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Metabolite Profiling By GC-MS Analysis Enhanced Alpha Amylase, Alpha Glucosidase Inhibitory Activity Of Dictyota Bartayresiana From Gulf Of Mannar

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ABSTARCT

The Phytocompounds and functional groups in the methanolic extract of marine algae Dictyota bartayresiana (Phaeophyta) from the Gulf of Mannar, Southeast coast of India. Materials and This study was analyzed using GC-MS and FTIR, respectively. A comparative antibacterial activity and alpha-amylase and alpha-glucosidase enzyme inhibitory activity of the methanolic extract was also studied. The maximal antibacterial activity of methanolic extract of D. bartayresiana was found against P. aeruginosa, followed by Streptococcus spp, S. aureus, and E. coli. The methanolic extract of D. bartayresiana showed alpha-amylase and alpha-glucosidase inhibitory activity with IC_{50} 43.12 µg/ml and 24.18 µg/ml, respectively, whereas acarbose, a standard drug shows 47.29 µg/ml and 38.53 µg/ml, respectively. FT-IR analysis showed major peaks indicating major O-H and C-H vibrations which indicated the presence of functional groups of marine compounds. The GCMS analysis identified thirteen biologically active marine compounds. The results obtained in the present study indicate that medicinally important marine algae D. bartayresiana methanolic extract could be considered a potential natural alpha amylase and alpha glucosidase enzyme inhibitor and an antibacterial agent.

Keywords: Dictyota bartayresiana, FTIR, GC-MS, antibacterial activity, alpha-amylase inhibitory activity, alpha-glucosidase inhibitory activity

INTRODUCTION

Marine algae possesses great resource of medicinal compounds with various pharmaceutical activities and also with distinctive structural arrangements (Leandro et al., 2020; Aguilar-Briseño et al., 2015; Alves et al., 2016). Interest on these marine phytocompounds has peaked in recent times, importantly, the developed marine drugs have reached a height of importance among the global communities for their efficient disease-curing ability with less or no adverse effects (Malve et al., 2016; James et al., 2012). A few drugs of marine algal compounds have also been approved for human trials and used in different parts of the world (Blunt et al., 2018).

Although there are several existing scientific methods for drug discovery including computational-based molecular modeling and design, none of the drugs developed can eventually compete with the reliability of nature-derived drugs (Felhi et al., 2017) the recent scientific investigations have reported various medicinally important phytocompounds derived from nature, especially from marine algae, and their uses in treating various diseases without side effects (Mohamed et al., 2012).

Gas Chromatography-Mass Spectroscopy (GC-MS) is a very desirable technique for the quantification and identification of compounds of organic materials especially from plant sources. With a well-refined methodology, several available libraries, and better interpretation, the identification of novel organic compounds in a crude mixture of plant extract is made easy (Ghosh et al., 2015).

Dictyota Bartayresiana (Class: Phaeophyceae, Order: Dictyotales, Family: Dictyotaceae) is a medicinally important growing brown marine algae in the inter-tidal Southeast coasts of the Gulf of Mannar, India. These phytocompounds derived from algae are extensively used for applications as antifungal, antibacterial, cytotoxic, and larvicides activity. This algae also used for the synthesis of silver nanoparticles which showed significant antifungal activity (Antonysamy et al., 2015) Considering its biological properties, GC-MS analysis of methanolic extract of D. bartayresiana conducting first time for the identification of phytochemical compounds. This study also reported for the antibacterial and alpha-amylase and alpha-glucosidase inhibitor activity of methanolic extract of D. bartayresiana.

MATERIALS AND METHODS

Sample Collection

Fresh Dictyota bartayresiana (J.V. Lamoureux) brown algae were collected from the Mandapam coastal region (78°8’E, 9°17’N), in the Gulf of Mannar, Tamilnadu, South India in December 2021 and were immediately transported to the laboratory. D. bartayresiana washed several times with marine water to remove debris and the epiphytes were removed with distilled water. After cleaning, they were dried in shade at 36°C for two weeks. The dried D. bartayresiana were ground in to a fine powder and further used to extraction.

Preparation of Extract

The methanolic extract was prepared from 1kg of the powdered D. bartayresiana using the Soxhlet apparatus. The methanol extract was further concentrated using a rotary evaporator to obtain their corresponding residues. The methanolic extract was further analyzed for FTIR and GC-MS analysis.

Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

The methanolic extracts of D. bartayresiana were done FTIR analysis. 1 mg of dry methanolic extract powder was mixed in a mortar with 99 mg of KBr. A thin KBr disc was obtained by pressing the powder in a French press and Fourier Transform Infrared (FTIR) Spectra using Shimadzu IR-Prestige-21, read at the range of 400–4000 cm^{-1}.

