Biomass Conversion and Biorefinery (2024) 14:15587–15595
https://doi.org/10.1007/s13399-022-03708-2

ORIGINAL ARTICLE

Antioxidant and anticoagulant activity of crude polysaccharide and α-L-Rhamnose from *Grateloupia lithophila*

Palaniappan Seedevi[1]

Received: 27 September 2022 / Revised: 8 December 2022 / Accepted: 22 December 2022 / Published online: 29 December 2022

Abstract

The present study was to discover the antioxidant and anticoagulant activity of crude polysaccharide and α-L-Rhamnose from *Grateloupia lithophila*. Both the crude and purified polysaccharide contained higher portion of α-L-rhamnopyranosyl residues. The crude polysaccharide and α-L-Rhamnose showed the antioxidant activities in concentration manner, while increasing the concentration, the activity level was increased. The crude polysaccharide and α-L-Rhamnose displayed the total antioxidant activity (31.1–64.22% and 39–74.11% at 50–250 µg/ml), DPPH radical scavenging activity (22.5–69.91% and 29.89–83.31% at 10–160 µg/ml), ABTS scavenging activity (25.04–74.46% and 33.01–87.32% at 25–125 µg/ml), reducing power (1.23–16.11% and 2.54–21.92% at 50–400 µg/ml). Additionally, crude polysaccharide and α-L-Rhamnose have anticoagulant properties, through the APTT and PT assays on human blood coagulation were assessed. The APTT anticoagulant activity of crude polysaccharide and α-L-Rhamnose were 23 IU and 28.2 IU at 25 µg/ml; following the PT assay of the crude polysaccharide and α-L-Rhamnose reported 1.92 IU and 3.58 IU at 25 µg/ml. Above results, the crude polysaccharide and α-L-Rhamnose had higher portion of rhamnose and their molecular weight may possess the antioxidant and anticoagulant activity. So, the crude polysaccharide and α-L-Rhamnose from *G. lithophila* could be used as a potential natural source of antioxidant and anticoagulant, as a food supplement, or as a feature in the pharmaceutical industries.

Keywords Crude polysaccharide · α-L-Rhamnose · Antioxidant · Anticoagulant

1 Introduction

Polysaccharides derived from seaweeds are important free-radical scavengers and anti-oxidants in the prevention of oxidative stress in living animals [1]. Positive anti-oxidant systems are known to shield marine algae from oxidative stress, and among them, marine algae remain one of the richest potential anti-oxidant sources [2]. Furthermore, byproducts of marine food production can be easily transformed into nutraceuticals and functional food ingredients with antioxidant properties [3]. The majority of research on marine-derived antioxidants has only examined the efficacy of crude extracts, with only a minimal amount of purification and characterization of such antioxidants [4].

Several studies were then conducted to confirm the antioxidant properties of seaweeds [5,6]. However, seaweed polysaccharides are thought to be a moral foundation of anti-oxidants [7]. Sulfated polysaccharides from the marine algae (seaweed) *Laminaria japonica* [8], *Ecklonia kurome* [9], *Fucus vesiculosus* [2], *Porphyra haitanesis* [10], and *Ulva pertusa* [11] have been shown to have antioxidant properties over the years. There have been few studies on the relationship amid the structure and antioxidant activity of sulfated polysaccharides derived from seaweeds. Glucan and non-glucan polymer free radical scavenging activities discovered that polyelectrolytes like glucan sulfate or phosphate may boost scavenging activity [12].

Furthermore, seaweed-derived sulfated polysaccharides have been shown to have anticoagulant activity comparable to or greater than heparin [13]. Furthermore, an anticoagulant sulfated polysaccharide from *Monostroma nitidum* (Chlorophyceae) was reported to have six times the activity of heparin [14]. In comparison, red and green seaweed

✉ Palaniappan Seedevi
seedevisalem@gmail.com

[1] Department of Product Development, Institute of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 602105, Tamil Nadu, India

extracts have lower anticoagulant activity than seaweed extracts [15, 16]. Recently, methanol extract of the red seaweed *G. lithophila* reported the antibacterial activity against some clinical pathogenic bacteria [17]. In addition, rhamnose-enriched polysaccharide from *G. lithophila* proved potent anti-diabetic activity against STZ-induced rats [18]. Keeping in mind the importance of seaweed polysaccharide as an antibacterial, antioxidant, anticoagulant, and antidiabetic activity, the current study attempted to determine the in-vitro antioxidant and anticoagulant effects of crude polysaccharide and α-L-Rhamnose derived from *G. lithophila*.

2 Material and Methods

2.1 Seaweed collection, identification, and pre-treatment

The Seaweed *G. lithophila* was collected during the low tide period from the intertidal region (up to 0.5 to 2 m depth) from the coastal area of Tranquebar (Lat. 11°01′ N; Long. 79°51′ E) in Tamil Nadu, Southeast coast of India. The seaweed was identified using the standard systematic key described by Umamaheswara rao [19]. The seaweed was washed with seawater followed by tap water, distilled water, and then shadow-dried. The dried seaweed was powdered in a mixer grinder for further extraction.

