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Research Article

GC-MS ANALYSIS AND PREDICTION OF BIOLOGICAL ACTIVITIES USING MOLECULAR DOCKING OF *CODIUM DECORTICATUM* (WOODWARD) M. HOWE AGAINST CANCER

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ABSTRACT

Objective: The objective of this study was to analyze the phytochemicals and the prediction of biological activities in the petroleum ether extract of marine green macro alga *Codium decorticatum* (Woodward) M. Howe (Green algae) collected from Mandapam, Tamil Nadu, India.

Methods: The characterization of biochemical was done by gas chromatography–mass spectroscopic (GC-MS) spectra analysis and the biological activities were analyzed using the Prediction of Activity Spectra for Substances (PASS) technique.

Results: GC-MS spectral analysis showed a prevailing compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (100%) with retention time of 22.33 min. The PASS result showed 1,933 different kinds of biological activities, namely, anti-cancer (93.5%), anti-seborrheic (89.4%), antieczematic (81.2%), anti-hypercholesterolemic (81.5%), antiviral (81.2%), insulin promoter (79.4%), and anti-inflammatory (77.8%).

Conclusion: The present study confirmed the inter-molecular hydrogen bonding of the bioactive compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester with the active site of ARF6 (PDB ID 2w83) and the binding energy is −7.49 kcal/mol. Four hydrogen bond interactions were present at GLY80, THR81, GLY80, and GLY80 at the active site.

Keywords: Green algae, *Codium decorticatum*, Biochemical, GC-MS, PASS

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INTRODUCTION

The marine algae have high level of species diversity. *Codium* includes 109 species that dominate the subtropical and temperate regions [1]. The marine environment also represents a largely unexplored source for isolation of new microbes that are potent producers of bioactive secondary metabolites with strong anti-coagulant, anti-viral, anti-oxidative, anti-tumor, anti-thrombotic, fungicidal, neuroprotective, immunomodulating, anti-hyperlipidemic, anti-hepatotoxic activities, anti-bacterial, and anti-fungal activities are being intensely used as anti-biotics and may be effective against infectious diseases such as HIV [2]. Therefore, marine algae derived SPs have great potential for further development as healthy food and medical products [3]. A number of biologically active compounds with varying degrees of action, such as antidepressants include anticoagulants, Inhibitor, neurotoxic, hyperthermic, apoptosis agonist, dirt expression inhibitor, chemopreventive, hypokalemia, anti-microtubule, anti-proliferative, cytotoxic, photo protective, as well as antibiotic and antifouling compounds have been isolated from the algae [4].

The marine algae giving immune stimuli enhance cellular and humoral immune response mechanisms in fish. Immunostimulants are an alternative to chemical antibiotics, which are effective in controlling disease for aquatic organisms, as well as being safe for consumers and environment friendly [5]. The extract of *Codium* species in different doses for 14 days increased the total hemocytes of tiger shrimp that indicated the bioactive compound of *Codium* species may act and enhance as an immune system [6]. Few marine green algal species contain a lot of sulfate polysaccharides that have been appeared to catch the replication period of wrapped contaminations [7]. The concept of biological function spectrum was introduced to describe the properties of biologically active substances. Prediction of Activity Spectra for Substances (PASS) software product predicts 300 pharmacological effects and biochemical instructions [8]. Among the various biological activities, cancer is also one of the deadly disease and the search is ongoing to identify potential anti-cancer drugs. The human body contains different cancer macromolecules in different ways, and this is very difficult a single molecule or drug that blocks all molecules at once [9]. The high value of predictive accuracy shows that the chemical explanations and methods used in PASS provide very strong structural-functional relationships and reliable predictions. It has been demonstrated that the very specific drug information used in PASS's training package can make a very specific distinction between drug and nondrug [10].

The objective of the present study is to analyze the phytochemicals and the prediction of biological activities in the petroleum ether extract of marine green macro alga *Codium decorticatum* (Woodward) M. Howe (Green algae) collected from Mandapam, Tamil Nadu, India, followed using AutoDock 4.2 tool, that prediction to confirm the efficiency of the active compound against cancer.

METHODS

Sample collection

Fresh samples of *C. decorticatum* (Woodward) M. Howe, a green algal species, were collected from the intertidal region of Mandapam (Lat. 9° 16' 48.00" N Long. 79° 07' 12.00" E), Ramanathapuram district, Southeast coast of Tamil Nadu, India. The collected algal species was authenticated and deposited in Xavier's College Herbarium, Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai-627002 and the Voucher number (XCH20508) was also given for the green algal species. Samples were collected by hand picking during low tide and flushed with marine water to remove debris and epiphytes. The entire epiphytes were expelled using soft brush. In the laboratory, the samples are again washed in freshwater and stored in refrigerator for further analysis [11].

