included *Cymodocea serrulata, Thalassia hemprichii, Halodule uninervis, Thalassodendron ciliatum, Enhalus acoroides*, *Cymodocea rotundata* and *Syringodium isoetifolium* from the Tanzanian coast. The results obtained provides baseline information to corroborate indigenous knowledge and may be useful in development of novel antibacterial drugs that will help to solve the problems of antibiotic resistance of the pathogenic bacteria *S. typhi*.

Materials and methods

Sample collection

Samples of the seagrasses *C. serrulata, T. hemprichii, H. uninervis, T. ciliatum, E. acoroides*, *C. rotundata* and *S. isoetifolium* were handpicked during low tide from the intertidal beaches of Mjimwema (06°50'S, 39°21'E), 4 km south of Dar es Salaam harbor, and Bagamoyo (6°27'32"S, 38°56'E) between Bagamoyo fish landing site and Kaole ruins. Seagrasses were identified in the field using standard identification guide books such as Richmond (2011). The seagrass leaves and roots were separated and transported to the Department of Molecular Biology and Biotechnology (DMBB), University of Dar es Salaam (UDSM) for further laboratory analysis.

Preparation of extraction

The collected seagrass samples were kept on laboratory benches to dry for 10 – 14 days away from direct sunlight until a constant weight was achieved. The dried leaves and roots of seagrasses were then ground to a fine powder using a grinding machine (Laboratory mill model 4).

Extraction of crude extracts from seagrasses

Crude extracts were obtained as described by Rengasamy *et al.* (2010), where 100 g of the seagrass powder was soaked in 500 ml of hexane (non-polar), dichloromethane (less polar) and methanol (polar) by increasing the order of polarity for 48 hours at room temperature in a shaker (Edmund Buhler 7400). The solvents with extracts were concentrated by using a rotary evaporator (BUCHI Rota vapor model R-210). The concentrated crude extract was stored at 4 °C for antibacterial sensitivity assay against *S. typhi* and identification of compounds.

Test organism

S. typhi (ATCC 14028) obtained from the Department of Molecular Biology and Biotechnology (DMBB), University of Dar es Salaam (UDSM), was used as a test organism. The bacteria were grown in nutrient broth and incubated at 37 °C for 24 hours to obtain fresh culture prior to analysis.

Screening of antimicrobial activities of crude extracts from seagrasses species

The antibacterial activities of seagrass extracts against *S. typhi* were determined by the disc diffusion method as explained by Sosovele *et al.* (2012). The test organism was cultured separately on a Muller-Hinton agar plate. A 100 mg/ml concentration of the extracts were made using 99.9 % Dimethyl sulfoxide (DMSO) and loaded onto a sterilized paper disc, dried and placed on agar plates inoculated with freshly grown colonies of the test organism. Thereafter, the diameter of the inhibition zone (IZ) was measured. A Tetracycline disc (1.0 mg/ml) was used as a positive control and a DMSO disc without plant extract was used as a negative control.

Minimum Inhibitory Concentration (MIC) assay

The micro dilution technique using 96-well microtitre plates was used to determine the minimum inhibitory concentration (MIC) of the crude extracts obtained from the seagrass species. The plate was pre-loaded with 25 µl of Muller-Hinton broth in each well, then 25 µl of 100 mg/ml crude extract was added to each well in the first row to make a total volume of 50 µl with a concentration of 50 mg/ml of crude extract. After thorough mixing, serial two-fold dilution was carried out by drawing 25 µl from each well of the first row and placed in the next row of wells. The process were repeated downward the columns to the last wells, resulting in concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 mg/ml. Thereafter, 25 µl of *S. typhi* bacterial suspension prepared, equivalent to 0.5 McFarland standard turbidity was added to each well to make a final volume of 50 µl, and halving the concentrations in the first row throughout. Thus, the starting concentration was 25 mg/ml and the final concentration was 0.195 mg/ml (Nondo *et al.*, 2011). The rows that contained only broth and bacterial suspension were used to monitor the growth of bacteria, while the rows containing the 25 µg/ml of tetracycline were used as positive control and rows containing DMSO (10 %) as a negative control. The 96-well microtitre plates were then incubated at 37 °C for 24 hours. The MIC of each tested extract was determined by the addition of 30 µl of 0.02 % p-iodonitrotetrazolium (INT) chloride in each well an hour before reading the results (i.e. 23 hours of incubation). Thereafter the plates were incubated for the remaining incubation period at 37 °C. After 24 hours of incubation, bacterial growth was indicated by the presence of pink coloration. The minimum inhibitory concentration was determined as the lowest concentration which showed no bacterial growth (Nondo *et al.* 2011).