GC-MS Analysis

GC-MS analysis was done on a GC system containing a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument, Elite-5MS capillary column fitted to a Perkin Elmer Clarus 680 equipped with Mass spectrometer Clarus 600 (EI) employing the following conditions: Retention time. Inial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min, Total Run Time: 32.00 mint, auto=260°C, Volume=1 µL, Split=10:1, Flow Rate: 1 mL/mint Carrier Gas=He. The peak was measured from the base to the tip. Turbo Mass version 5.4.2 software was used for the spectral analysis. Structure determination was done by comparison of mass spectral patterns to the NIST library.

Microbial Cultures

The clinical bacterial isolates, Staphylococcus aureus (gram-positive), Escherichia coli, Streptococcus spp,and Pseudomonas aeruginosa (gram-negative), were got from the Aravind eye hospital, Coimbatore, Tamilnadu, India. The isolated bacteria were cultured in nutrient agar (High Media, India) slants and were sub-cultured before use at 37°C. The obtained bacterial strains were serially diluted and were swabbed on MHA agar plates in order to acquire the desired Colony Forming Units, i.e., 1.5 x 10^7 CFU/mL.

Minimum Inhibitory Concentration (MIC) Determination

Protocols specified by CLSI-Clinical and Laboratory Standards Institute were followed to determine the MIC of D. bartayresiana extract against Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus spp, and Escherichia coli (Wayane., 2016). Mueller–Hinton Broth (MHB) was used to culture the pathogens overnight at 37°C and the culture was diluted to produce 5×10^7 CFU/ml. Sterile culture media (200 µL) served as the control. The extract of D. bartayresiana at different concentrations (0.5, 1, 10, 25, 50, 75, 100 µg/ml) was added to 200 µL of overnight grown cultures in a 96-well polypropylene microplate and was incubated at 37°C overnight. The MIC was determined using the absorbance difference between before and after incubation at 550 nm. Similarly, the lowest concentration helped to determine the Minimum Bactericidal Concentration (MBC).

Antibacterial Activity Determination

The extract of D. bartayresiana was checked for its antibacterial activity against the bacterial strains using the agar disc diffusion method (Senthilkumar et al., 2013). Briefly, the bacterial strains were swabbed on MHA agar plates and algal extract of D. bartayresiana was diluted to 1mg/1mLconcentration using sterile distilled water. A well of 5mm in diameter was made on MHA agar plates and was loaded with 20, 30, and 50µL concentrations of methanolic extract of D. bartayresiana extract and was incubated for 24 h at 37 °C. The zone of inhibition around the wells was measured and the antibacterial activity was determined.

Alpha-amylase inhibitory activity

The alpha-amylase inhibitory assay of the methanolic extract of D. bartayresiana was performed in by the modified method of Khadaya, et al., 2020. The alpha-amylase enzyme solution was prepared by dissolving 0.1mg of porcine pancreatic alpha-amylase in 20 mM phosphate buffer (100 mL, pH 6.9). The starch azure solution (0.5% w/v) was used as the substrate. A series of concentrations from 10 to 100 µg/mL of methanolic extract of D. bartayresiana was prepared using DMSO. The total volume was made up to 2.0 mL with distilled water. The DNS solution (20 ml) was used as the coloring reagent in the reaction. The experiment was conducted in triplicates with blank and control. 1 mL of the series of the methanolic extract with different concentrations (10 to 100 µg/mL) was incubated with alpha-amylase enzyme solutions at 25°C for 30 min. After incubation, 1 mL of starch solution was added and the mixture was incubated at 25 °C for 3 min. lastly, 1 mL of the DNS solution was added to the reaction mixture. Further, all the tubes were heated in a boiling water bath at 85° C for 15 min and it was cooled. The reaction was mixed well and the absorbance was recorded at 540 nm. A reaction mix with DNS solution and the starch solution was used as the blank and the reaction mixture with algal extract substituted by 1 mL of DMSO served as control. In this study. Acarbose was used as a positive control. The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = [(A_c – A_s) /A_c] \times 100$$

Where, Ac-absorbance for control; as-absorbance for standard. The IC_{50} values were determined graphically.

Alpha-glucosidase inhibitory assay

The α-glucosidase inhibition assay was performed using the method of Bräunlich et al., 2013. The methanolic extract of D. bartayresiana (0.2 mL) was added with 2.9 mM p-nitrophenylα-glucopyranoside (pNPG) at different concentrations (10 to 100 mg/mL). To this mixture, 1.0 U/mL of α-glucosidase in sodium phosphate buffer (pH 6.9) was added. A mixture of DMSO, enzyme, and substrate was used as control, while in positive control the D. bartayresiana extract was substituted with acarbose. Blank contains reaction mixtures without enzyme and acarbose. The test tubes with the reaction mixtures were incubated at 25 ºC for 5 min, after which the reaction was stopped by boiling for 2 min. The absorbance of the subsequent p-nitro phenol (pNP) was confirmed at 405 nm using a UV spectrophotometer which is directly proportional to the activity of the enzyme. Finally, the IC_{50} values were calculated.