Bioactive compound extraction

The plant specimens were washed with distilled water and placed on blotting paper and spread out at room temperature in the shade

condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The fine-powdered samples were then stored in the refrigerator for further analysis. 30 g of the fine powdered samples were packed in the Soxhlet apparatus and extracted with petroleum ether for 8 h separately [12].

Gas Chromatography–Mass Spectroscopic (GC-MS) Spectra Analysis spectrum analysis

GC-MS analysis of the selected petroleum ether extracts of C. decorticatum (Woodward) M. Howe. was carried out using Elite-5MMS, (100% dimethyl ply siloxane), 30.0 m × 250µm DF capillary column Spectrometer. Initial temperature set to 60° C maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised to 300°C at an increase rate of 10°C/min and maintained for 6 min. The injection port temperature was fixed at 250°C and the helium flow rate at 1 ml/min. The ionization voltage was 70eV. The samples were injected in a split manner as 10:1. The mass spectral level solvent delay was set at 2 min, the transfer temperature at 240°C, the source temperature at 240°C, and the scanning range at 50–600Da. The substance constituents were distinguished by GC-MS.

Demonstration of GC-MS mass spectrum using the National Institutional Standards and Technology (NIST) database of over 62,000 formats. The spectrum of known components was compared with the spectrum of known components stored in the NIST library. The name of the components of the test material, the retention time (RT), the molecular weight, and the molecular formula were determined [13].

PASS prediction

PASSs are a web-based application that predicts the biological functional spectrum of a compound based on its structure. It operates on the principle that the biological function of a compound is equal to its structure. PASS forecasting tools are built using 20,000 primary compounds from the MDDR database. The molecule is predicted by "comparing" the structure of the new compound with the structure of the well-known biologically active substrate present in the database. The methodology of action range assessment depends on Bayesian methodology. The PASS prediction will anticipate the Pa: Pi (active, inactive ratio). The average accuracy of the estimate is about 95% according to the leave-one-out cross-validation estimate [14].

AutoDock

Docking was performed with AutoDock 4.2 (Scripps Research Institute, USA). The 3D structure of 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester was downloaded in SDF format from pub chem. The open Bable tool is used to convert the SDF file to PDBQT and Gasteiger charge was assigned to the ligand. X-ray crystal structures of protease 2w83 were obtained from the RCSB Protein Data Bank. Receptors were prepared for docking in such all heteroatoms such as water and ions, which were removed. Kollman charges, polar-H atoms, and docking parameters were added to the macromolecule. The program was carried out with a spacing of 0.375 A° and grid dimensions of 90, 90, 90 A°. Gridbox center was set to coordinate −0.074, 0.083, and −0.013 in x, y, and z, respectively [15].

RESULTS AND DISCUSSION

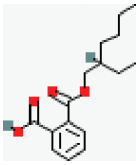
GC-MS analysis

The compounds present in the petroleum ether extract of the selected species *C. decorticatum* (Woodward) M. Howe, which were identified after the comparison of the mass spectra with NIST library by GC-MS analysis. The active principles with their RT, name, structure, molecular formula, and molecular weight are presented in Table 1 and Fig. 1. GC-MS spectrum of petroleum ether of *C. decorticatum* (Woodward) M. Howe revealed one major peak which indicated the presence of the major compound. The prevailing compound in petroleum ether extract was the major component 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (100%) with RT of 22.33 min.

PASS prediction

The identifying compound in petroleum ether extract from GC-MS analysis was 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (100%). 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester occupy 100% of area, which were analyzed for biological activity by PASS software. In Pa>0.7, their 1,933 different activities are predicated. All the 1,933 activities are fallen in the four categories that are mechanism of action, metabolic terms, pharmacotherapeutic effects, and adverse

Table 1: GC-MS profile of petroleum ether extract of *Codium decorticatum* (Woodward) M. Howe

Serial number	Retention time	Name of compound	Molecular formula	Molecular weight (g/mol)	Percentage	Structure
1	22.33	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.34	100.000	

