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# Phytochemical and molecular docking analysis of the ethanolic extract of *Codium decorticatum* (Woodward) M. Howe.

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**ABSTRACT**

*The present study was carried out to investigate the potential of anti-diabetic properties of the ethanolic extract of Codium decorticatum (Woodward) M. Howe by in silico method using AutoDock 4.2. Codium decorticatum (Woodward) M. Howe was collected from the intertidal region of Idinthakarai coast, Tirunelveli District, the southeast coast of Tamil Nadu, India. The phytochemical compounds were identified and isolated by using GC-MS, Preparative HPLC, FTIR and NMR. GC-MS spectrum of ethanolic extract prevailing six major compounds such as 1-hexyl-2-nitrocyclohexane, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, 9-octadecenal, (z), pentadecanal, 2-dodecen-1-yl(-) succinic anhydride and bicyclo[4.1.0]heptanes 7-pentylape. 2-dodecen-1-yl(-) succinic anhydride obtained high percentage area and it has the potential of anti-diabetic activity due to that, it was isolated. Preparative HPLC revealed the one prominent peak at the retention time of 5.427min with 100% peak area. The fractions of the peaks were selected for further analysis. The Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR) were used to identify and determine the structure of the isolated compound. The FTIR confirmed the presence of functional groups such as alkynes, alkenes, esters and tertiary amides. $^{1}H$ NMR analysis for determine the types and number of hydrogen atoms present in the compound and the chemical shifts indicates the presence of vinylic compounds, benzylic compounds, tertiary compounds, secondary compounds and primary compounds. $^{13}C$ NMR analysis was done to determine the types and number of carbon atoms present in a molecule and the chemical shifts indicates the presence of carboxylic acid and derivatives, alkene, aromatic compounds, tertiary compounds, secondary compounds and primary compounds. The isolated compound 2-dodecen-1-yl(-) succinic anhydride was docked against with aldose reductase. It confirmed the inter-molecular hydrogen bonding of the bioactive compound 2-dodecen-1-yl(-) succinic anhydride with the active site of aldose reductase (PDB ID: 1EF3), with the binding energy of -6.11 kcal/mol. Two hydrogen bond interactions are present at ARG217:HE and LYS221:HZ2 at the active site.*

***Keywords:*** *Codium decorticatum, 1EF3, GC-MS, HPLC, FTIR and NMR.*

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## INTRODUCTION

Diabetes mellitus is a chronic, endless disease. It is caused because of the sugar level is high in the circulatory system. This is due to improper work Pancreatic of beta cells. It will affect different parts of the body, including pancreatitis, increased risk of heart disease, high blood pressure, kidney failure, nerve damage, leg problems and eye problems[1,2]. Algae are a mixed group of plants with extensive fossil history and to form secondary metabolites of a wide range of biological functions because they contain biological compounds[3]. They are one of the renewable resources of the oceans with potential food uses. Consumption of algae as seafood in the human diet is a common practice in many Asian countries[4]. They can exist from unicellular microscopic organisms to multicellular organisms[5]. Evaluation of the components of phytochemicals in macro algal extracts has received attention for the important role in the prevention of human diseases. Because algae contain a variety of phytochemicals such as alkaloids, phenols, tannins, phlorotannins, terpenoids, pigments, glycosides, and steroid[6]. Algal bioactive compounds are used in traditional and complementary medicine antioxidant, antiviral, antifungal, antimicrobial, cytotoxic and larvicidal potentials. The secondary metabolites demonstrate a wide range of biological functions ranging from neurotransmitters to algicidal, nematicidal, insecticidal and

ichthyotoxicity and they can help to reduce swollen or inflamed lymph nodes and used to treat autoimmune diseases including chronic fatigue, HIV, arthritis and chronic allergies[7] and other pharmacological and cosmetic values[8]. In recent years, GC-MS, Preparative HPLC, FTIR and NMR were widely used due to its great performance and high recovery of the secondary metabolites from plant extracts. Prep-HPLC helps to separate even minor compounds[9] and most of the preparative separations organic solvents used as mobile phase[10]. Docking is widely used to anticipate the alignment of small molecular therapeutic compounds with respect to their protein targets in anticipation of the interaction and activity. Docking plays a vital role in drug design[11]. With this background, the present study was designed to isolate and identify the phytochemical compounds of the ethanolic extract of *Codium decorticatum* (Woodward) M. Howe using GC-MS, Preparative HPLC, FTIR and NMR. The isolated compounds docked against with of aldose reductase (PDB ID: 1EF3) using AutoDock 4.2.