Statistical Analysis

All the above-mentioned experiments were done in triplicates, and the results were presented as mean ± standard deviation (n=4). SPSS was used to analyze the experimental data. (SPSS version 22. IBM Corporation). $P<0.05$ value was considered statistically significant.

RESULTS AND DISCUSSION

Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

The FTIR spectra of the methanolic extract of D. bartayresiana a marine algae is presented in Figure 4. The characteristic peak at 3235.06 cm^{-1} corresponds to O-H stretching alcohol and phenols. The strong bands at 1608.24 cm^{-1} exhibited the presence of the carbonyl group and the C-H vibrations showed at 1443.84 cm^{-1}. The peaks at 999.39, 856.90, and 645.23 cm^{-1} are attributed to the sulphate esters. In discussion with the works of Tew et al., 2022. Similar peaks were found in the range of 3500-3300 cm^{-1} with the O-H stretching and other characteristic peaks.

GC-MS analysis

The algal Phytocompounds in the methanolic extract of D. bartayresiana were analyzed using GC-MS. Figure 5 shows the chromatogram of methanolic extract of D. bartayresiana and Thirteen algal Phytocompounds are identified in this extract, presented between retention times 2 to 30. The bioactive algal compounds are identified and these compound names, molecular formulas, molecular weight, structure, nature, and uses are tabulated in Table 2.

The 2, 6, 6-Trimethylbicyclo [3.1.1] heptan-3-amine (18.094) reported to be an Insecticidal (Sathya et al., 2016). 1, 6;3,4-Dianhydro-2-Deoxy-. Beta. -d-Lyxo-Hexopyranose (18.605), Glutaraldehyde (18.670), Dodecanal (23.432) and Dodecanedioic acid (27.778) exhibited antibacterial activity (Jafari et al., 2017; El-Din et al., 2016). 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin (24.707) shows cytotoxicity against human MDA-MB-231 cells (Razak et al., 2019). Few other compounds of 11-Tridecen-1-ol (18.194), 2S,3S) - (-)-3-propyloxiranemethanol (20.755) and 2-(Aminooxy) propionic acid (27.218) Heptanal (18.289) Heterogeneous catalyst and Hexadecane, 1-chloro- (26.523) have no reported activity.

Minimum Inhibitory Concentration (MIC) Determination

The MIC and MBC of methanolic extract of D. bartayresiana against P. aeruginosa, S. aureus, Streptococcus spp, and E. coli were found to be 2.1, 1.5, 1.2, and 1.7 µg/Ml, respectively (Table 1). The MIC results exhibited that the extract of D. bartayresiana was active against all tested bacteria, making it a good agent for treatment against pathogenic bacteria.

Antibacterial activity determination

The antibacterial activity of the algal extract against the pathogenic bacteria using the standard agar well diffusion method is shown in Figure 1 (a-d). The maximal antibacterial activity (zone of

inhibition) of the algal extract was found against P. aeruginosa at 32.2 mm, followed by Streptococcus spp at 25.7 mm, S. aureus at 24.1 mm, and E. coli at 21.4 mm at the concentration of 50 µL. 20 and 30 µL concentrations exhibited moderate activity of 12.1, 10.4, 8.2, 7.1, and 14.8, 13.2, 12.6 7.9 mm, respectively. The results of the present study evidently showed that D. bartayresiana marine algae extract exhibited antibacterial activity against the tested pathogenic bacterial strains.

The efficiency of the bioactive compounds present in the extracts of D. bartayresiana marine algae inhibits the growth of the bacteria. The study is in accordance with the previous reports of Manivannan et al., 2011. stating that the methanolic extract of the marine algae showed inhibitory activity against pathogenic bacteria. The present study also agrees with the previous reports on the high antibacterial activity of D. bartayresiana extract against selected pathogens (Ahbib et al., 2020; Ibrahim et al., 2015).

Alpha-amylase inhibitory activity

The alpha-amylase inhibitory activity of methanol extracts of D. bartayresiana is shown in Figure 2. Methanolic extract of D. bartayresiana exhibited a strong inhibitory potential with an IC_{50} value of 43.12 µg/ml, while acarbose showed activity with an IC_{50} value of 47.29 µg/ml against the alpha-amylase enzyme. The α-amylase and glucosidase are important for the conversion of carbohydrates to absorbable monosaccharides in the human system and these enzymes postpone the absorption of monosaccharides, thus reducing the postprandial glucose and insulin levels (Hwang et al., 2015). Marine flora has always been an excellent source of alpha-amylase and alpha-glucosidase inhibitors (Teixeira et al., 2007). The inhibitory results of alpha-amylase showed that methanol extracts of D. bartayresiana may be used for treating type II diabetes.

Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity of D. bartayresiana extract and acarbose is shown in Figure3. The methanolic extract of D. bartayresiana has the highest alpha-glucosidase inhibitory activity with an IC_{50} of 24.18 µg/ml, whereas acarbose exhibited an IC_{50} value estimated at 38.53 µg/ml against the alpha-glucosidase enzyme. Alpha-glucosidase enzyme inhibitors show a major character in the control of postprandial hyperglycemia of the blood glucose level in human beings (Dong et al., 2012).

The Gulf of Mannar, southeast coast of Tamil Nadu in India is an exceptional marine diversity hosting various marine algae and this sustainable resources have been used as medicine and industrial product (Senthilkumar et al., 2012).To the best of our knowledge, there is no previous report on alpha-glucosidase inhibitory activity D. bartayresiana marine algae. A previous study reported that several marine algae are known to have alpha-glucosidase and alpha-amylase inhibiting property (Reka et al., 2017).

CONCLUSION

This present study was done to analyze the antibacterial activity and alpha-amylase and alpha-glucosidase enzyme inhibitory activity of methanolic extract of marine brown algae D.

bartayresiana. The methanolic extract of D. bartayresiana exhibited strong antibacterial activity against P. aeruginosa, S. aureus, Streptococcus spp, and E. coli. The alpha-amylase and alpha-glucosidase assay showed the highest inhibition against alpha-amylase and alpha-glucosidase enzymes. Several mechanisms of the compounds in the extract may be attributed to inhibiting the carbohydrate metabolic enzymes and also controlling bacterial growth. The functional groups of marine algal compounds were analyzed using FT-IR analysis and it showed major characteristic peaks. The individual compounds present in the crude methanolic extract were identified using GC-MS analysis. Since D. bartayresiana contains significant numbers of bioactive compounds based on GC-MS analysis. The conclusion of this work is valuable to further research to identify, isolate and characterize the specific compounds which are accountable for higher antidiabetic and antibacterial activity.

ACKNOWLEDGMENT

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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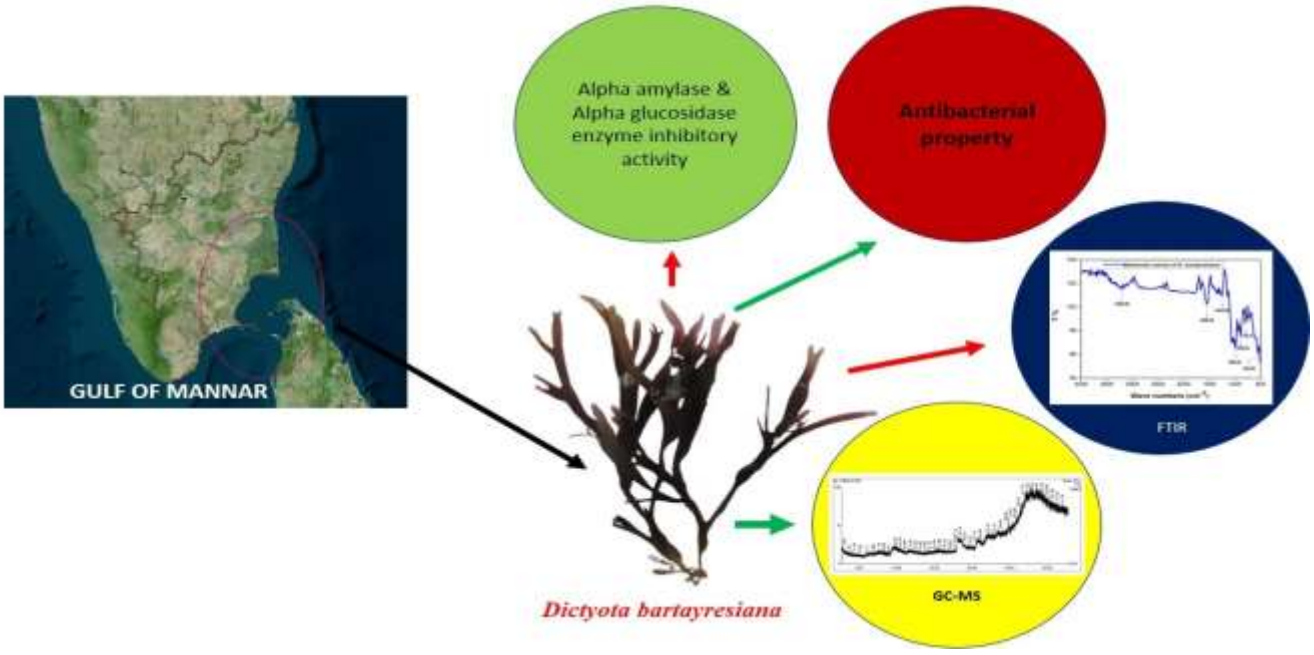