Brine shrimp lethality assay (Cytotoxicity)

The brine shrimp lethality assays of different seagrass crude extracts were carried out at DMBB following standard procedures (Meyer *et al.*, 1982). This test was done to determine whether the seagrass crude extracts possessed toxic compounds that could cause cell damage or death. One teaspoon full of brine shrimp eggs were hatched in 1000 ml filtered seawater in a container and incubated for 24 hours under illumination using an electric bulb (120 Volts). A stock solution of 40 mg/ml concentration of each crude extract was prepared by dissolving the extract with 99.9 % DMSO, from which different concentrations (240, 120, 80, 40 and 24 µg/ml) were made and kept in vials containing ten brine shrimp larvae. Each concentration of the extract was tested in duplicate. The vials containing brine shrimp larvae, DMSO (0.6 %) and seawater were set as a negative control. Incubation was carried out for 24 hours at room temperature after which incubation vials were observed against a light background.

The number of dead larvae were counted and the mean obtained was subjected to analysis using Microsoft Excel. The graph of the brine shrimp percentage mortality rate against log concentration was plotted, and the regression equation was obtained. From the equation, LC_{50} (µg/ml) was determined as described by Throne *et al.* (1995). The obtained data were interpreted as follows: LC_{50} <1 µg/ml were regarded as highly toxic; LC_{50} > 1.0 and < 10.0 µg/ml as toxic; LC_{50} > 10 and <30 µg/ml as moderately toxic; LC_{50} > 30 and < 100 µg/ml as mildly toxic; and LC_{50} > 100 µg/ml as non-toxic (Meyer *et al.*, 1982; Bastos *et al.*, 2009).

Phytochemical analysis

The qualitative test for the identification of phytochemical constituents of alkaloids, glycosides, flavonoids, phenols, tannins, phytosterols, saponins and diterpenes were determined from methanol and dichloromethane extracts only since there were insufficient samples from hexane extracts. The analyses were carried out according to standard procedures described by Junaid and Patil (2020), with minor adjustment. In order to get seagrass extract solutions for phytochemical analysis, the methanol and dichloromethane extracts were dissolved in their respective solvents. The procedures for each constituent is as described below:

Test for Alkaloids: 0.5 ml of extracts was mixed with 1.0 ml dilute HCl. The mixture was filtered before addition of 2 drops of Wagner's reagent. Formation of a brown/reddish precipitate indicates the presence of alkaloids.

Test for Flavonoids: One drop of 10 % ferric chloride solution was added to a drop of extract aqueous solution and appearance of a green precipitate indicated the presence of flavonoids.

Test for Glycosides: One drop of extracts was mixed with one drop of bromine water. Formation of a yellow precipitate indicates the presence of Glycosides.

Test for Phenols: A few drops of seagrass extract aqueous solution was added to a few drops of 5 % ferric chloride solution. The presence of Phenol was observed by a dark green/bluish black colour.

Test for Tannins: 0.4 ml of seagrass extract was mixed with 4.0 ml of 10 % NaOH and shaken well. The presence of tannis was determined by the formation of an emulsion (Hydrolysable tannins).