## MATERIALS AND METHODS

### Collection of Sample

Fresh samples of *Codium decorticatum* (Woodward) M. Howe were collected from the intertidal region of Idinthakarai coast, Tirunelveli District, Southeast coast of Tamil Nadu, India. The collected algal sample was washed with marine water and transported to the laboratory in a plastic cover and washed thoroughly with tap water followed by distilled water until the salts epiphytes and debris were removed. The *Codium decorticatum* (Woodward) M. Howe was authenticated and deposited in St. Xavier's College Herbarium, Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu. And the voucher number was given as XCH20528.

### Preparation of extract

The collected samples were shade-dried and then milled into fine powdered using the tissue blender. The extraction was done by the soxhlet method. 30g powdered samples were packed in Soxhlet apparatus and extracted with ethanol extract for 8h separately[12, 13].

### Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was carried out using GC model Clarus 680, Mass Spectrometer Clarus 600 (EI) Perkin Elmer, Gas Chromatography was equipped and coupled to a mass detector TurboMass 5.4.2 spectrometer with an Elite-5MS, (100% Dimethyl ply siloxane), 30.0m × 250µm df capillary column. The instrument was set to an initial temperature of 60°C and maintained at this temperature for 2min. At the end of this period, the oven temperature was raised upto 300°C, at the rate of an increase of 10°C/min and maintained for 6min. Injection port temperature was ensured as 250°C and Helium flow rate as 1ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass Spectral condition solvent delay 2min, transfer temperature 240°C, source temperature 240°C and scanning range was set at 50-600Da. The chemical constituents were identified by GC-MS. Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The retention time, compound name, molecular formula and molecular weight and area percentage of the test materials were ascertained [14].

### Preparative HPLC

A Preparative HPLC system is associated with large columns and high flow rate. The objectives of the preparative HPLC are isolation and purification of the crude extracts. The phytochemical was purified using a Shimadzu HPLC fitted with a fraction collector (FRC-10A). Preparative reversed-phase HPLC was performed Shimpack PRC-ODS (250X20mm id, 5µm, Shimadzu). The instrument is consisted of an auto sampler, two pumps (main pump and make up pump) and a photodiode array detector connected with the fraction collector. The column configuration consists of C18 reversed-phase column with 100 mm. The temperature of the auto sampler tray was maintained at 35°C and the column was kept at room temperature. HPLC grade methanol as 80 %v/v and 20% of water made up the mobile phase. With a total flow rate of 5.0 mL min-1, the detection wavelength was set to 190-370 nm. Injection volumes were 5.0ml. The fraction collector was used to isolate and collect fractions. A photodiode array detector was used to detect phytoconstituents at a wavelength of 254nm and an individual peak was collected and the solvent was removed using rotary evaporation [15].

### FTIR analysis

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each analysis was repeated twice and confirmed the spectrum [16].

**NMR spectroscopy**

The NMR spectrometer is made up of three main parts: (1) The probe, which holds the sample to be tested, is attached to the superconducting magnet; (2) The console, which includes all of the electronics required for transmitting and receiving radio frequency (rf) pulses to the probe through the preamplifier and (3) The computer on which the experiment is executed and the collected NMR data is processed. The Bruker model AVANCE III HD (Switzerland) was used to analyze the $^{1}H$ NMR and the DRX-300 Mega Hz Bruker (Switzerland) was used to analyze the $^{13}C$NMR. All NMR measurements were acquired at 298K (25°C). Topspin 3.1 software was used to examine the data. Chemical shifts (δ) were calculated using residual solvent signals can evolve only in the state of magnetization and it expressed in ppm. For the recording of $^{1}H$ and $^{13}C$ NMR spectra, the following conditions were used: 30° pulse experiment; acquisition time 4.1 seconds; relaxation time 1.0 second; sweep width 15.1ppm (8012Hz); data points 65536 and dummy scan 2. Line broadening 0.1Hz was used to process the data. A total of 16 scans were acquired for each sample. For improved resolution, free induction decays were Fourier processed with a line widening factor of 0.1Hz. Manual phase adjustment was performed on the spectra. For quantitative analysis, peak integration was performed. At least triplicate measurements were taken in each experiment.