2.2 Isolation crude polysaccharide

The isolation of crude polysaccharides was carried out following the procedure described by Seedevi et al. [20]. Briefly, 50 g of seaweed powder was dipped in 1:50 volumes (2.5 L) of distilled water and kept at room temperature for 2 h, then homogenized and refluxed at 100 °C for 2 h. After cooling, the mixture was filtered using cheesecloth; further the supernatant was separated from the algal residue by centrifugation at 6000 rpm for 20 min. The residue was further extracted thrice (three times) with double-distilled water at 100 °C for 2 h. All extracts were pooled and concentrated under reduced pressure in a rotary evaporator and dialyzed in cellulose membrane (molecular weight cut off 12,000 kDa) against distilled water for three successive days. The retained fraction was freeze-dried in a lyophilizer (Penguin Plus −4 kg, Lark India) and stored in a refrigerator

2.3 Isolation of α-L-Rhamnose

Seedevi et al. [20] described the isolation of α-L-Rhamnose from *G. lithophila*. The crude polysaccharide was dissolved in distilled water (10 mg/10ml), centrifuged at 6000 rpm for 10 min, and the supernatant was applied to a Q-Sepharose™ (GE-Heathcare) Fast Flow column (EX 150 mm × 50 cm) equilibrated with distilled water. The column was eluted stepwise with distilled water, with a linear gradient of 0–3 mol/ NaCl at a flow rate of 0.55 ml/min and 15 fractions were pooled together, dialyzed against distilled water, and then freeze-dried. Furthermore, the semi-purified polysaccharide was further purified by gel permeation chromatography using a High-Resolution Sepharose 4-LB Fast Flow column (EX 100 mm × 25 cm), equilibrated with distilled water. The column was eluted stepwise with distilled water; with extend of 0.2 mol/l sodium acetate buffer at an elution rate of 0.33 ml/min. The purified fraction was dialyzed against distilled water and then freeze-dried

2.4 Chemical and structural characterization of crude polysaccharide and α-L-Rhamnose

The chemical and structural characterization of crude polysaccharide and α-L-Rhamnose of *G. lithophila* were described previously by Seedevi et al. [20]

2.5 Antioxidant activity

2.5.1 Total antioxidant activity

Total antioxidant activity was assessed using the described method of Arivuselvan et al. [21]. In a flash, 2.0 ml of crude polysaccharide and α-L-Rhamnose at various concentrations (50–250 µg/ml) were combined with 1.0 ml of 0.6 M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate. The mixture was incubated in a water bath at 95 °C for 90 min. Then the mixture was cooled to room temperature and the absorbance of each solution was measured at 695 nm. As standards, L-ascorbic acid and BHT were used.

$$\text{Total antioxidant (\%)} = 1 - \frac{\Delta A \text{ sample} - \Delta A \text{ blank}}{\Delta A \text{ control}} \times 100$$

2.5.2 Scavenging ability on DPPH radicals

The DPPH free radical scavenging activity of crude and α-L-Rhamnose was inspected using the Zhang et al. [22] method. In brief, a 0.1mM DPPH solution in 100% methanol was prepared, and 1ml of this solution was added to 4 ml of sample in 40% methanol at various concentrations (10–160 µg/ml). The mixture was vigorously shaken and incubated at 30

°C in the dark for 15 min. The reduction of the DPPH radical was measured by continuously monitoring the decrease in absorption at 517 nm. As standards, L-ascorbic acid and BHT were used.

$$\text{DPPH scavenging effect (\%)} = 1 - \frac{\Delta A \text{ sample} - \Delta A \text{ blank}}{\Delta A \text{ control}} \times 100$$

2.5.3 Scavenging ability on ABTS radicals

The ABTS radical scavenging activity of crude polysaccharide and α-L-Rhamnose was determined using the method of Giao et al. [23]. This method is based on antioxidant molecules' ability to satisfy the long-lived ABTS+ species, a blue-green chromophore with a typical absorption wavelength of 734 nm. The addition of antioxidants to the preformed radical cation reduces it to ABTS, resulting in color loss. The results were expressed as a percentage of inhibition and the L-ascorbic acid and BHT serving as standards.

$$\text{ABTS scavenging effect (\%)} = 1 - \frac{\Delta A \text{ sample} - \Delta A \text{ blank}}{\Delta A \text{ control}} \times 100$$

2.5.4 Reducing power assay

The reducing power was calculated using the method described by Zhang et al. [22]. In a nutshell, 1ml of sample solution (50–400 µg/ml) in phosphate buffer (0.2 M pH 6.6) was combined with 1ml of potassium ferricyanide (1%, w/v) and incubated at 50 °C for 20 min. The reaction was then stopped by adding 2.0 ml of TCA (10% w/v) and the solution was mixed with 1.25 ml of ferric chloride (0.1% w/v) before measuring at 700 nm. As standards, L-ascorbic acid and BHT were used.

2.6 Anticoagulant activity

2.6.1 APTT assay

The anticoagulant activity of the crude polysaccharide and α-L-Rhamnose was determined using the activated partial thromboplastin time (APTT) coagulation assay. In brief, citrated normal human plasma (90 µl) was mixed with 10 µl of sample separately and incubated for 1 min at 37 °C in separate glass vials. Then, 100 µl of bovine cephalin was added and incubated at 37 °C for 3 min. After the incubation, 100 µl of pre-warmed 0.25 M $CaCl_2$ solution was added, and the clotting time was measured and compared to the standard; the activity was expressed as IU/mg.

2.6.2 PT assay

For crude polysaccharide and α-L-Rhamnose, the Prothrombin Time (PT) assay was used. 90 µl of citrated normal human plasma was mixed with 10 µl of sample separately and incubated at 37 °C for 10 min. The pre-incubated PT assay reagent (200 µl) was added after the incubation period, and the clotting time was recorded.

2.7 Statistical analysis

One-way analysis of variance (ANOVA) was performed on all experimental data, and Dunnett's multiple comparison test (GraphPad Software, USA) was also employed to find differences in means at the level of $p < 0.05$.