GC-MS: Gas chromatography–mass spectroscopic

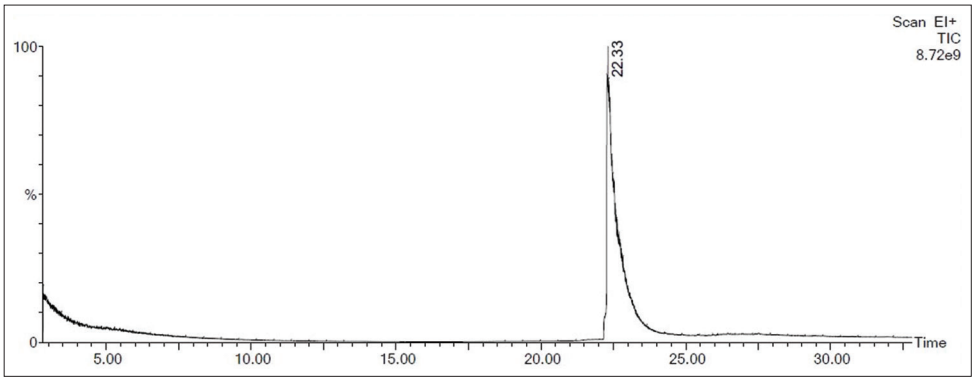


Fig.1: Gas chromatography–mass spectroscopic spectra analysis profile of petroleum ether extract of *Codium decorticatum* (Woodward) M. Howe

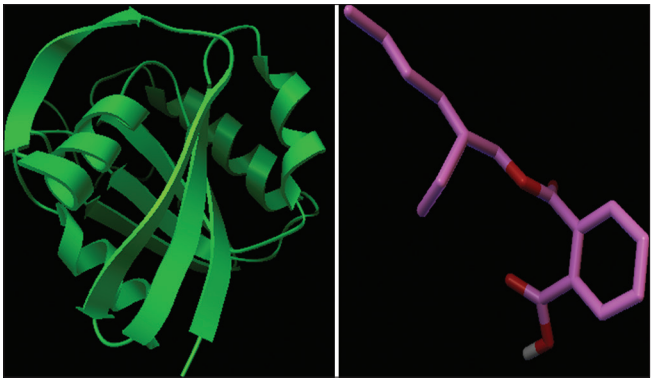


Fig. 2: 3D View of ARF6 (PDB ID 2w83) and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester

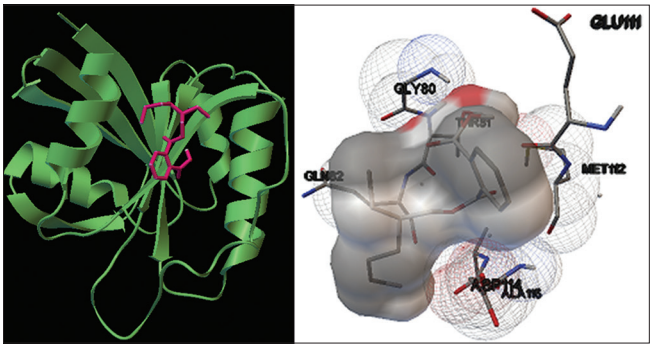


Fig. 3: 3D and 2D view of target – Ligand Interatction: 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester against ARF6 (PDB ID 2w83)

Table 2: Prediction activity spectra for 1,2-benzenedicarboxylic acid and mono(2-ethylhexyl) ester by prediction of activity spectra for substances system

Pa	Pi	Activity
0.980	0.002	Anti-cancer
0.963	0.003	Skin irritation, inactive
0.928	0.003	Sugar-phosphatase inhibitor
0.908	0.004	Lipid metabolism regulator
0.884	0.004	Pullulanase inhibitor
0.882	0.003	Cutinase inhibitor
0.836	0.005	Exoribonuclease II inhibitor
0.829	0.004	Lipoprotein lipase inhibitor
0.792	0.005	Cholesterol antagonist
0.815	0.028	Phobic disorders treatment
0.794	0.008	Anti-seborrheic
0.791	0.021	Antiseborrheic
0.782	0.013	Membrane permeability inhibitor
0.763	0.011	Carboxypeptidase Taq inhibitor
0.760	0.012	Ribulose-phosphate 3-epimerase inhibitor
0.752	0.004	5 Hydroxytryptamine release inhibitor
0.755	0.008	Fibrinolytic
0.769	0.025	Antieczematic
0.761	0.031	Anti-hypercholesterolemic
0.721	0.003	Antiviral
0.741	0.024	Insulin promoter
0.726	0.012	Oxidoreductase inhibitor
0.715	0.008	Anti-inflammatory

and toxic effects. There are 72 different types of mechanism of action including eye irritation inactive, skin irritation inactive, cholesterol antagonist, phobic disorders treatment, anti-seborrheic, anti-eczematic, preneoplastic conditions treatment, anti-hypercholesterolemic, anti-viral, anti-septic, insulin promoter, anti-infective, venombin inhibitor, anti-secretoric, antiviral, anti-myopathies, anti-convulsant, mucositis treatment, anti-pruritic, allergic, anti-inflammatory, kidney function stimulant, anti-hypoxic, respiratory analeptic, anti-uremic, intestinal, anti-anginal, anti-hypertensive, anti-pyretic, anti-fungal, anti-thrombotic, and anthrax lethal factor inhibitor (Table 2).