**MOLECULAR DOCKING ANALYSIS**

**Preparation of target protein**

The X-ray crystal structures of aldose reductase (PDB ID: 1EF3) were retrieved from RCSB Protein Data Bank. The protein is found in complex structure with fidarestat. The fidarestat is removed by using PyMol. The target protein were prepared for docking in such all heteroatoms like water, ions, etc. were removed. Kollman charges, polar-H atoms and docking parameters were added to the macromolecule.

**Preparation of ligands**

The 3D structure of 2-Dodecen-1-Yl(-)Succinic Anhydride was downloaded in sdf format from pub chem. The open babel computational tool is used to convert the sdf file to pdbqt. Finally the ligand was docked into the active site of the selected using the AutoDock 4.2 [17].

**Docking performance**

Docking was performed with AutoDock 4.2 (Scripps Research Institute, USA). A computational docking program based on an empirical free-energy force field and rapid Lamarckian genetic algorithm search method. The grid was run with a spacing of 0.436 A° and grid dimensions of 114, 122, 96 A°. The Grid box center was set to coordinate -26.036, 13.591 and -43.967 in x y, and z respectively [18].

**RESULTS AND DISCUSSION**

**GC-MS analysis**

GC-MS spectrum of ethanol extract of *Codium decorticatum* (Woodward) M. Howe revealed six major peaks with different retention time such as 21.66min 1-hexyl-2-nitrocyclohexane, 22.40min hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, 23.51min 9-octadecenal, (z), 24.57min pentadecanal, 27.09min 2-dodecen-1-yl(-) succinic anhydride and 27.74min bicyclo[4.1.0]heptane, 7-pentylape (Figure-1 & Table-1). Among these compounds 2-dodecen-1-yl(-) succinic anhydride obtain high area percentage of 54.937% and this compound was subjected to isolate and purify using prep. HPLC, FTIR and NMR.

**Figure-1: GC-MS spectrum of ethanolic extract of *Codium decorticatum***

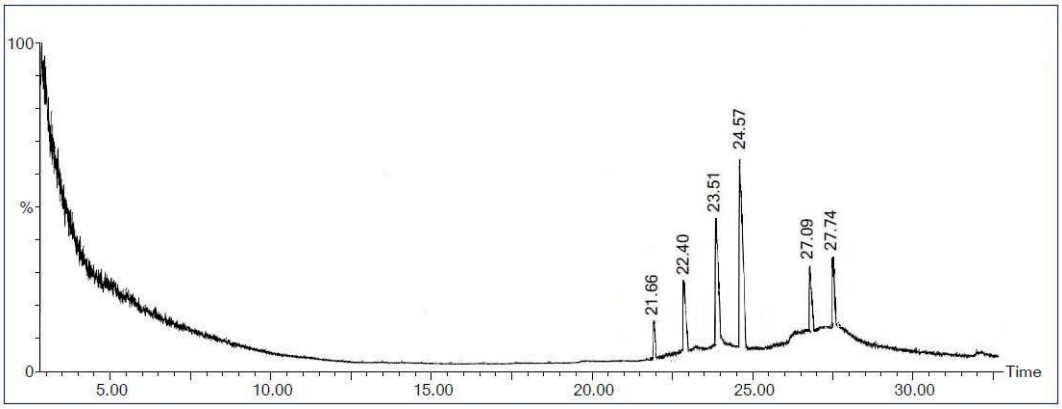


**Table-1: GC-MS spectral analysis of ethanolic extract of *Codium decorticatum***

| S. No | Retention time | Name of compound | Molecular Formula | Molecular Weight | % |
|---|---|---|---|---|---|
| 1 | 21.66 | 1-Hexyl-2-Nitrocyclohexane | $C_{12}H_{23}NO_2$ | 213.32 | 2.395 |
| 2 | 22.40 | Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester | $C_{35}H_{68}O_5$ | 568.9 | 9.731 |
| 3 | 23.51 | 9-Octadecenal, (Z)- | $C_{18}H_{34}O$ | 266.5 | 9.885 |
| 4 | 24.57 | Pentadecanal | $C_{15}H_{30}O$ | 226.4 | 2.029 |
| 5 | 27.09 | 2-Dodecen-1-Yl(-)Succinic Anhydride | $C_{16}H_{26}O_3$ | 266.38 | 54.937 |
| 6 | 27.74 | Bicyclo[4.1.0]Heptane, 7-Pentylape | $C_{12}H_{22}$ | 166.30 | 21.023 |

