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In vitro anti-diabetic and anti-inflammatory activities of metabolites isolated from Marine Sponge, *Heteronema erecta* (Keller, 1889) and its *in silico* studies

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Abstract
Phenylalanine, aromatic amino acid and clupadonic acid, a long-chain fatty acid were purified from methanol and methanol-dichloromethane extracts of marine sponge H. erecta Keller. It was characterized by using UHPLC- Triple quadrupole ESI/MS. The clupadonic acid isolated possessed good inhibition towards α-amylase and α-glucosidase with IC_{50} of 17µg/ml and 22µg/ml respectively. Apart from that, phenylalanine gave better anti-inflammatory compared to clupadonic acid with 16µg/ml. The interaction between phenylalanine and clupadonic acid against the three receptors IXU7, 1SMD and 4Y14 has been studied using molecular docking.

Keywords: LC-MS, Sponges, Bioactive Metabolites, Anti-diabetic, *in-silico*.

Introduction

Sponges are spineless animals belonging to phylum, "the pore bearers" (Porifera), serve as most primitive multi-celled animals existing for millions of year ago. Marine sponges are soft-bodied, sessile and channel feeders gathering little particles of nourishment from ocean water ascending through their bodies[11,24]. This is an exceptional storehouse of novel bioactive natural products with structural and chemical features generally not found in terrestrial natural products. The marine organisms also provided a rich source of nutraceuticals and potential candidates for the treatment of several human diseases. Some of the natural merchandise isolated from marine invertebrates are shown to be or are suspected to be of microbial origin and this is now thought to be the case for the majority of such molecules[13].

Bioactive compounds from the marine habitat have been represented as the greatest under an exploited source of potentially active pharmaceutical agents. They produce a variety of metabolites, some of which can be used for drug development[8]. The relationship between sponges and medicines goes back to conqueror physicians and was totally described by the Roman scholar Plinius.

Physicians used sponges that were drenched with iodine to kindle coagulation of the blood, or with bioactive plant extracts to anesthetize patients. Also, they were soaked with pure wine and put on the left part of the chest in case of heartaches and soaked in urine to treat bites of poisonous animals. Plinius recommended the use of sponges against sunstrokes and they were used against all kinds of wounds, bone fractures drops, stomach aches, infectious diseases and testicular tumors[14], or perhaps as implants once breast operations[6].

Metabolic disorders (i.e. any of the diseases or disorders that disrupt traditional metabolism) square measure common pathologies and particularly polygenic diseases. In 2013, it was estimated that over 382 million people throughout the world have diabetes and this number is expected to increase up to 500 million in 2030 when it is expected that this disease will be the 7th leading cause of death. Diabetes is sometimes caused by the interaction of genetic and environmental factors and is characterized by absence of hormone secretion (relative and absolute) and hormone resistance, always leading to metabolism disorders of fat, protein and carbohydrate.

Insulin could be an amide internal secretion created by beta cells of the exocrine gland islets. It has two crucial functions without which the body would cease to function: (1) insulin stimulates glucose uptake and lipid synthesis and (2) insulin inhibits the breakdown of lipids, proteins and glycogen and also inhibits the glucose pathway (gluconeogenesis).

Many people affected by diabetes will eventually have a series of diabetic complications like nephropathy, neuropathy, retinopathy, diabetic foot, ketoacidosis and even increased risk of cardiovascular diseases and hypertension. Square measure 2 sorts of polygenic disease, type-1 and type-2. Many secondary metabolites isolated from sponges were tested for anti-diabetic activity.

One of the most significant problems that has hindered research on secondary metabolites in sponges is their low concentration; for marine invertebrates varied compounds square measure were found at concentrations of but zero.00001% of their weight [24], creating necessary the employment of techniques like natural process coupled with mass spectrometry, which has proven to be a powerful analytical tool for the fast and effective investigation of complex biomolecules[10]. So the current study aims the investigation of marine sponge derived metabolites towards anti-diabetic and anti-inflammation.

Material and Methods

Sample Collection: The sponge, *H. erecta* was collected from Mandapam coast situated on the southeast coast of India. The marine sponge was handpicked at a depth of 2 ft. The collected samples were rinsed in seawater repeatedly to remove any debris or epiphytes and were transported to the laboratory. Then, it was washed thoroughly in tap water to remove unwanted particles and dried in room temperature for 4 days. The samples were air-dried and initially chopped roughly using scissor. Later, they were ground to achieve a fine powder and stored in an air-tight zip lock cover for further use.