3 Results and discussion

3.1 Chemical and structural characterization of crude polysaccharide and α-L-Rhamnose

The chemical and structural characterization of the crude polysaccharide and α-L-Rhamnose from *G. lithophila* were reported in our previous study by Seedevi et al. [18], following, the yield (70% and 39.3% (w/w)), total carbohydrate (75.7 and 89.7%), protein (0 and 0%), ash (18.2 and 3.2 %), moisture (14.8 and 1.3 %), molecular weight

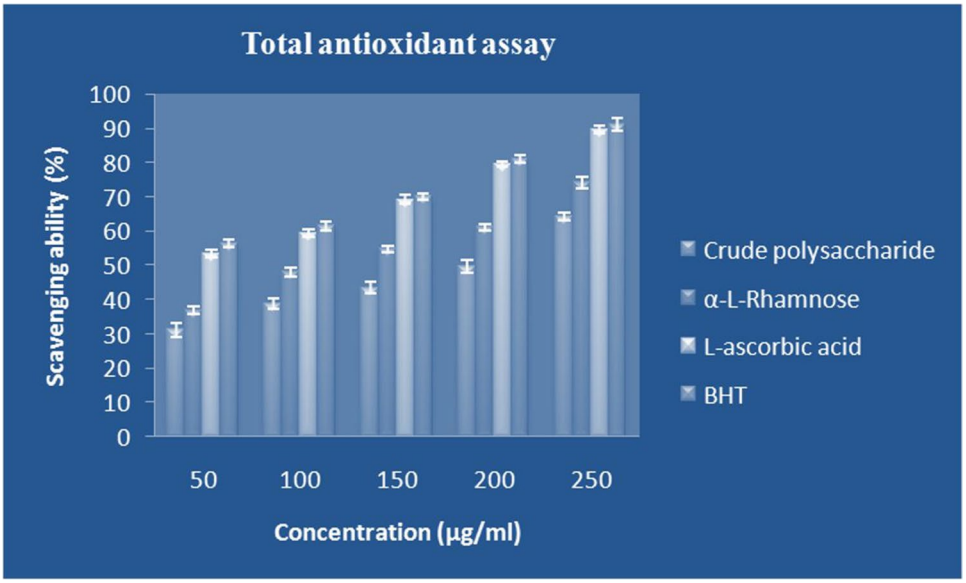

Fig. 1 Total antioxidant activity of crude polysaccharide and α-L-Rhamnose

(37 kDa and 24 kDa) and the monosaccharide composition of the crude polysaccharide reported rhamnose (79.82%), fructose (8.38%), galactose (3.95%), xylose (3.31%), and glucose (1.48%) and the α-L-Rhamnose reported 95.88% of rhamnose, 1.13% of xylose, and 2.21% of fructose. Furthermore, the crude polysaccharide and α-L-Rhamnose were structurally characterized via UV-vis, HPLC, FT-IR, NMR, DSC, and Polarimeter analysis [18].

3.2 Antioxidant activity

3.2.1 Total antioxidant activity

The total antioxidant capacity (TAC) assay determines the antioxidant capacity of natural compounds. At different concentrations (50–250 µg/ml), crude polysaccharide and α-L-Rhamnose demonstrated total antioxidant activity ranging from 31.1 to 64.22% and 39 to 74.11%, respectively (Fig. 1). At a concentration of 250 µg/ml of α-L-Rhamnose,