Test for Saponins: Distilled water (2.5 ml) was added to 0.5 ml of seagrass extract in a test tube and shaken vigorously. The presence of Saponin was determined by the formation of persistent frothing.

Test for Diterpenes: Three drops of seagrass extract was dissolved in distilled water and three drops of copper acetate solution was added. Formation of an emerald green colour showed the presence of diterpenes.

Test for Phytosterols: 0.4 ml of chloroform was treated with the seagrass extract. The mixture was filtered before addition of a few drops of concentrated H_2SO_4, shaken well and allowed to stand to observe if a red color developed in the lower layer in the test tube.

Typhoid data

Typhoid case data (outpatient and inpatient) for all Tanzania mainland regions were obtained from the then Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC) in September 2021. Currently, the name of the minitry is the Ministry of Health (https://www.moh.go.tz).

Data analysis

Inhibition zones were presented as the mean plus/minus (±) standard deviation, while minimum inhibition concentration and typhoid fever data were presented as a whole number, with the phytochemical compounds reported as present (+) or absent (-). Statistics were carried out using Graph Pad Instant t_m 1990-1993 software, where a P-Value less than 0.05 was considered significant. Prior to analysis the data were subjected to normality test.

Results

Antibacterial activity of seagrass extracts against *S. typhi*

Extracts obtained using three different solvents (hexane, dichloromethane and methanol) revealed different antibacterial activities against *S. typhi* at a concentration of 100 mg/ml, as shown in Table 1. For each solvent, there were 14 extracts (i.e. from roots and leaves of the seven seagrass species), and hexane showed activity in all extracts, dichloromethane showed activity in five extracts, while methanol showed activities in only one extract. Hexane extracts showed maximum inhibition zone diameters (IZ) ranging from 7.0 ± 0.0 to 10.0 ± 0.0 mm (Table 1). The IZ of negative and positive controls were 0.0 and 22 mm, respectively. Statistically (using repetitive measure ANOVA) the results revealed that hexane extracts had significantly

Table 1. Zones of inhibition (diameter) (IZ) of seagrass extracts at 100 mg/mL concentration against *S. typhi*. The IZ for negative and positive controls were 0.0 and 22.0 mm, respectively.

Seagrass species	Solvents	Zone of inhibition (mm)	
		Roots	Leaves
Cymodocea rotundata	Hexane	10.0 ± 0.0	7.7 ± 0.6
	Dichloromethane	0.0 ± 0.0	8.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Cymodocea serrulata	Hexane	8.5 ± 0.7	7.7 ± 0.6
	Dichloromethane	8.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Halodule uninervis	Hexane	9.0 ± 1.4	8.0 ± 0.0
	Dichloromethane	9.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	7.5 ± 0.7
Thalassia hemprichii	Hexane	8.5 ± 0.7	7.0 ± 0.0
	Dichloromethane	7.0 ± 0.0	7.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Thalassodendron ciliatum	Hexane	8.0 ± 0.0	9.0 ± 0.0
	Dichloromethane	0.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Syringodium isoetifolium	Hexane	8.0 ± 0.0	9.0 ± 0.0
	Dichloromethane	0.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Enhalus acoroides	Hexane	9.0 ± 0.0	9.5 ± 0.7
	Dichloromethane	0.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0

Key: 0.0 = no zone of inhibition

stronger antimicrobial activities against *S. typhi* than other solvent extracts ($P < 0.0001$). In terms of seagrass species, *H. uninervis* and *T. hemprichii* had more antimicrobial activities than others, whereas out of six extracts, four showed activity, followed by *C. rotundata* and *C. serrulata* which showed activity in three extracts. The least antibacterial activity was shown by *T. ciliatum*, *S. isoetifolium* and *E. acoroides*, and with hexane extracts only (Table 1). However, there were no significant differences in antimicrobial activities against *S. typhi* among the seagrass species ($P = 0.448$). Furthermore, there were no significant differences in antibacterial activities against *S. typhi* between roots and leaves (Wilcoxon matched-pairs signed-ranks test the P value = 0.289).