Preparation of extracts: Two different solvent extraction methods were employed to prepare the sponge extract. In the first set, the sponge powder was macerated with methanol (MeOH) at a ratio 1:5 (w/v) and incubated for 24 hours. The second method involved the maceration of sponge powder with MeOH-Dichloromethane (DCM) [MeOH/DCM; 1:1] for 24 hours. After maceration, the solutions were decanted, filtered and evaporated to dryness in a rotary vacuum evaporator at 40^0 C. This process was repeated several times until the solution appeared colorless. Thus, the two extracts of sponge namely MeOH and MeOH/DCM were subsequently used for the purification and identification of target-specific bioactive molecules.

Ultra High-Pressure Liquid Chromatography-Electron Spin Ionization /Mass Spectrometry (UHPLC-ESI/MS) analysis of sponge metabolites: UHPLC–ESI/MS analyses were performed at 254 nm at 30 °C using RP C_{18} column (150 × 4.6) × 1.8µm equipped with a binary pump coupled to a UV detector and a Triple Quadra pole (ESI) mass spectrometer (Shimadzu, Japan). The sample was injected as a 3 µL volume. The mobile phase consisted of solvent A as water and solvent B as methanol at a flow rate of 0.4 mL/min. The binary gradient was applied, conditions were as follows as 7.5% of B from 0^{th} min increased gradually to 90%; 95% B for 2 min and then decreased to 50% B for 3 min, finally reduced to 7.5% B back and the total run time was 30 min. ESI ionization conditions were spray voltage 4 kV, capillary 350 °C, 15 V. Pure nitrogen was the sheath gas and pure helium was the collusion gas. The full scan mass data m/z was obtained in negative mode and ranged from 100 to 2000 Da.

Preparative - Reverse Phase High-Pressure Liquid Chromatography (RP-HPLC) fractionation of sponge metabolites: The crude MeOH and MeOH-DCM extract of *H. erecta* were subjected to preparative reverse phase HPLC (Phenomenex, 250mm x 10mm Luna C_{18} (2) 10µ 100A˚) using a gradient mixture. The HPLC conditions were standardized as linear gradient from 7.5% of B from 0^{th} min increasing gradually to 90%; 95% B for 2 min and then decreased to 50% B for 3 min, finally reduced to 7.5% B back and the total run time was 30 min. The major fraction was collected and its mass value was detected through mass spectrometry.

Fourier Transforms Infrared Spectroscopy (FTIR) analysis of sponge metabolites: The infrared spectra were recorded on Shimadzu IR-470 model in the range of spectra 400–4000 cm^{-1}. The samples were subjected to FT-IR analysis through pressed KBr pellet technique. The spectra were plotted as intensity versus wave number.

***In Vitro* Anti -Inflammatory Activity using Albumin Denaturation:** The anti-inflammatory activity of the purified compound was performed by the method[20] with albumin denaturation. The reaction mixture was consisting of test sample at different concentrations and 1% aqueous solution of bovine albumin solution. The pH of reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37 °C for 20 min and then heated at 57 °C for 20 min. After cooling the tubes, the turbidity was measured at 660 nm in a UV spectrophotometer. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = Abs_{control} - Abs_{sample} / Abs_{control} \times 100$$

α-Amylase Inhibitory Activity: The amylase inhibitory activity was measured by following method[22]. The starch solution (0.5% w/v) was obtained by stirring and boiling 0.25 g of soluble potato starch in 50 ml of deionized water for 15 min. The enzyme solution was prepared by mixing 0.001 g of α-amylase in 100 ml of 20 mmol/l sodium phosphate buffer (pH 6.9) containing 6.7 mmol/l sodium chloride. The purified compound was dissolved in dimethylsulfoxide to give suitable concentrations for the assay. The color reagent was a solution containing 96 mmol/l 3,5-dinitrosalicylic acid (20 ml), 5.31 mol/l sodium potassium tartrate in 2 mol/L sodium hydroxide (8 ml) and deionized water (12 ml).