Graphical Abstarct

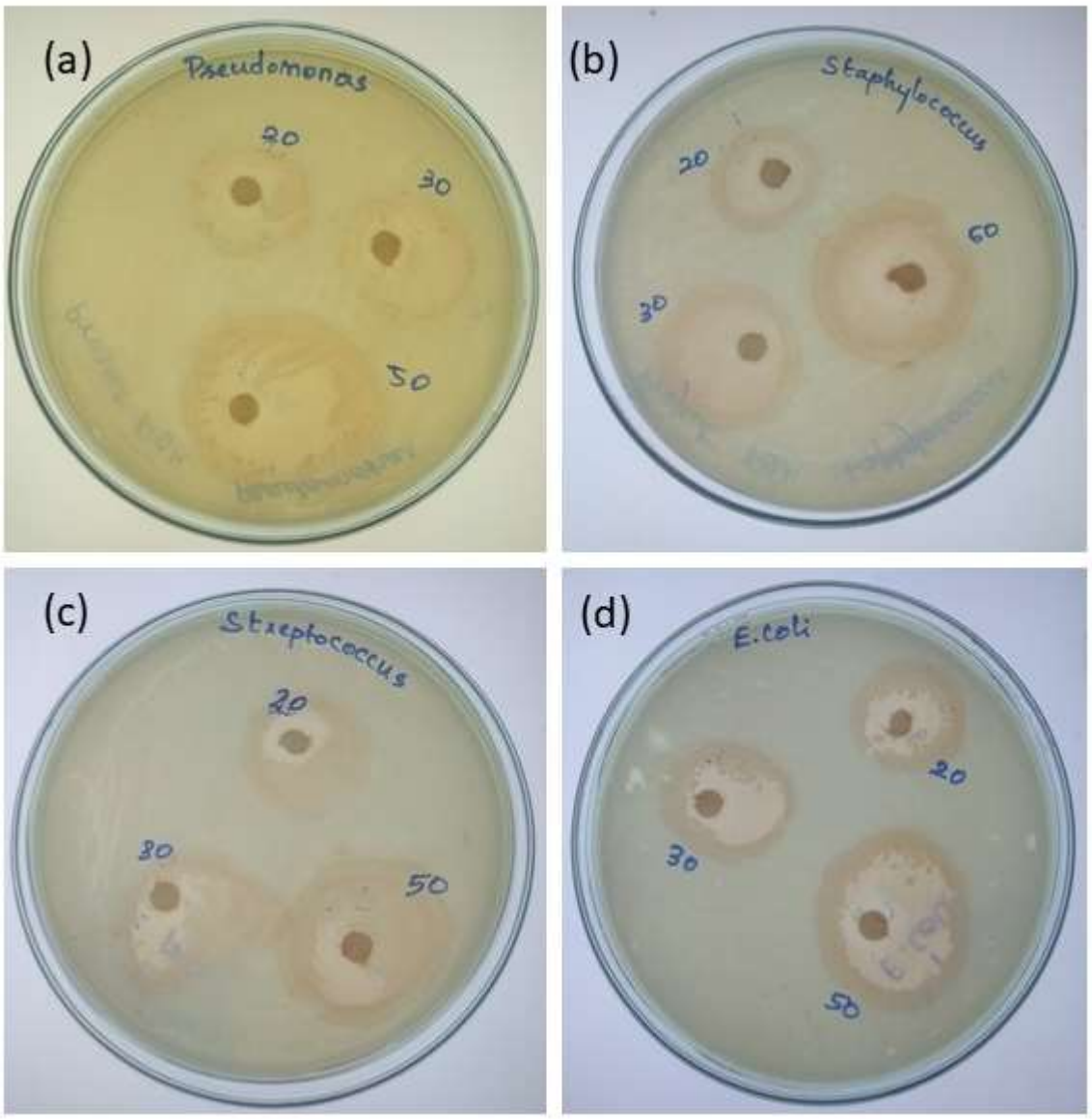


Figure 1: Antibacterial activity of methanolic extract (a). P. aeruginosa (b). S. aureus, (c). Streptococcus spp
(d) E. coli.