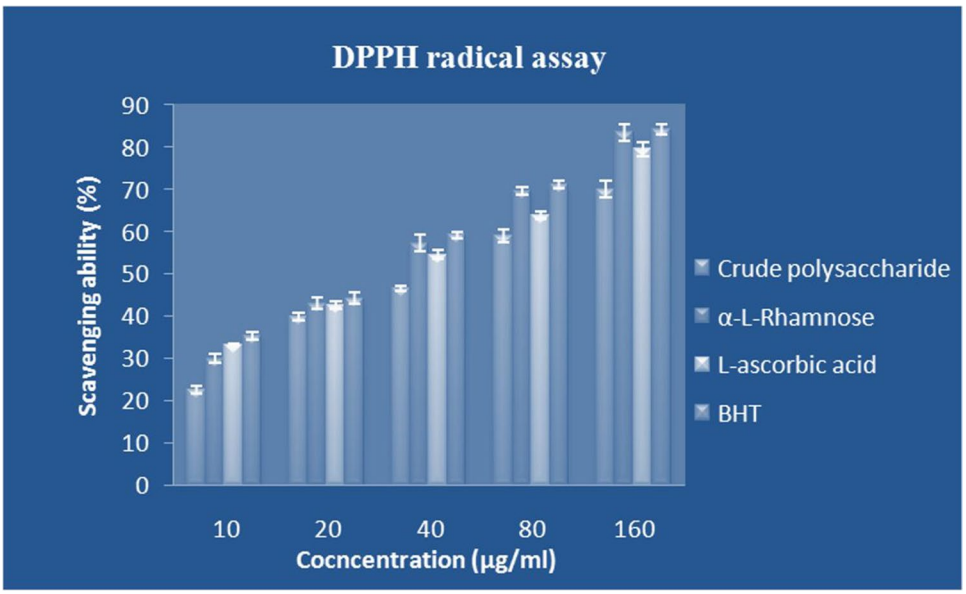

Fig. 2 DPPH radical scavenging activity of crude polysaccharide and α-L-Rhamnose

the maximum inhibition was 74.11%. The total antioxidant activity was found to increase with increasing concentration. In comparison, at the highest concentration of 250 µg/ml, the standards BHT and L-ascorbic acid stated 91.11 and 89.6% total antioxidant activity, respectively. Similarly, at 1000 µg/ml, the total antioxidant activity of purified sulfated polysaccharide from *S. tenerrimum* was found to be 62.55% [24]. The crude polysaccharide of *G. lithophila* demonstrated maximum total antioxidant activity at the lowest concentration than the crude polysaccharide of *S. tenerrimum* was 62.55% at 1000 µg/ml [24]. Similarly, at a concentration of 1000 µg/ml, *T. ornata* crude polysaccharide showed 88.17% activity [24]. However, Costa et al. [13] reported that of the five heterofucans such as SF-0.5v, SF-0.7v, SF-1.0v, SF-1.0v, and SF-2.0v, SF-0.7v and SF1.0v from *S. filipendula* reported 77.3% and 90.7% antioxidant activity at 0.5mg/ml, respectively. In the current study, however, α-L-Rhamnose from *G. lithophila* demonstrated 74.11% total antioxidant activity even at a lower concentration of 250 µg/ml. Thus, the total antioxidant activity of α-L-Rhamnose from *G. Lithophila* was higher than in all of the previous studies.

3.2.2 DPPH radical scavenging assay

The inhibitory effect of crude polysaccharide and α-L-Rhamnose on DPPH radical was found to be significant and concentration-dependent, with inhibition levels ranging from 22.5 to 69.91% and 29.89 to 83.31% (Fig. 2). In 160 µg/ml concentration of α-L-Rhamnose noted the highest activity as 83.31%. The antioxidant activity of the standards BHT and L-ascorbic acid at their highest concentrations was 79.42% and 83.95%, respectively. In the present study a maximum DPPH scavenging activity was shown by the crude polysaccharide (69.91%) at the concentration of 160 µg/ml, which is higher when compared to the results of previous study on two acid protein-containing crude heteropolysaccharides from *Ampelopsis from*, i.e., at the concentration range of 0.25 to 8 mg/ml, the scavenging effort of BHT, ALPS and ASPS ranging from 24 to 99%, 3.9 to 19.3%, and 5.1 to 56.7%, respectively [25]. In an earlier study, the crude polysaccharide from *S. tenerrimum* reported the DPPH scavenging activity of 64.66% at the maximum concentration of 1000 µg/ml [24]. Likewise, the DPPH scavenging activity of three crude polysaccharides from the brown seaweed *S. pallidum* was found to be 17.8%, 19.1%, and 10.2% at the concentration of 3.8 mg/ml [26]. In the present study, α-L-Rhamnose showed 83.31% of DPPH radical scavenging activity at 160 µg/ml concentration. The DPPH scavenging activity may be considered that α-L-Rhamnose which radical the hydrazine on reacting with the hydrogen donors in it. In addition to that DPPH scavenging activity may be attributed the occurrence of monosugars. The DPPH scavenging activity of the two high rhamnose containing purified sulfated polysaccharides (S1, S2) and de-sulfated polysaccharides (DS-1, DS-2) from *Undaria pinnitafida* showed 30.87%, 35.01%, 56.12%, and 60.88% at 1.4mg/ml concentration [27]. Similarly, the DPPH antioxidant activity of four purified polysaccharides from the brown seaweed *S. pallidum* was 3.55%, 3.26%, 6.60%, and 4.94% at the concentration of 5 mg/ml, respectively [26]. Likewise, the DPPH scavenging activity of the four polysaccharides such as SMP-1, SMP-2, SMP-3, and SMP-4 extracted from *Salvia miltiorrhiza* showed 86.7%, 89.4%, 76.8%, and 81.9% at 4mg/ml concentration [28]. The difference in antioxidant activity of

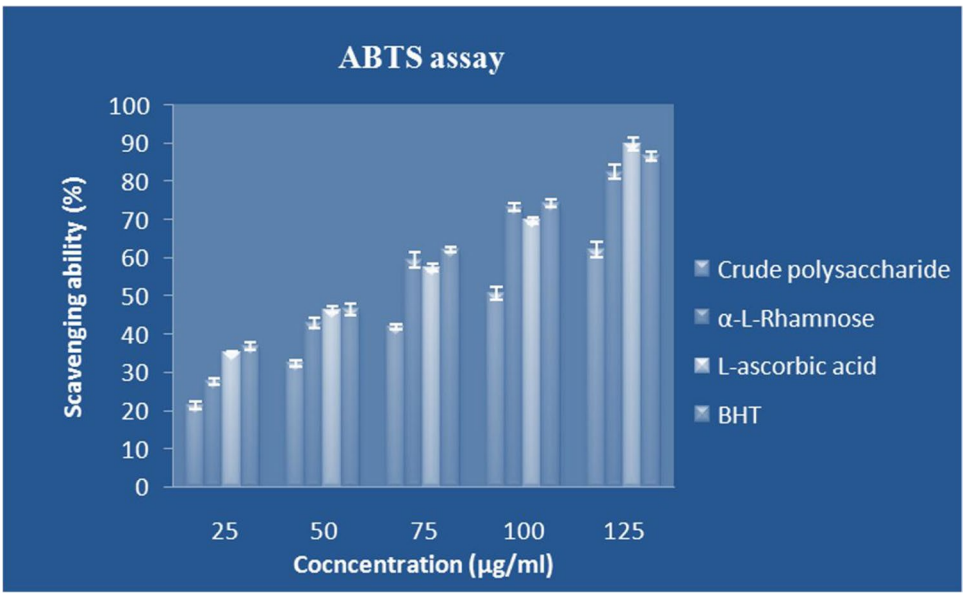

Fig. 3 ABTS scavenging activity of crude polysaccharide and α-L-Rhamnose

the polysaccharides could be attributed to a variety of factors, including molecular weight (MW) and chemical structure [22].