## ISOLATION AND IDENTIFICATION OF PHYTOCHEMICALS

### Preparative High Pressure Liquid Chromatography analysis

The ethanolic extract of *Codium decorticatum* (Woodward) M. Howe was subjected to preparative HPLC for the purification and isolation of the compound. It revealed the one prominent peak at the retention time of 5.427min with 100% peak area. The fractions of the peaks were selected for further analysis. The Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR) were used to identify and determine the structure of the isolated compound (Figure-2 & Table-2).

**Figure-2: Prep. HPLC spectrum of ethanol extract of *Codium decorticatum***

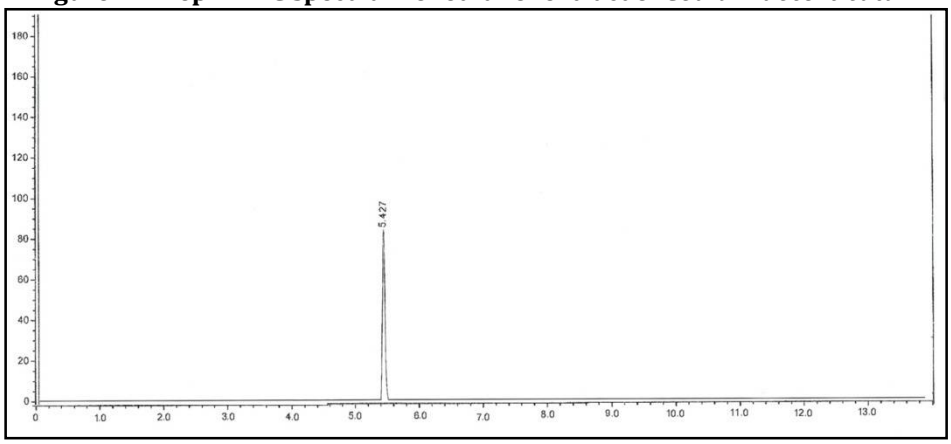



**Table-2: Prep. HPLC spectrum analysis of ethanol extract of *Codium decorticatum***

| Name | Retention Time (min) | Peak Area (mV.s) | Peak Height (mV) | Peak Area % |
|---|---|---|---|---|
| 1 | 5.427 | 1253.06 | 85.06 | 100 |

### FTIR spectrum analysis

The FTIR results of ethanol extract of *Codium decorticatum* (Woodward) M. Howe showed different peaks at 651.89, 678.90, 895.87, 1000.99 and 1636.49cm$^{-1}$. It was confirmed the presence of functional groups such as alkynes, alkynes, alkenes, ester and tertiary amides respectively (Figure-3 & Table-3).

**Figure-3: FTIR spectrum of ethanol extract of *Codium decorticatum***

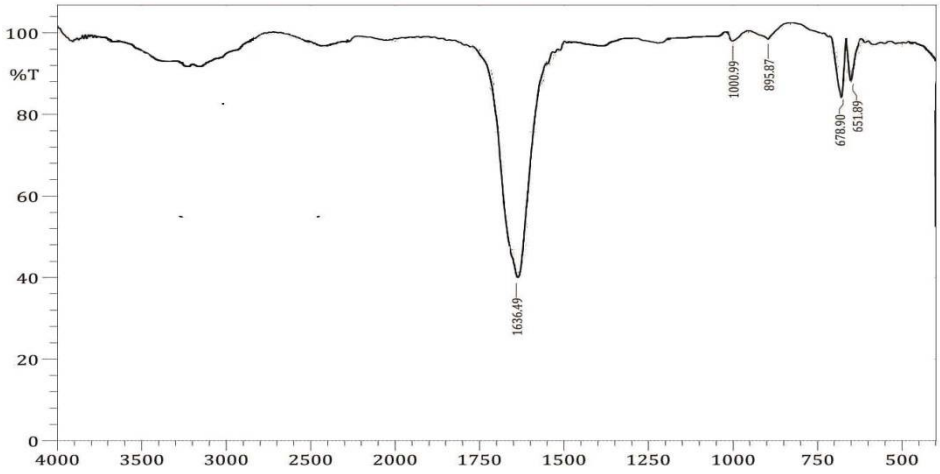


**Table-3: FTIR spectrum of ethanol extract of *Codium decorticatum***

| S.No | Peak value ($cm^{-1}$) | Functional group | Type of vibration | Possible compound |
|---|---|---|---|---|
| 1 | 651.89 | C-H | Bend | Alkynes |
| 2 | 678.90 | C-H | Bend | Alkynes |
| 3 | 895.87 | =CH | Bend | Alkenes |
| 4 | 1000.99 | C-O | Stretch | Ester |
| 5 | 1636.49 | C=O | Stretch | Tertiary amides |