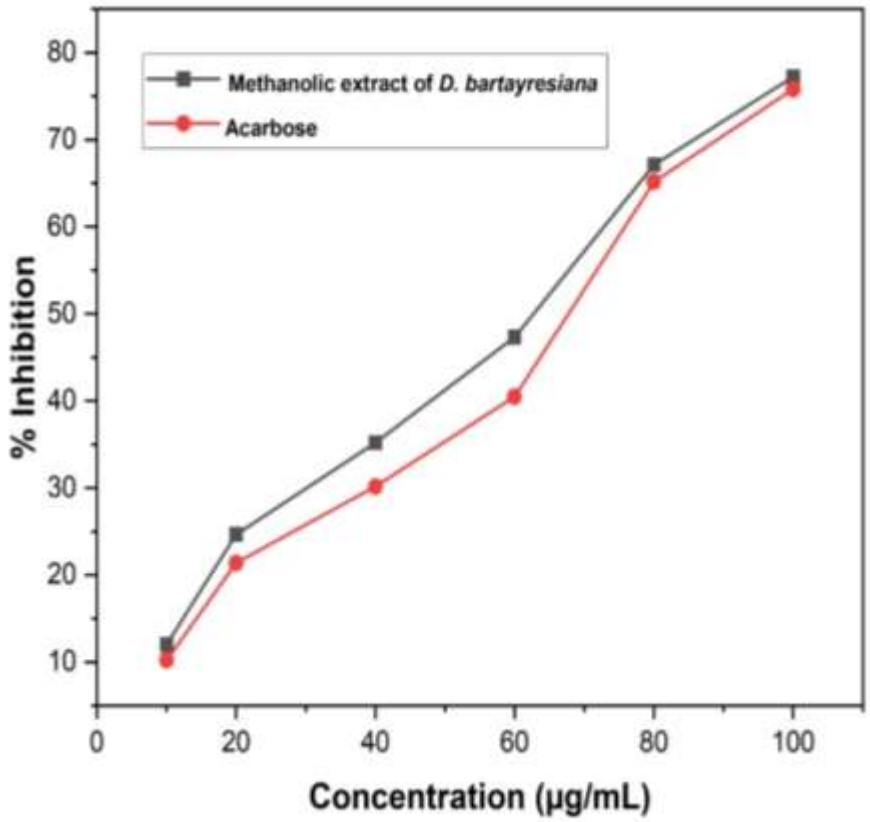


Figure 2: Alpha-amylase inhibitory activity of methanol extracts of D. bartayresiana compared with Acarbose.

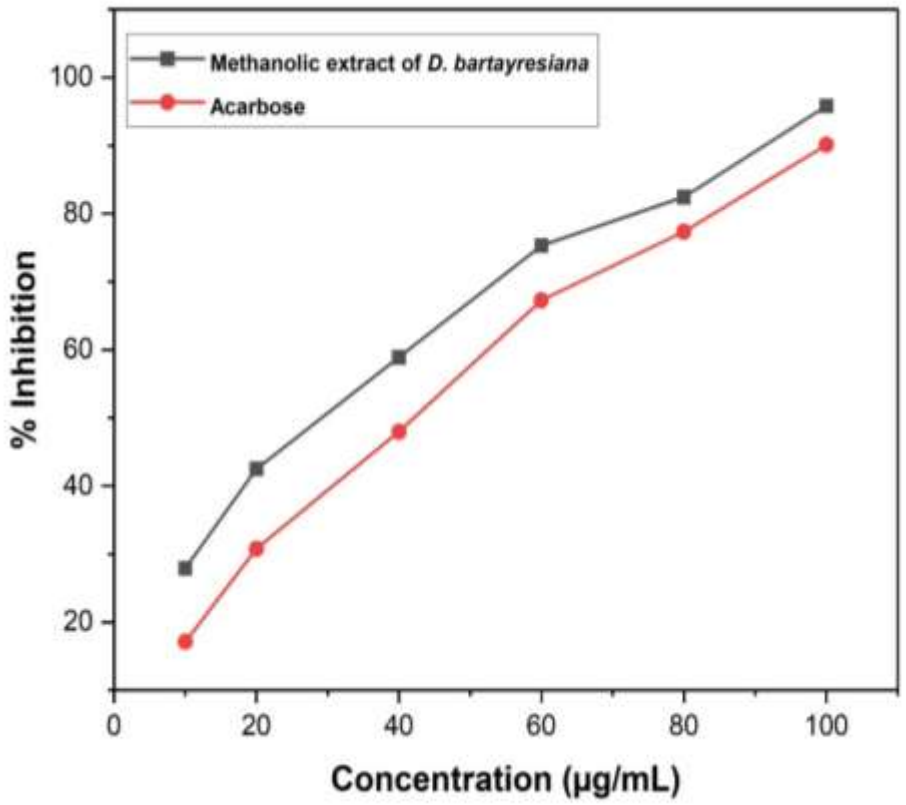


Figure 3: Alpha-glucosidase inhibitory activity of D. bartayresiana extract compared with Acarbose.