About 1 ml of each compound and 1 ml of the enzyme solution were mixed in a test tube and incubated at 25 °C for 30 min. About 1 ml of this mixture was added to 1 ml of the starch solution and the tube was further incubated at 25 °C for 3 min. Then, 1 ml of the color reagent was added and the tube was placed into 85 °C water bath. After 15 min, the reaction mixture was cooled and diluted with 9 ml distilled water and the absorbance value determined at 540 nm using a Shimadzu UV-visible Spectrophotometer (Japan). Individual blanks were prepared for correcting the background absorbance.

In this case, the color reagent solution was added before the addition of starch solution and then the tube was placed into the water bath. Controls were conducted in an identical manner, replacing extracts with 1 ml dimethylsulfoxide. Acarbose solution was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$$\alpha\text{-amylase}\% = 100(A_{Control}-A_{Sample})/A_{Control}$$

where A Control=A Test-A Blank, A Sample=A Test-A.

α-Glucosidase Inhibitory Activity: The purified compound (100 µl) was mixed with 100µl of 0.1 mol/l phosphate buffer (pH 6.9) and 100 µl α-glucosidase solution (1 unit/ml/min) and incubated at 25 °C for 5 min. After the pre-incubation, 100 µl *p*-nitrophenyl-α-*D*-glucopyranoside (5 mmol/l) solution was added and the reaction mixture was incubated at 25°C for 10 min. After the incubation, the absorbance was recorded at 405 nm and α-glucosidase inhibition (%) was calculated:[29]

$$\alpha\text{-amylase\%} = 100(A_{Control} - A_{Sample})/A_{Control}$$

where A Control=A Test-A Blank, A Sample=A Test-A.

Molecular Docking Studies

***In silico* analysis of enzyme-inhibitor complex:** *In silico* analysis of the enzyme-inhibitor complex was performed following the procedure described[5].

Determining target protein sequence: The 1XU7, 1SMD and 4Y14 receptors were selected for studying the efficacy of the targeted molecules against the type 2 Diabetes Mellitus (T2DM). Hence, these three receptors were obtained from the RCSB PDB and pre-processed using the Swissdock tools.

Ligand identification from databases: The 2D structures of phenylalanine and clupanodonic acid were imported from Pubchem and Zinc database.

Docking Studies: The Swissdock tool was used to conduct the docking between the target compounds and the receptors. The docking parameters were in the Chimera with view dock tools.

Results

Yield calculations: The yields of the two different sponge extracts were illustrated in table 1. The yield of MeOH+DCM extract was greater than the yield of MeOH extract.

Ultra High-Pressure Liquid Chromatography - Electron Spin Ionization /Mass Spectrometry (UHPLC_ESI/MS): The MeOH and MeOH-DCM extracts of sponge H.erecta were subjected to UHPLC-ESI/MS for initial screening of metabolites. From MeOH extract, phenylalanine as major compound and doxepin were found and from another extract of Clupadonic acid, Cyclotrisilaxane hexamethyl and along with phenylalanine were found.

On observing, phenylalanine was considered to be present in both the extracts due to influence of solvents used and considered to be major compound from MeOH extract. Simultaneously clupadonic acid was considered as major compound from MeOH-DCM. The compound phenylalanine, an aromatic amino acid which was isolated from the MeOH extract of *H. erecta*, had a molecular formula of $C_9H_{11}NO_2$, deduced by ESI-MS at *m/z*165.19 $[M^+]$ with an addition of oxygen molecule.

It shows the characteristic nature of tyrosine and clupadonic acid from MeOH-DCM had a molecular formula of 353Da $[M+Na]^+$ considered to be major compound having high intensity which results in better yield taken for purification and further biological activities. The compounds detected in this study are represented as chromatogram and spectrum below.