3.2.3 ABTS scavenging assay

The crude polysaccharide and α-L-Rhamnose showed excellent ABTS scavenging activity in a concentration-dependent manner. The scavenging ability was stated to be 25.04–74.46% and 33.01–87.32% at the numerous concentrations of 25–125 µg/ml used in the present study for crude polysaccharides and α-L-Rhamnose (Fig. 3). The maximum of 87.32% scavenging capacity was detected at the concentration of 125 µg/ml of α-L-Rhamnose. It showed owing ABTS scavenging capability, with increased concentration; whereas the BHT and L-ascorbic acid presented 83.29% and 84.99% of scavenging ability at 125 µg/ml concentration. In the present study, the ABTS free radical activity of crude polysaccharide was maximum when compared to the previous crude polysaccharide from *S. tenerrimum* stated 70.33% at the concentration of 1000 µg/ml [24]. The ABTS radical scavenging ability of sulfated polysaccharide from brown alga *S. tenerrimum* was calculated as 70.33% at 1000 µg/ml [24]. The methanolic extract from the red alga *Compsopogon helwanii* reported an increasing trend in its antioxidant activity, i.e., 55.8% and 74.3% at 50 and 100 µg/ml concentration respectively [29]. Similarly, sulfated fucoidan from *L. japonica*, agar-like sulfated galactan from *Nori* sp., and sulfated polysaccharide from *Fucus vesiculosus* showed ABTS antioxidant activity of 81%, 75.8%, and 66.9% at the concentration of 100 µg/ml [30 – 33].

3.2.4 Reducing power

Reducing properties are frequently associated with the presence of reductones, which have been shown to exhibit antioxidant effects by breaking the free radical chain by donating a hydrogen atom [34]. According to reports, reductones interact with specific peroxide precursors to stop the generation of peroxide [35, 36]. The reducing power of crude polysaccharide and α-L-Rhamnose from *G. lithophila* was assessed based on the measurement of Fe^{3+}–Fe^{2+} transformation. At concentrations of 50–400 µg/ml, the crude polysaccharide and α-L-Rhamnose showed reducing powers of 1.23–16.11% and 2.54–21.92%, respectively (Fig. 4). The compound α-L-Rhamnose had the greatest recorded reducing the power of 21.9% and shown a consistent rise in reducing power through increasing concentration. At the highest concentration of 400 µg/ml, the (BHT and L-ascorbic acid) standards contributed 18.32% and 20.67% of the reducing power, respectively.

The crude polysaccharide in the current investigation demonstrated a maximum reducing power capability than the crude sulfated polysaccharide from *S. Tenerrimum* reported a 0.99% concentration at 1000 µg/ml [24]. The α-L-Rhamnose showed higher reducing power ability than that of the purified polysaccharide, obtained from fresh persimmon fruit *Diospyros kaki* namely PFP and its derivatives (PF-SI, PF-SII, and PF-SIII) which showed the reducing power of 0.981, 0.987, 0.871, and 0.966% at 400 µg/ml concentration [36]. Similarly, the reducing power of polysaccharide from the brown seaweed *S. graminifolium* was calculated as 0.21, 0.37, 0.42, 0.57, and 0.89% at the concentrations of 0.2, 0.4,

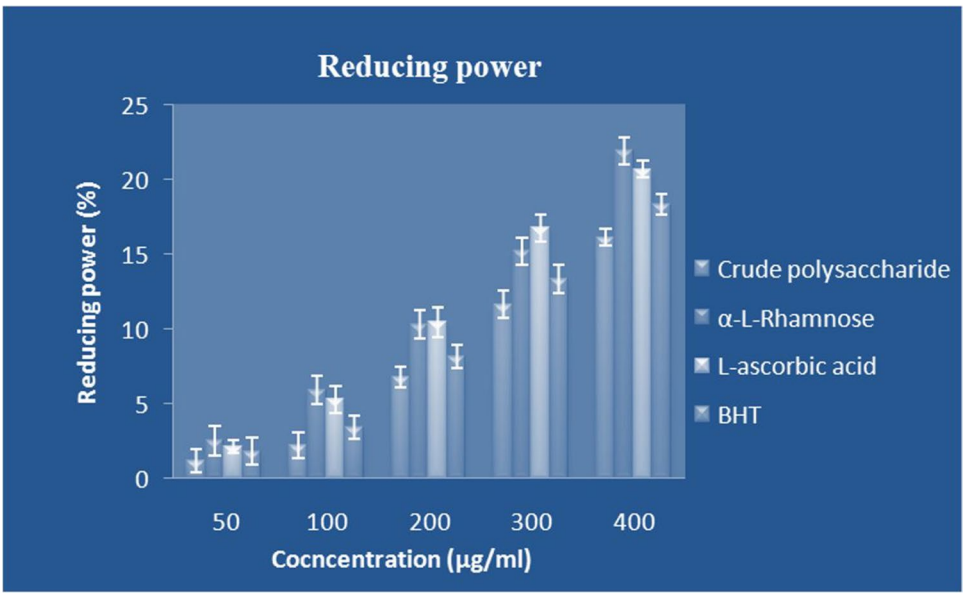

Fig. 4 Reducing power ability of crude polysaccharide and α-L-Rhamnose

Table 1 Anticoagulant activity of crude polysaccharide and α-L-Rhamnose from *G. lithophila* on human blood