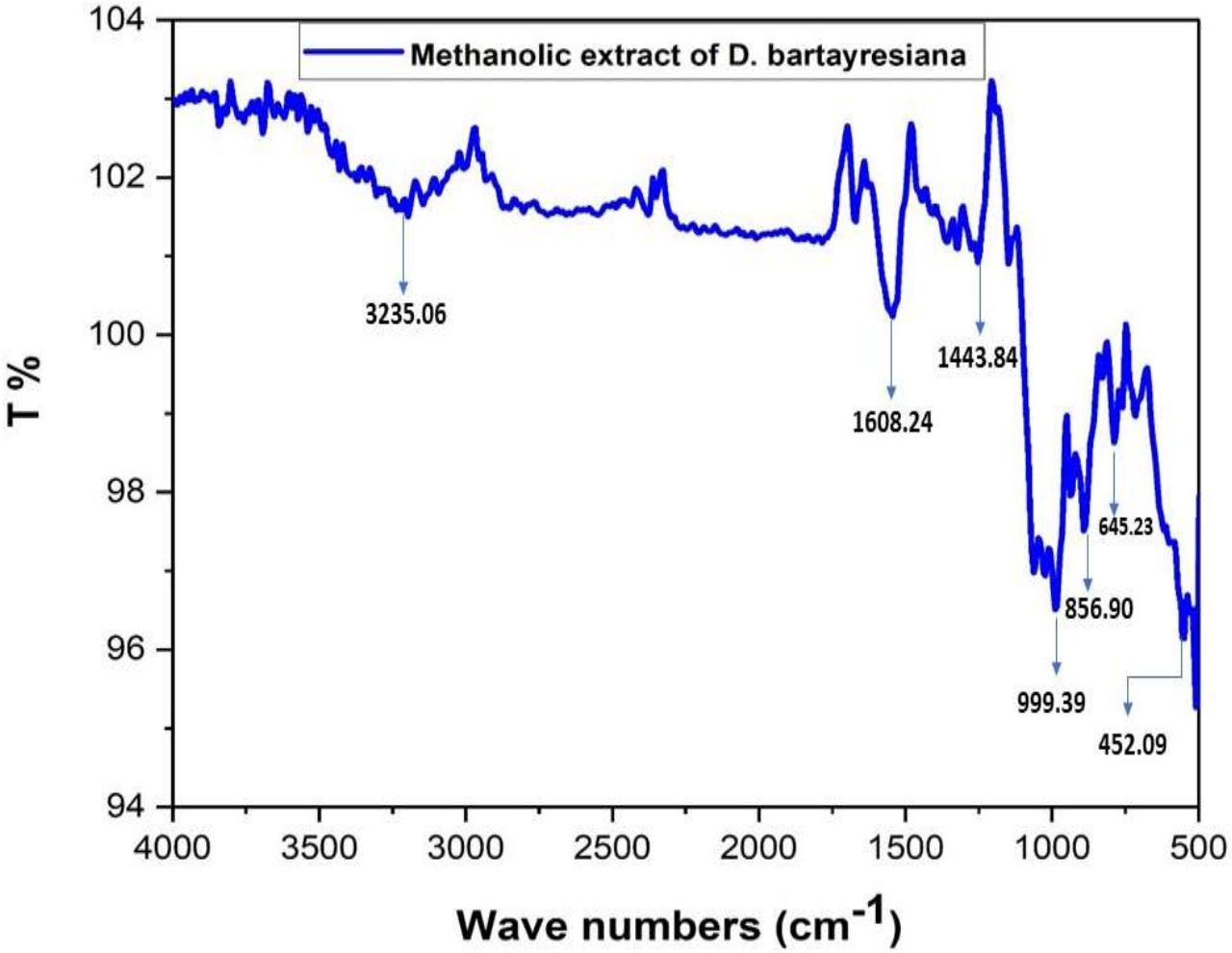


Figure 4: The FTIR spectra of the methanolic extract of D. bartayresiana marine algae.