**NMR analysis**

**$^{1}$H NMR analysis of 2-Dodecen-1-Yl(-)Succinic Anhydride**

The isolated compound of the ethanol extract of *Codium decorticatum* (Woodward) M. Howe was subjected to $^{1}$H NMR analysis for determine the types and number of hydrogen atoms present in the compound. The isolated compound shows the chemical shift values (δ) at 5.32, 4.99, 1.59, 1.56, 1.25 and 0.88ppm respectively. Among these chemical shifts, 5.32 and 4.99ppm indicates Vinylic compounds, 1.59 and1.56ppm indicates Tertiary compounds, 1.25ppm indicates Secondary compounds, 0.88ppm to indicates the presence of Primary compounds (Figure-4 & Table-4).

**Figure-4: $^{1}$H NMR analysis of 2-Dodecen-1-Yl(-)Succinic Anhydride**

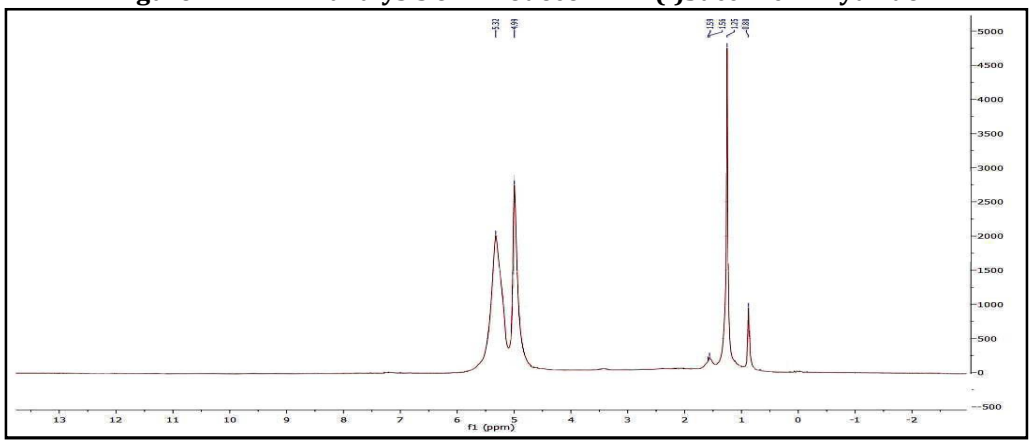



**Table-4: $^{1}$H NMR analysis of 2-Dodecen-1-Yl(-)Succinic Anhydride**

| S. No | Assign | Shift (ppm) | Range (ppm) | Type of bond | Description |
|---|---|---|---|---|---|
| 1 | A | 5.32 | 4.5-6.5 | $R_2C=H$ | Vinylic |
| 2 | B | 4.99 | 4.5-6.5 | $R_2C=H$ | Vinylic |
| 3 | E | 1.59 | 1.4-1.7 | $R_3C-H$ | Tertiary (methine) |
| 4 | F | 1.56 | 1.4-1.7 | $R_3C-H$ | Tertiary (methine) |
| 5 | G | 1.25 | 1.2-1.4 | $R-CH_2-R$ | Secondary (methylene) |
| 6 | H | 0.88 | 0.8-1.0 | $R-CH_3$ | Primary (methyl) |

**$^{13}$C NMR analysis of Compound: 2-Dodecen-1-Yl(-)Succinic Anhydride**

The isolated compound of the ethanol extract of *Codium decorticatum* (Woodward) M. Howe was subjected to $^{13}$C NMR analysis for determine the types and number of carbon atoms present in a molecule. The isolated compound shows the chemical shift values (δ) at the following ppm 174.56, 164.24, 135.34, 122.84, 44.87, 38.79, 38.09, 27.70, 24.15, 22.72, 20.26, 17.01 and 13.85ppm. Among these chemical shifts 174.56 and 164.24ppm indicates the presence of Carboxylic acid and derivatives, 135.34 and 122.84ppm indicates Alkene, aromatic compounds, 44.87, 38.79 and 38.09ppm indicates tertiary compounds 27.70, 24.15, 22.72, 20.26 and 17.01ppm secondary compounds, 13.85ppm indicates primary compounds (Figure-5 & Table-5).