S. No.	Sample	Clotting time (IU)	
		APTT	PT
1	Crude polysaccharide	23	1.92
2	α-L-Rhamnose	28.2	3.58

0.8, 1.6, and 3.2 mg/ml, respectively [37]. Furthermore, the reducing power capacity of two sulfated polysaccharide fractions (F1 and F2) and de-sulfated polysaccharide fractions (DF-1 and DF-2) from *Corallina officinalis* showed moderate reducing power (0.424%, 0.303%, 0.53%, and 0.348%) at higher concentration of 1.4 mg/ml [38]. When compared to other studies, the reducing power of the crude polysaccharide and α-L-Rhamnose was higher in the current investigation, since the antioxidant activity of the polysaccharide derivatives with different molecular weights varied. The molecular weight was found to boost antioxidant activity. However, Zhao et al. [39] discovered that in terms of antioxidant activity, low molecular weight compounds outperform high molecular weight products. Regardless, the findings of all of the preceding studies indicate that the molecular weight plays an important role in the antioxidant properties of polysaccharides. Along with molecular weight, sulfur group and mono-sugar composition influence the antioxidant activity.

3.3 Anticoagulant activity

3.3.1 APTT and TT anticoagulant activity

The many problems of blood coagulation and fibrinolysis have made it a high priority in recent years biomedical research to examine anticoagulant, thrombolytic, and antithrombic reagents from various sources [40]. The APTT test, which precisely identifies the anticoagulant potency of the substance, is the most popular [41]. The anticoagulant properties of crude polysaccharide and α-Rhamnose are discussed. Through the use of APTT and PT assays, *G. lithophila* effects on human blood coagulation were assessed. The APTT anticoagulant activity of crude polysaccharide and α-L-Rhamnose proved 23 IU and 28.2 IU at 25 µg/ml; whereas the heparan sulfate exposed highest clotting time at 170 IU. Following the, PT assay of the crude polysaccharide and α-L-Rhamnose disclosed the clotting time of 1.92 IU and 3.58 IU at 25 µg/ml. Heparan sulfate comparison revealed 124 IU (Table 1).

Nardella et al. [42] well-defined the anticoagulant activity of low molecular weight fraction (F21, F22, D21, and D22) from brown seaweed *Ascophyllum nodosum* which showed 2.2, 25.3, 0.2, and 7.6 IU/mg. In the present study, the α-L-Rhamnose showed the APTT activity of 28.20 IU/mg which coincides with the result of APTT activity of *A. nodosum* polysaccharide fraction F22. In the present study, much higher activity exhibited by crude polysaccharide of APTT at 506 (s) and α-L-Rhamnose 620 (s) and for PT showed 26.88 and 50.12 s, respectively. Similarly, Mao et al. [43] reported the sulfated polysaccharide from *Monostroma latissimum* showed anticoagulant capacity as APTT > 200 s and PT 17.6 s at highest concentration of 50 µg/ml. Providentially, in the present study α-L-Rhamnose showed better anticoagulant activity in lower concentrations. The variation in the anticoagulant potential may be due to the difference in the sugar residue and the position of sulfate group in the polysaccharides [41, 44]. While the PT test revealed that the anticoagulant polysaccharide derived from *Caulerpa cupressoides* did not affect extrinsic pathways on the coagulation fall, the APTT test suggested that the drug functioned on intrinsic and common routes [45, 46].

The current study results show that α-L-Rhamnose has the highest anticoagulant activity, though it is weaker than heparin. Matsubara et al. [37] observed similar results for the anticoagulant proteoglycans purified from *Codium pugniformis*, a marine green alga. Besides, the different molecular weights of sulfated polysaccharides showed different anticoagulant activities, in which higher molecular weight (216.4–61.9 kDa) had difficult anticoagulant actions [47]. These results propose that the crude polysaccharide and α-L-Rhamnose from *G. lithophila* have a reflective outcome on the anticoagulant activity, and the molecular weight plays a significant role in the anticoagulant action.

4 Conclusion

In conclusion, crude polysaccharide and α-L-Rhamnose from *G. lithophila* established strong antioxidant activities such as total antioxidant activity, DPPH radicals, ABTS radicals and reducing power, and good anticoagulant activity, particularly APTT, and PT. This biological activity of the crude polysaccharide and α-L-Rhamnose had higher portion of rhamnose and their molecular weight may possess the antioxidant and anticoagulant activity. So, the crude polysaccharide and α-L-Rhamnose from *G. lithophila* could be used as a potential natural source of antioxidant and anticoagulant, as a food supplement, or as a feature in the pharmaceutical industries.

Acknowledgements I would like to acknowledge the Director, Saveetha School of Engineering, Saveetha Institute of Medical and Technical sciences, Tamil Nadu, India.

Availability of data and materials The data used to support the finding of this study are included within the manuscript.

Author contribution PS designed experiments and manuscript preparation.

Declarations

Ethical approval Not Applicable.

Competing interests The author declares no competing interests.