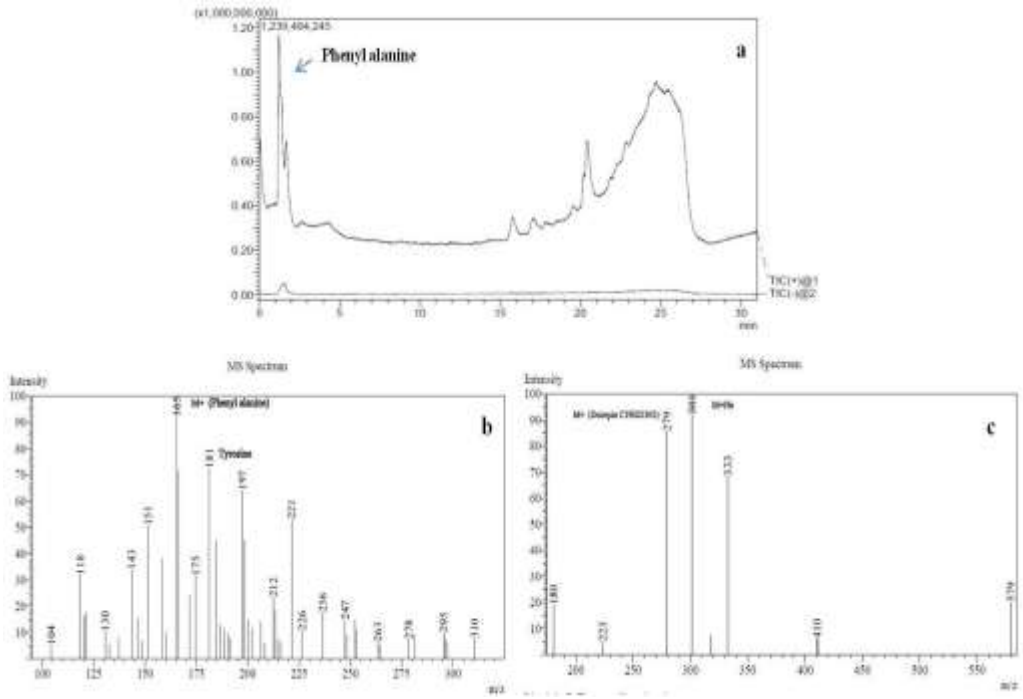

Figure 1: (a) showing the UHPLC-ESI/MS on positive and negative TIC for MeOH Extract; (b) Mass Spectrum (m/z) for Phenyl alanine from MeOH Extract; (c) Mass Spectrum (m/z) for Doxepin from MeOH Extract

RP- HPLC purification and mass spectrum analysis of Phenylalanine and Clupadonic acid: The major peak was collected and the compounds present were identified and reconfirmed through the mass spectrum as phenylalanine (4.8mg) from MeOH and other as Clupadonic acid (6.2mg) from MeOH-DCM extract respectively. The purified sample spectrum signifies the elimination of junk peaks from crude extract that was screened before and after is given below (fig 7a & 7b). The HPLC conditions were followed as same as earlier.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectrum revealed that the presence of specific functional groups corresponding to the purified fraction of phenyl alanine and clupadonic acid. The major groups detected that were 3199.33 cm^{-1} (Carboxylic acid), 3066.87 cm^{-1} (Aromatics), 1542.11 cm^{-1} (Amides) 1542.11 cm^{-1} (Aromatics nitro) for confirming the presence of amino acid whereas the clupodonic acid has the functional group of 3199.33 cm^{-1}(carboxylic group) and other certain groups (Figure 8a &8b)