Out of 42 seagrass extracts only 26 were found to have a MIC at or below the cut off value (3.13 mg/ml), in which for each seagrass species at least one extract was sensitive to *S. typhi* with a MIC at or below the cutoff point (Table 2). Hexane and dichloromethane extracts showed a MIC value within the cut off range in all of the seagrasses while methanol extracts were sensitive at 3.13 mg/ml in only two seagrass species (*H. uninervis* and *T. hemprichii*) (Table 2). The MIC of the positive control (Tetracycline) was below 0.019 mg/ml while the negative control was ≥ 25 mg/ml. Statistically, hexane extracts were extremely sensitive to *S. typhi* ($P < 0.0001$) as compared to dichloromethane and methanol extracts. Furthermore, the seagrass *H. uninervis* and *C. serrulata* extracts were more sensitive to *S. typhi* with the MIC value of 0.39 mg/ml. However, there were no significant differences ($P = 0.663$) in MIC among the seagrasses. In addition, both leaves and roots of all the seagrasses showed more or less similar minimum inhibitory activities in all the tested solvents (Table 2).

Generally, the results showed that most of the seagrass extracts had low a cytotoxicity level (non toxic), with *C. rotundata* having the lowest level (LC_{50} = 2521.31 mg/ml), while *T. hemprichii* extracts had the highest level (LC_{50} = 0.038 mg/ml) (Table 3), regarded as mildly toxic. Statistically, the results revealed significant differences in toxicity among the seagrasses ($P = 0.018$), with the post hoc test Turkey Kramer multiple comparison test showing the differences were between *C. rotundata* and *S. isoetifolium* ($P < 0.05$), and between *C. serrulata* and *S. isoetifolium* ($P < 0.01$). On comparing extracts from different solvents, dichloromethane extracts of all the seagrasses showed a lower cytotoxicity level compared to hexane and methanol extracts (Table 3). Furthermore, on comparing below and above ground parts, all seagrasses had more or less similar trends in cytotoxicity level (Table 3). There were no significant differences ($P > 0.05$) in toxicity among the extracts from different solvents as well as between the roots and shoots.

Table 2. Minimum inhibitory concentrations (MIC) of seagrass extracts against *S. typhi*. The MIC value for negative and positive controls were ≥ 25 and 0.019 mg/ml, respectively.

Seagrass species	Solvent	Minimum inhibitory concentrations (mg/ml)	
		Roots	Leaves
Cymodocea rotundata	Hexane	1.56	3.13
	Dichloromethane	3.13	1.56
	Methanol	12.5	12.5
Cymodocea serrulata	Hexane	1.56	0.39
	Dichloromethane	1.56	3.13
	Methanol	>25	6.25
Halodule uninervis	Hexane	1.56	1.56
	Dichloromethane	3.13	0.39
	Methanol	6.25	3.13
Thalassia hemprichii	Hexane	1.56	0.78
	Dichloromethane	0.78	3.13
	Methanol	>25	3.13
Thalassodendron ciliatum	Hexane	3.13	>25
	Dichloromethane	1.56	6.25
	Methanol	>25	6.25
Syringodium isoetifolium	Hexane	1.56	1.56
	Dichloromethane	1.56	1.56
	Methanol	>25	12.5
Enhalus acoroides	Hexane	3.13	3.13
	Dichloromethane	NT	NT
	Methanol	>25	12.5

Key: NT = Not Tested

The findings revealed that out of eight phytochemical groups examined, seven groups (except phytosterol) were detected in the studied seagrasses (Table 4). Six groups, namely alkanoids, saponins, tannis, diterpenes, flavonoids and cardiac glycosides were found in both the seagrass root and leaf extracts, while the phenolic group was exclusively found in leaf extracts (Table 4). The results further revealed that alkaloids, tannins and diterpenes were the most common groups found in almost all extracts. Among the studied seagrasses, all the seven detected phytochemical groups were found in *C. serrulata* and *T. hemprichii*, six groups were found in *T. ciliatum* and *E. acoroides*, while five groups were found in *H. uninervis*, *C. rotundata* and *S. isoetifolium.*

Data on the typhoid fever records in mainland Tanzania for five years (2016-2020) indicated that there

Table 3. Cytotoxicity of selected seagrass crude extracts to brine shrimp larvae (LC_{50} values in mg/ml).