References

1. Choi D-S, Athukorala Y, Jeon Y-J, Senevirathne M, Cho K-R, Kim S-H (2007) Antioxidant activity of sulfated polysaccharides isolated from sargassum fulvellum. Preventive Nutrition and Food Science 12(2):65–73. https://doi.org/10.3746/jfn.2007.12.2.065
2. Rupérez P, Ahrazem O, Leal JA (2002) Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed fucus vesiculosus. J Agric Food Chem 50(4):840–845. https://doi.org/10.1021/jf010908o
3. Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39(4):383–393. https://doi.org/10.1016/j.foodres.2005.10.010
4. Shahidi F, Alasalvar C, Liyana-Pathirana CM (2007) Antioxidant phytochemicals in hazelnut Kernel (Corylus avellana L.) and hazelnut byproducts. J Agric Food Chem 55(4):1212–1220. https://doi.org/10.1021/jf062472o
5. Zhang Q, Li N, Zhou G, Lu X, Xu Z, Li Z (2003) In vivo antioxidant activity of polysaccharide fraction from Porphyra haitanesis (Rhodephyta) in aging mice. Pharmacol Res 48(2):151–155. https://doi.org/10.1016/S1043-6618(03)00103-8
6. Yuan YV, Bone DE, Carrington MF (2005) Antioxidant activity of dulse (Palmaria palmata) extract evaluated in vitro. Food Chem 91(3):485–494. https://doi.org/10.1016/j.foodchem.2004.04.039
7. Nagai T, Yukimoto T (2003) Preparation and functional properties of beverages made from sea algae. Food Chem 81(3):327–332. https://doi.org/10.1016/S0308-8146(02)00426-0
8. Xue CH, Fang Y, Lin H, Chen L, Li ZJ, Deng D, Lu CX (2001) Chemical characters and antioxidative properties of sulfated polysaccharides from Laminaria japonica. J Appl Phycol 13:67–70. https://doi.org/10.1023/A:1008103611522
9. Hu JF, Geng MY, Zhang JT, Jiang HD (2001) An in vitro study of the structure-activity relationships of sulfated polysaccharide from brown algae to its antioxidant effect. J Asian Nat Prod Res 3(4):353–358. https://doi.org/10.1080/10286020108040376
10. Zhang Q, Li N, Liu X, Zhao Z, Li Z, Xu Z (2004) The structure of a sulfated galactan from Porphyra haitanensis and its in vivo antioxidant activity. Carbohydr Res 339(1):105–111. https://doi.org/10.1016/j.carres.2003.09.015
11. Qi H, Zhang Q, Zhao T, Chen R, Zhang H, Niu X, Li Z (2005) Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta) in vitro. Int J Biol Macromol 37(4):195–199. https://doi.org/10.1016/j.ijbiomac.2005.10.008
12. Tsiapali E, Whaley S, Kalbfleisch J, Ensley HE, Browder IW, Williams DL (2001) Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. Free Radic Biol Med 30(4):393–402. https://doi.org/10.1016/S0891-5849(00)00485-8
13. Costa MG, Batista PR, Shida CS, Robert CH, Bisch PM, Pascutti PG (2010) How does heparin prevent the pH inactivation of cathepsin B? Allosteric mechanism elucidated by docking and molecular dynamics. BMC Genomics 11(S5):S5. https://doi.org/10.1186/1471-2164-11-S5-S5
14. Maeda M, Uehara T, Harada N, Sekiguchi M, Hiraoka A (1991) Heparinoid-active sulphated polysaccharides from Monostroma nitidum and their distribution in the chlorophyta. Phytochemistry 30(11):3611–3614. https://doi.org/10.1016/0031-9422(91)80076-D
15. Patankar MS, Oehninger S, Barnett T, Williams RL, Clark GF (1993) A revised structure for fucoidan may explain some of its biological activities. J Biol Chem 268(29):21770–21776. https://doi.org/10.1016/S0021-9258(20)80609-7
16. Chevolot L, Foucault A, Chaubet F, Kervarec N, Sinquin C, Fisher A-M, Boisson-Vidal C (1999) Further data on the structure of brown seaweed fucans: relationships with anticoagulant activity. Carbohydr Res 319:154–165. https://doi.org/10.1016/S0008-6215(99)00127-5
17. Priya P, Murugesan S, Kotteswari M, Shanthi N, Sivamurugan V (2018) Antibacterial activity of marine red alga Grateloupia lithophila Boergesen. Int J Pharm Bio Sci 8(3):1125–1129
18. Seedevi P, Ramu Ganesan A, Moovendhan M, Mohan K, Sivasankar P, Loganathan S, Vairamani S, Shanmugam A (2020) Anti-diabetic activity of crude polysaccharide and rhamnose-enriched polysaccharide from G. lithophila on Streptozotocin (STZ)-induced in Wistar rats. Sci Rep 10(1):1–12
19. Umamaheshwara Rao M (1987) Key for identification of economical important seaweeds. CMFRI Bull 41:116
20. Seedevi P, Moovendhan M, Sudharsan S, Sivasankar P, Sivakumar L, Vairamani S, Shanmugam A (2018) Isolation and chemical characteristics of rhamnose enriched polysaccharide from Grateloupia lithophila. Carbohydr Polym 195:486–494. https://doi.org/10.1016/j.carbpol.2018.05.002
21. Natarajan A, Moorthy R, Perumal A (2011) In vitro antioxidant and anticoagulant activities of sulphated polysaccharides from brown seaweed (Turbinaria ornata) (Turner) J.Agardh. Asian Journal of Pharmaceutical & Biological Research 1(3):232–239
22. Zhang Y, Shi S, Wang Y, Huang K (2011) Target-guided isolation and purification of antioxidants from Selaginella sinensis by offline coupling of DPPH-HPLC and HSCCC experiments. J Chromatogr B 879(2):191–196. https://doi.org/10.1016/j.jchromb.2010.12.004
23. Gião MS, González-Sanjosé ML, Rivero-Pérez MD, Pereira CI, Pintado ME, Malcata FX (2007) Infusions of portuguese medicinal plants: dependence of final antioxidant capacity and phenol content on extraction features. J Sci Food Agric 87(14):2638–2647. https://doi.org/10.1002/jsfa.3023
24. Vijayabaskar P, Vaseela N (2012) In vitro antioxidant properties of sulfated polysaccharide from brown marine algae Sargassum tenerrimum. Asian Pacific Journal of Tropical Disease 2:S890–S896. https://doi.org/10.1016/S2222-1808(12)60287-4
25. Wang Y, Bian X, Park J, Ying L, Qian L, Xu P (2011) Physicochemical properties, in vitro antioxidant activities and inhibitory potential against α-glucosidase of polysaccharides from ampelopsis grossedentata leaves and stems. Molecules 16(9):7762–7772. https://doi.org/10.3390/molecules16097762
26. Ye H, Wang K, Zhou C, Liu J, Zeng X (2008) Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed Sargassum pallidum. Food Chem 111(2):428–432. https://doi.org/10.1016/j.foodchem.2008.04.012
27. Hu T, Liu D, Chen Y, Wu J, Wang S (2010) Antioxidant activity of sulfated polysaccharide fractions extracted from Undaria pinnatifida in vitro. Int J Biol Macromol 46(2):193–198. https://doi.org/10.1016/j.ijbiomac.2009.12.004
28. Wu W, Zhu Y, Zhang L, Yang R, Zhou Y (2012) Extraction, preliminary structural characterization, and antioxidant activities of polysaccharides from Salvia miltiorrhiza Bunge. Carbohydr Polym 87(2):1348–1353. https://doi.org/10.1016/j.carbpol.2011.09.024