Figure 5: The GC-MS spectra of the methanolic extract of D. bartayresiana

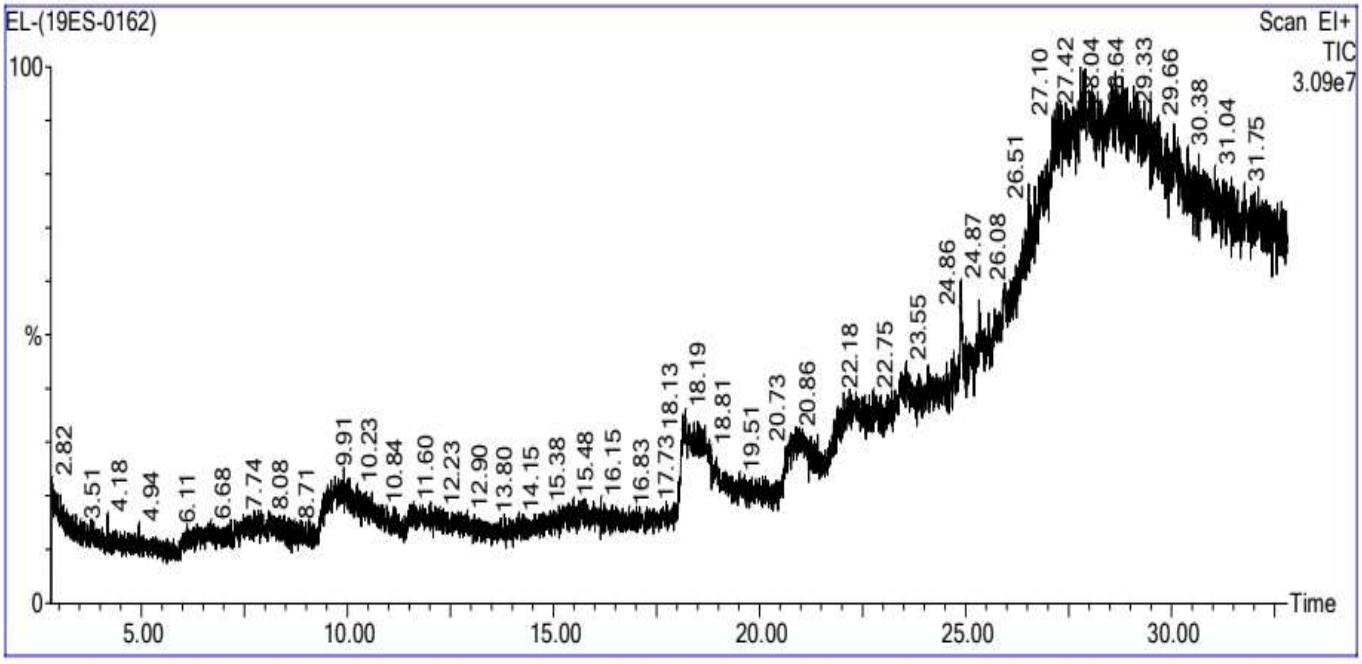
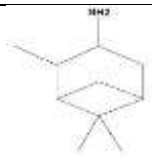
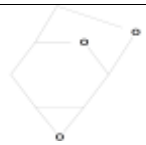
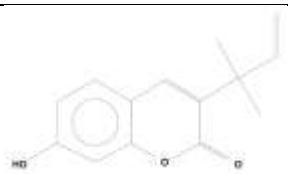
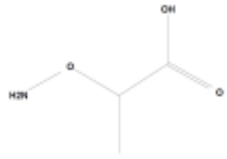


Table: 1. MIC value of tested bacterial samples

S.no	Bacterial sample	MIC (µg/mL)
1.	P. aeruginosa	2.1
2.	S. aureus.	1.5
3.	Streptococcus spp.	1.2
4.	E. coli	1.7

Table 2: Phytocomponents identified in the methanolic extract of D. bartayresiana by GC-MS analysis

Peak	R. Time	IUPAC name	Molecular formula	Molecular weight	Chemical Structure	Nature/reported activity
1	18.094	2,6,6-Trimethylbicyclo [3.1.1] heptan-3-amine	$C_{10}H_{19}N$	153.26 g/mol		Insecticidal [18]
2	18.194	11-Tridecen-1-ol	$C_{17}H_{32}O_2$	268.4 g/mol		No reported activity
3	18.289	Heptanal	$C_7H_{14}O$	114.19 g/mol		Heterogeneous catalyst [24]
4	18.605	1,6;3,4-Dianhydro-2-Deoxy-. Beta. -d-Lyxo-Hexopyranose	$C_6H_8O_3$	164.16 g/mol		Antimicrobial agent [19]
5	18.670	Glutaraldehyde	$C_5H_8O_2$	100.11 g/mol		Antimicrobial agent [20]
6	20.755	(2S,3S) - (-)-3-propyloxiranemethanol	$C_6H_{12}O_2$	116.16 g/mol		No reported activity
7	23.432	Dodecanal	$C_{12}H_{24}O$	184.32 g/mol		Antimicrobial agent [21]
8	24.707	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	$C_{14}H_{14}O_3$	230.26 g/mol		Cytotoxicity against human MDA-MB-231 cells (Fang-Rong et al., 2018)
9	24.872	1,6;3,4-Dianhydro-2-Deoxy-. Beta. -d-Lyxo-Hexopyranose	$C_6H_8O_3$	164.16 g/mol		Antimicrobial agent [19]

10	25.317	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	$C_{14}H_{14}O_3$	230.26 g/mol		Cytotoxicity against human MDA-MB-231 cells (Fang-Rong et al., 2018)
11	26.523	Hexadecane, 1-chloro-	$C_{16}H_{33}Cl$	260.886 g/mol		Ovulation inhibitor [25]
12	27.218	2-(Aminooxy)propanoic acid	$C_3H_7NO_3$	105.09 g/mol		No reported activity
13	27.778	Dodecanedioic acid	$C_{12}H_{22}O_4$	230.3 g/mol		Antibacterial agent [22]