Seagrass species	Extracts	Toxicity (mg/ml)	
		Leaves	Roots
Cymodocea rotundata	Hexane	0.80	0.11
	Dichloromethane	2521.31	2.80
	Methanol	0.86	1.24
Cymodocea serrulata	Hexane	10.38	0.07
	Dichloromethane	2.93	25.44
	Methanol	0.12	0.05
Halodule uninervis	Hexane	0.24	0.06
	Dichloromethane	3.82	0.14
	Methanol	NT	NT
Thalassia hemprichii	Hexane	0.22	0.04
	Dichloromethane	7.16	16.44
	Methanol	0.16	0.12
Thalassodendron ciliatum	Hexane	0.15	0.84
	Dichloromethane	1.60	2.94
	Methanol	0.06	NT
Syringodium isoetifolium	Hexane	NT	NT
	Dichloromethane	722.39	2.89
	Methanol	NT	NT
Enhalus acoroides	Hexane	0.175	NT
	Dichloromethane	5.767	0.91
	Methanol	NT	NT

Key: NT = Not Tested due to insufficient amount of extract

Table 4. Phytochemical analysis of the selected seagrass methanol and dichloromethane extracts.

Seagrass species		Alkaloids	Flavonoids	Saponins	Tannins	Phenolic	Phytosterol	Cardiac glycosides	Diterpenes
Methanol									
Cymodocea rotundata	Leaves	-	-	+	-	-	-	-	-
	Roots	+	-	+	+	-	-	+	-
Cymodocea serrulata	Leaves	-	+	-	+	+	-	-	+
	Roots	-	-	+	+	-	-	+	-
Halodule uninervis	Leaves	-	-	+	-	-	-	+	+
	Roots	-	-	+	-	-	-	+	+
Thalassia hemprichii	Leaves	-	-	-	+	+	-	-	+
	Roots	+	+	+	+	-	-	+	+
Thalassodendron ciliatum	Leaves	-	+	+	+	+	-	-	-
	Roots	+	-	-	-	-	-	-	-
Syringodium isoetifolium	Leaves	+	-	-	+	-	-	-	+
	Roots	+	-	-	+	-	-	-	-
Enhalus acoroides	Leaves	+	+	+	+	-	-	-	+
	Roots	+	-	-	-	-	-	-	+
Dichloromethane									
Cymodocea rotundata	Leaves	-	-	+	+	-	-	-	+
	Roots	+	-	-	+	-	-	+	-
Cymodocea serrulata	Leaves	-	-	-	+	-	-	+	-
	Roots	+	-	+	+		-	+	-
Halodule uninervis	Leaves	NT	NT	NT	NT	NT	NT	NT	NT
	Roots	+	-	+	+	-	-	+	+
Thalassia hemprichii	Leaves	+	-		-	+	-	-	+
	Roots	+	-	-	+	-	-	+	+
Thalassodendron ciliatum	Leaves	-	-	-	+	+	-	+	-
	Roots	+	-	+	+	-	-	+	-
Syringodium isoetifolium	Leaves	-	+	-	+	+	-	+	-
	Roots	-	-	-	+	-	-	+	-
Enhalus acoroides	Leaves	NT	NT	NT	NT	NT	NT	NT	NT
	Roots	-	-	+	+	-	-	+	-