29. Shanab SMM, Shalaby EA (2012) The first record of biological activities of the Egyptian red algal species Compsopogon helwanii. International Journal of Bioscience, Biochemistry and Bioinformatics 2:291–296. https://doi.org/10.7763/IJBBB.2012.V2.119
30. Rupérez P, Saura-Calixto F (2001) Dietary fibre and physicochemical properties of edible Spanish seaweeds. Eur Food Res Technol 212(3):349–354. https://doi.org/10.1007/s002170000264
31. Duan X-J, Zhang W-W, Li X-M, Wang B-G (2006) Evaluation of antioxidant property of extract and fractions obtained from a red alga. Polysiphonia urceolata Food Chemistry 95(1):37–43. https://doi.org/10.1016/j.foodchem.2004.12.015
32. Zhao X, Xue CH, Li BF (2008) Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*. J Appl Phycol 20(4):431–436
33. Gordon MH (1990) The mechanism of antioxidant action in vitro. Food antioxidants. Springer, pp 1–18
34. Xing R, Yu H, Liu S, Zhang W, Zhang Q, Li Z, Li P (2005) Antioxidant activity of differently regioselective chitosan sulfates in vitro. Bioorg Med Chem 13(4):1387–1392
35. Xing R, Liu S, Guo Z, Yu H, Wang P, Li C, Li P (2005) Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. Bioorg Med Chem 13(5):1573–1577
36. Zhang Y, Lu X, Fu Z, Wang Z, Zhang J (2011) Sulphated modification of a polysaccharide obtained from fresh persimmon (Diospyros kaki L.) fruit and antioxidant activities of the sulphated derivatives. Food Chem 127(3):1084–1090
37. Zhang C-Y, Wu W-H, Wang J, Lan M-B (2012) Antioxidant properties of polysaccharide from the brown seaweed Sargassum graminifolium (Turn.), and its effects on calcium oxalate crystallization. Mar Drugs 10(1):119–130
38. Yang Y, Liu D, Wu J, Chen Y, Wang S (2011) In vitro antioxidant activities of sulfated polysaccharide fractions extracted from Corallina officinalis. Int J Biol Macromol 49(5):1031–1037
39. Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L, Gu G (2006) Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (Hordeum vulgare L.). J Agric Food Chem 54(19):7277–7286
40. Matsubara K, Hori K, Matsuura Y, Miyazawa K (2000) Purification and characterization of a fibrinolytic enzyme and identification of fibrinogen clotting enzyme in a marine green alga, Codium divaricatum. Comp Biochem Physiol B: Biochem Mol Biol 125(1):137–143
41. Mourão PAS, Pereira MS (1999) Searching for alternatives to heparin: sulfated fucans from marine invertebrates. Trends Cardiovasc Med 9(8):225–232
42. Nardella A, Chaubet F, Boisson-Vidal C, Blondin C, Durand P, Jozefonvicz J (1996) Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed Ascophyllum nodosum. Carbohydr Res 289:201–208
43. Mao W, Li H, Li Y, Zhang H, Qi X, Sun H, Guo S (2009) Chemical characteristic and anticoagulant activity of the sulfated polysaccharide isolated from Monostroma latissimum (Chlorophyta). Int J Biol Macromol 44(1):70–74
44. Melo FR, Pereira MS, Foguel D, Mourao PAS (2004) Antithrombin-mediated anticoagulant activity of sulfated polysaccharides: different mechanisms for heparin and sulfated galactans. J Biol Chem 279(20):20824–20835
45. Athukorala Y, Kim K-N, Jeon Y-J (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga. Ecklonia cava Food and chemical toxicology 44(7):1065–1074
46. Pushpamali WA, Nikapitiya C, De Zoysa M, Whang I, Kim SJ, Lee J (2008) Isolation and purification of an anticoagulant from fermented red seaweed Lomentaria catenata. Carbohydr Polym 73(2):274–279
47. Zhang H-J, Mao W-J, Fang F, Li H-Y, Sun H-H, Chen Y, Qi X-H (2008) Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from Monostroma latissimum. Carbohydr Polym 71(3):428–434