AutoDocking

The present study confirmed the inter-molecular hydrogen bonding of the bioactive compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester with the active site of adenosine diphosphate ribosylation factor 6 (ARF6, PDB ID 2w83), with the binding energy of -7.49 kcal/mol. Four hydrogen bond interactions are present at GLY80, THR81, GLY80 and GLY80 at the active site. Macromolecule was performed using an empirical free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, there will be at most 2,500,000 function evaluations used. GA will run for at most 27,000 generations, a mutation rate of 0.02, and a crossover rate of 0.80. Hundred independent docking runs were performed for each ligand. Results differing by 2.0 A° in positional root-mean square deviation were clustered together and represented by the result with the most favorable free energy of binding (Table 3 and Figs. 2 and 3).

DISCUSSION

1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester was analyzed by PASS for the different biological functions, and Pa (active) and Pi (inactive) are the probability estimates for each, respectively, from the biological spectrum. Their values vary from 0.000 to 1.000. The result of the present study predicted 1,933 different kinds of biological activities such as eye irritation inactive, skin irritation inactive, cholesterol antagonist, phobic disorders treatment, anti-seborrheic, fibrinolytic, anti-eczematic, preneoplastic conditions treatment, anti-viral, anti-septic, insulin promoter, anti-infective, venombin inhibitor, anti-secretoric, antiviral, anti-myopathies, anti-convulsant, mucositis treatment, anti-pruritic, allergic, anti-inflammatory, kidney function stimulant, anti-hypoxic, respiratory analeptic, anti-uremic, intestinal, anti-anginal, anti-hypertensive, anti-pyretic, anti-fungal, anti-thrombotic, anthrax lethal factor inhibitor, anti-pruritic, anti-psoriatic, oxidizing agent, dermatologic, retinal dehydrogenase inhibitor, diabetic nephropathy treatment, ophthalmic, lipoprotein disorders treatment, anti-fibrinolytic, anti-ulcerative, wound healing agent, ophthalmic drug, anti-diabetic (type 2), antitoxic, dementia treatment, DNA synthesis inhibitor, anti-mutagenic, hemostatic, antibacterial, HIV-2 reverse transcriptase inhibitor, antiperistaltic, diabetic neuropathy treatment, immunostimulant, prion diseases treatment, anti-cataract, anti-nephritic, insecticide, anti-helmintic, anti-tussive, atherosclerosis treatment, anti-neurogenic pain, renal failure treatment, hepatic disorders treatment, anti-infertility, female, anti-glaucomic, pancreatic disorders treatment, hair growth stimulant, urticaria treatment, anti-diabetic, menstruation disorders treatment, anti-acne, gastritis treatment, ovulation inhibitor, anti-mycobacterial, skin whitener, muscle relaxant, etc. Pa>0.7, this substance is likely to show efficacy in testing, but is more likely to match the analogue of a known pharmaceutical agent. 0.5<Pa<0.7, this substance is likely to show efficacy in testing, but the probability is low, and the evidence is unlike known pharmaceutical agents.

Table 3: Molecular docking result analysis of bioactive compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester against adenosine diphosphate ribosylation factor 6 (PDB ID 2w83)

Serial number	Compound	Binding energy (kcal/mol)	Number of hydrogen bonds	Hydrogen bond interactions	Total polar and nonpolar bonding
1	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	−7.49	4	GLY80, THR81, GLY80, GLY80	GLY82, GLY80, THR81, GLU111, MET112, ASP114, ALA115

Pa<0.5, the material is unlikely to reveal efficacy in the experiment. However, if the substance of the experiment is tested, the material will be a new synthetic element [16, 17].

CONCLUSION

In the present study, the bioactive compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester was isolated using petroleum ether from *C. decorticatum* (Woodward) M. Howe and the isolated compound was identified by GC-MS spectral analysis. The isolated compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester was analyzed by PASS prediction for the different biological functions. Totally 1,933 different kinds of biological activities such as anti-cancer antiviral, anti-bacterial, anti-fungal, anti-septic, anti-infective, anti-cataract, anti-diabetic, anti-mutagenic, anti-seborrheic, anti-hyper cholesterolemic, anti-secretoric, anti-inflammatory, anti-hypoxic, anti-thrombotic, and anti-infertility were predicted. In the molecular docking analysis, it was confirmed that the inter-molecular hydrogen bonding of the bioactive compound 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester in the active site of ARF6 (PDB ID 2w83) with the binding energy of −7.49 kcal/mol. ARF6 inhibitor drug was used for the treatment of lung cancer. The 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester affirm could be a ARF6 inhibitor. More experimental studies are needed to determine and clarify their underlying mechanism to develop anti-cancer drug.

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AUTHORS' CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Authors declare no conflicts of interests.

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