Key: + = present; – = absent; NT = Not tested

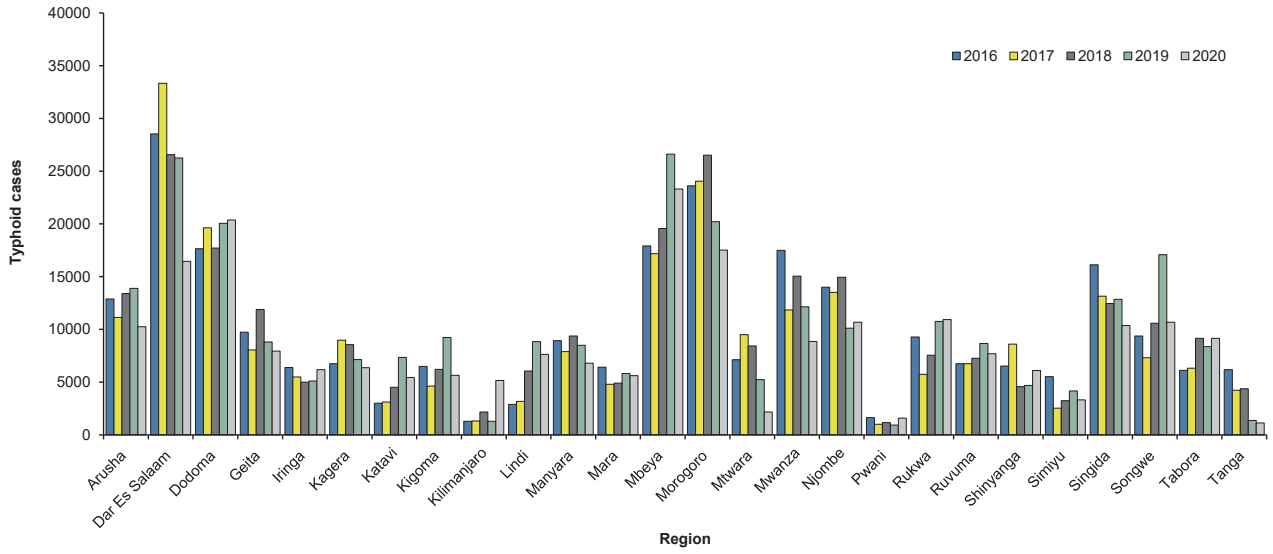

Figure 1. Total typhoid fever cases for each region in Tanzania Mainland from 2016 – 2020.

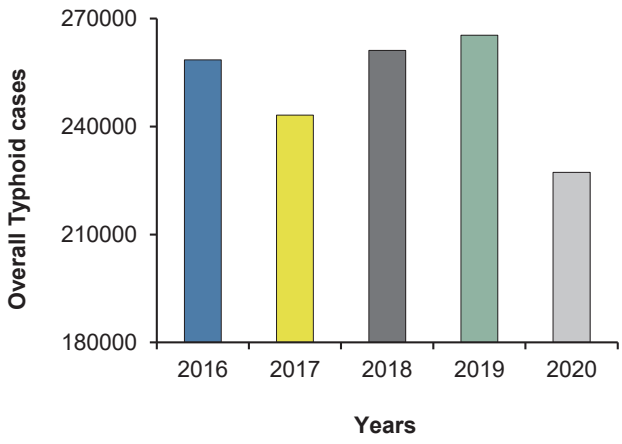

Figure 2. Overall typhoid fever cases for Tanzania Mainland from 2016 – 2020.

is a spatial and temporal fluctuating trend of typhoid occurrence. The numbers of cases were higher in the largest city Dar es Salaam, followed by Morogoro, Mbeya and Dodoma, with the Coast Region (Pwani) having the lowest typhoid cases (Fig. 1). In terms of yearly trends, the total number of typhoid fever cases showed that the disease burden was more or less constant in the five years studied although the numbers decreased from 258,560 cases in 2016 to 243,203 in 2017, followed by an increase in 2019 to 265,355, and finally further down in 2020 to 227,259 cases (Fig. 2). Likewise, the proportion of typhoid from all diagnoses (Out Patient Department/In Patient Department) was more or less constant over the five years (2016-2020) as shown in Table 5.