**Figure-5: $^{13}C$ NMR analysis of 2-Dodecen-1-Yl(-)Succinic Anhydride**

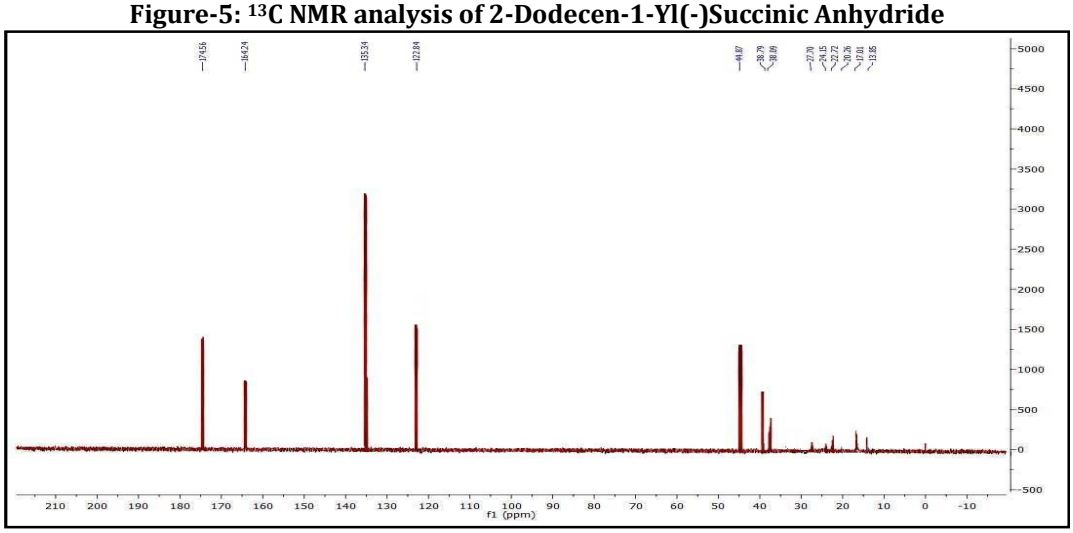
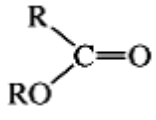
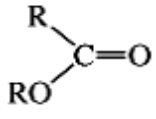



**Table-5: $^{13}C$ NMR analysis of 2-Dodecen-1-Yl(-)Succinic Anhydride**

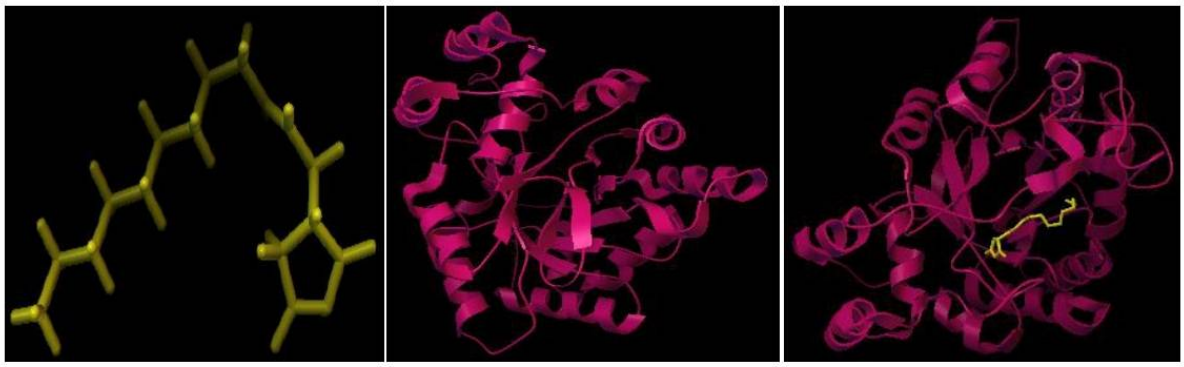
| S.No | Shift (ppm) | Range (ppm) | Type of bond | Description |
|---|---|---|---|---|
| 1 | 174.56 | 150-180 |  | Carboxylic acid and derivatives |
| 2 | 164.24 | 150-180 |  | Carboxylic acid and derivatives |
| 3 | 135.34 | 100-150 | C=C | Alkene, aromatic |
| 4 | 122.84 | 100-150 | C=C | Alkene, aromatic |
| 5 | 44.87 | 30-50 | $R_3C$-H | Tertiary |
| 6 | 38.79 | 30-50 | $R_3C$-H | Tertiary |
| 7 | 38.09 | 30-50 | $R_3C$-H | Tertiary |
| 8 | 27.70 | 15-50 | R-$CH_2$-R | Secondary |
| 9 | 24.15 | 15-50 | R-$CH_2$-R | Secondary |
| 10 | 22.72 | 15-50 | R-$CH_2$-R | Secondary |
| 11 | 20.26 | 15-50 | R-$CH_2$-R | Secondary |
| 12 | 17.01 | 15-50 | R-$CH_2$-R | Secondary |
| 13 | 13.85 | 5-22 | R-$CH_3$ | Primary |