Discussion

Seagrasses are rich in secondary metabolites; some of which have important pharmacological properties with potential for treatment of human diseases (De La Torre-Castro and Rönnbäck, 2004; Athiperumalsamy *et al.*, 2008; Nazar *et* al., 2009; Regalado *et al.*, 2012; Yuvaraj *et al.*, 2012; Rengasamy *et al.*, 2013; Kim *et al.*, 2021). In the present study, extracts from seven seagrass species, namely *C. serrulata, C. rotundata, T. hemprichii, H. uninervis, T. ciliatum, E. acoroides* and *S. isoetifolium* showed varying antibacterial activities against the tested Gram-negative pathogen *S. typhi*. The findings corroborate similar observations where the three seagrasses species *C. serrulata, Halophila ovalis* and *Zostera capensis* from the South Indian coast were effective against Gram-negative bacteria including *Salmonella* species as well as Gram-positive bacteria (Kumar *et al.*, 2008; Rengasamy *et al.*, 2013). Different studies show that extraction solvents have an effect on the yield (amount of extract) and the constituents of bioactive compounds, thus significantly affecting the biological activity of the extract (Alam *et al.*,

Table 5. Percentage occurrence of typhoid in diagnoses (Out Patient Department/In Patient Department) in Tanzania Mainland Regions.

Region	2016	2017	2018	2019	2020
Arusha	0.9	0.7	0.7	0.7	0.6
Dar es Salaam	0.6	0.6	0.5	0.4	0.3
Dodoma	1.3	1.4	1.0	1.2	1.1
Geita	1.0	0.8	0.8	0.5	0.5
Iringa	0.9	0.7	0.6	0.5	0.7
Kagera	0.4	0.4	0.4	0.3	0.3
Katavi	1.1	1.1	1.1	1.4	1.1
Kigoma	0.4	0.2	0.2	0.3	0.2
Kilimanjaro	0.1	0.1	0.1	0.1	0.3
Lindi	0.3	0.3	0.6	0.7	0.7
Manyara	1.1	0.9	0.9	0.8	0.7
Mara	0.5	0.4	0.4	0.4	0.4
Mbeya	1.8	1.6	1.6	1.8	1.7
Morogoro	1.4	1.3	1.1	0.8	0.7
Mtwara	0.6	0.7	0.6	0.3	0.2
Mwanza	1.1	0.7	0.7	0.5	0.4
Njombe	2.9	2.5	2.3	1.5	1.6
Pwani	0.1	0.1	0.1	0.0	0.1
Rukwa	2.0	1.1	1.2	1.5	1.6
Ruvuma	0.6	0.5	0.5	0.5	0.5
Shinyanga	0.6	0.8	0.4	0.4	0.5
Simiyu	0.8	0.4	0.4	0.5	0.5
Singida	1.6	1.3	1.0	1.0	0.9
Songwe	2.1	1.9	1.9	2.6	1.8
Tabora	0.5	0.5	0.5	0.4	0.4
Tanga	0.3	0.2	0.2	0.0	0.0
Average	0.8	0.7	0.6	0.6	0.8

1994; Sastry and Rao, 1994; Turkmen *et al.*, 2006; Rengasamy *et al.*, 2013; Truong *et al.*, 2019). In this study, hexane extracts had higher potential antimicrobial activities compared to dichloromethane and methanol extracts indicating that the extraction efficiency favoured the non-polar solvents. The results concurred with Sastry and Rao (1994), in which the non-polar extracts had more antibacterial activities than polar. However, they differ from some studies on marine plants that showed methanol (polar) extracts to have higher antimicrobial properties against both Gram-negative and Gram-positive bacteria compared to hexane extract (Alam *et al.*, 1994; Kumar *et al.*, 2008; Rengasamy *et al.*, 2013). Thus polarity of the extraction solvents could cause variations in the level of bioactive compounds of the extract. Apart from solvent, extraction method, temperature, extraction time and phytochemical composition are considered as factors that affect the efficacy of the extraction (Turkmen *et al.*, 2006).

Extracts from *H. uninervis* and *C. rotundata* in all the three solvents showed greater antibacterial activities against *S. typhi* compared to other seagrass species. The results presented here are consistent with some previous studies that different seagrass species might have varied antibacterial activities on different or similar microorganisms. For instance, *C. rotundata* extract was reported to be more effective against several strains of bacteria species as compared to other marine plants (Bhosale *et al.*, 2002), while *Halophila* sp. was reported to have more antimicrobial activity than *Cymodocea* sp. (Kumar *et al.*, 2008). Bushmann and Ailstock (2005) reported that although most seagrasses possess antibacterial compounds, their effects vary between species, locations and seasons.

The presence of the important phytochemical compounds such as tannins, saponins, phenolic, flavonoids, alkaloids, tannins and diterpenes in at least one of the seagrasses is reported, some of which were also reported in previous studies in marine extracts (Kumar *et al.*, 2008; Goda *et al.*, 2020; Kim *et al.*, 2021). As such, the result indicate that seagrasses from the Tanzania coast could be further studied for their active compound potential for the production of drugs against typhoid fever and other diseases.

Most of the studied seagrass extracts had low cytotoxicity properties (non toxic) with the higher value of LC_{50} ranging from 0.11 - 2,521.31 mg/ml. This is in agreement with results obtained from the study by Rengasamy *et al.* (2013) in the Gulf of Mannar, South India which showed low cytotoxicity properties from six seagrass species extracts, with *S. isoetifolium* exhibiting the lowest cytotoxicity level. The observed low toxicity activities of the seagrasses substantiate the usefulness and potential of using seagrasses for the development of pharmaceutical therapy against diseases caused by bacteria.

The typhoid data indicated that the disease is still a public health challenge facing Tanzania. This has also been shown in previous studies in the Singida region (from 2003 to 2007), where there were fluctuations in the incidence of typhoid, and where it was speculated that the reasons could be either water scarcity or lack of access to safe water, improper drainage systems and problems of unhygienic toilets (Uneke, 2008; Thriemer *et al.*, 2012). Despite being close to Dar es Salaam and Morogoro, the Coast Region (Pwani) was found to have a low incidence of typhoid cases. This could possibly be attributed to the use of plant extracts (including from seagrasses) for medical purposes against various illnesses. This may have reduced the number of cases reported in hospitals. As previously reported, people use seagrass as a treatment for fever and stomach ailments on the Tanzanian coast (De La Torre-Castro and Rönnbäck, 2004); symptoms similar to those shown by typhoid fever. Additionally, diet might be an important factor since it was previously reported that most of the coastal communities depend highly on the protein sources from the intertidal seagrass ecosystems (De La Torre-Castro and Rönnbäck, 2004).

In conclusion, the present study reports for the first time that seagrasses of the Western Indian Ocean, specifically Tanzania, contain promising antibacterial bioactive compounds against *S. typhi*. The seagrass *H. uninervis* and *C. rotundata* are of interest due to their strong antibacterial activities and low cytotoxicity levels. Thus they are recommended for further clinical assessment for pharmaceutical production of drugs. The phytochemical analyses of the extracts revealed the presence of phytochemicals such as saponins, tannins, alkaloids, cardiac glycosides, diterpenes and flavonoids that may account for the antibacterial activities observed. The current findings also support the local use of these seagrass for medicine. Further studies are required to corroborate the current findings for the development of a pharmaceutical industry using seagrasses as a therapy against typhoid fever and other diseases.

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