## MOLECULAR DOCKING ANALYSIS

### Docking of 2-dodecen-1-yl(-) succinic anhydride against aldose reductase

The docking analysis was confirmed the inter-molecular hydrogen bonding of the bioactive compound 2-dodecen-1-yl(-) succinic anhydride with the active site of aldose reductase (PDB ID: 1EF3), with the binding energy of -6.11 kcal/mol. Two hydrogen bond interactions are present at ARG217:HE and LYS221:HZ2 at the active site. Macromolecule was performed using an empirical free energy function and Lamarckian Genetic Algorithm. GA will run for at most 27000 generations and hundred independent docking runs were performed for ligand. Results differing by 2.0 A˚ in positional root-mean square deviation (RMSD) were clustered together and represented by the result with the most favorable free energy of binding (Figure-6 & Table-6 &7).

**Figure-7: Molecular docking analysis of 2-dodecen-1-yl(-) succinic anhydride against aldose reductase**



A B C

**A**: Structure of 2-dodecen-1-yl(-) succinic anhydride; **B:** Structure of aldose reductase; **C:** View of target-ligand interaction

**Table-6: Grid box coordinates for docking**

| Receptor (PDB ID) | Center grid coordinates (A°) | | | Spacing and Parameters of total gird (A°) | | | |
|---|---|---|---|---|---|---|---|
| | Centre X | Centre Y | Centre Z | Spacing | X Point | Y Point | Z Point |
| 1EF3 | -26.036 | 13.591 | -43.967 | 0.436 | 114 | 122 | 96 |

**Table-7: Docking of 2-dodecen-1-yl(-) succinic anhydride against aldose reductase**

| Target protein (PDB ID) | Binding energy (kcal/mol) | Ki (μM) | Inter mol. energy (kcal/mol) | vdW + H bond + desolv energy (kcal/mol) | Electrostatic energy (kcal/mol) | No. of H bonds | Hydrogen bond interactions |
|---|---|---|---|---|---|---|---|
| 1EF3 | -6.11 | 33.12 | -9.09 | -8.91 | -0.19 | 2 | ARG217:HE, LYS221:HZ2 |

## CONCLUSION

In the current study, an attempt was initiated to study the phytochemical analysis and the molecular docking of the phytochemicals in the ethanolic extract of *Codium decorticatum* (Woodward) M. Howe. GC-MS spectrum revealed six major compounds. 2-dodecen-1-yl(-) succinic anhydride was one among them which showed the highest percentage area and it has potential of anti-diabetic activity, due to that, it was isolated. 2-dodecen-1-yl(-) succinic anhydride and purified using by prep-HPLC and the functional groups were indentified with the help of FTIR and NMR which used to confirm the structure of the compound. The bioactive compound 2-dodecen-1-yl(-) succinic anhydride was docked with the active site of aldose reductase (PDB ID: 1EF3), with the binding energy of -6.11 kcal/mol. Thus, *Codium decorticatum* (Woodward) M. Howe is the best source of bioactive compounds and their bioactive compounds are utilized for the drug development. More experimental studies are needed to determine and clarify their underlying mechanism to develop drug design.

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