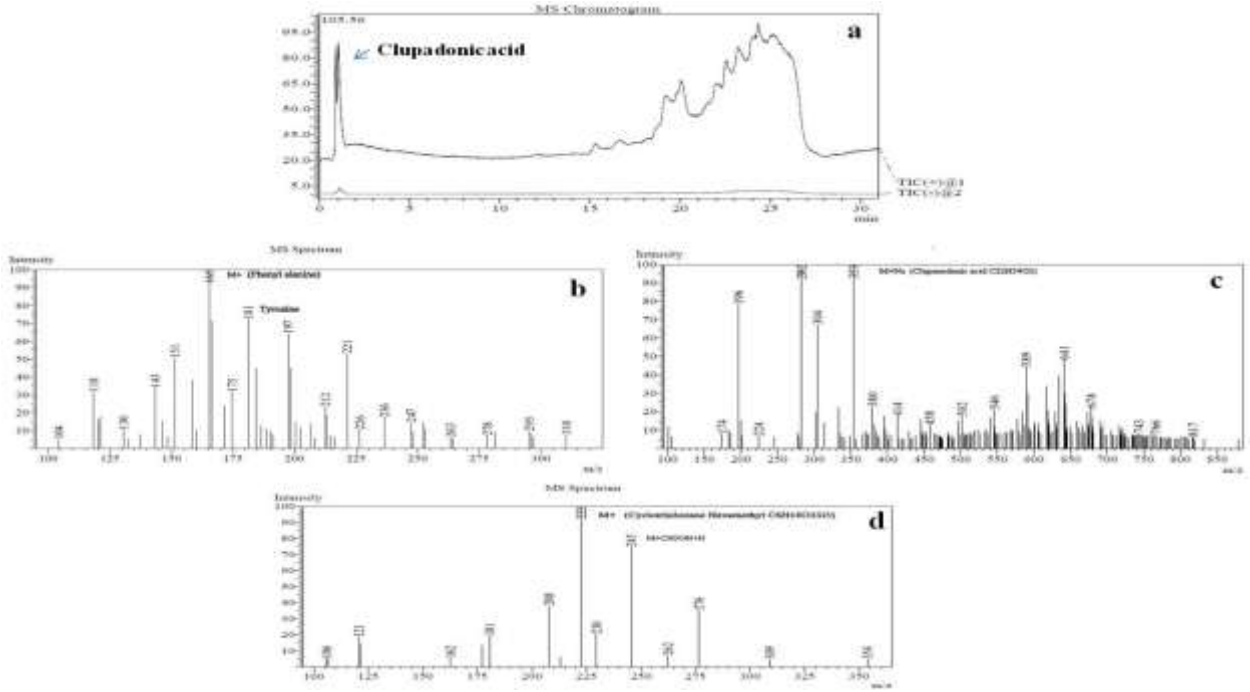

Figure 2: (a) showing the UHPLC-ESI/MS positive and negative TIC for MeOH-DCM Extract; (b) Mass Spectrum (m/z) for Phenyl alanine from MeOH-DCM Extract; (c) Mass Spectrum (m/z) for Cyclotrisilaxane hexamethyl from MeOH-DCM Extract; (d) Mass Spectrum (m/z) for Cyclotrisilaxane hexamethyl from MeOH-DCM Extract

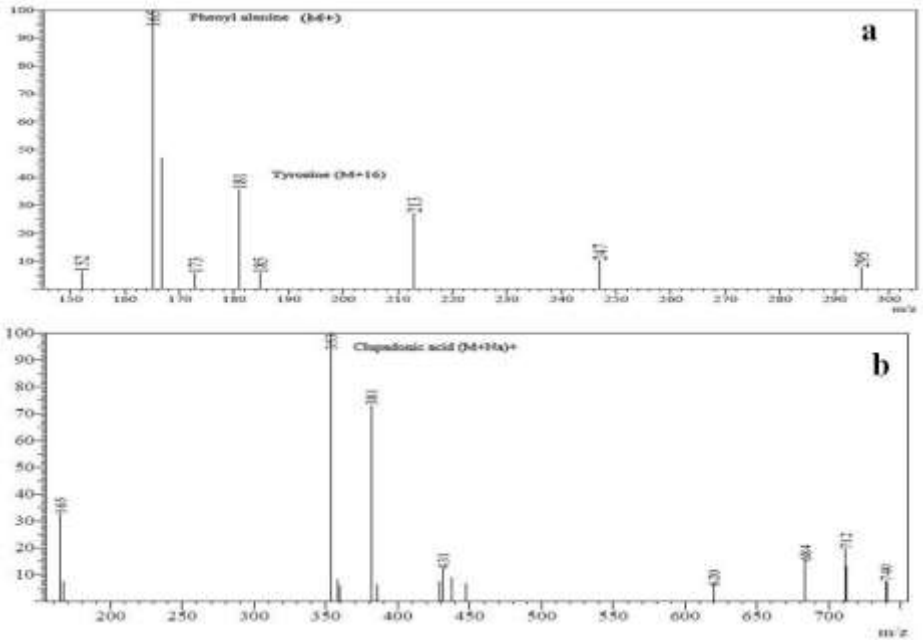

Figure 3: (a) showing the mass spectrum for purified Phenylalanine from MeOH Extract; (b) Mass spectrum for purified clupadonic acid from MeOH-DCM extract

Anti-inflammatory activity or Protein denaturation assay: The results indicated that the phenylalanine from MeOH extract of *H. erecta* possessed anti-inflammatory activity greater than clupadonic acid from MeOH+DCM extract of the same when compared with the standard anti-inflammatory drug, aspirin at a dose of 1mg/ml (Fig. 5a). The inhibition concentration (IC_{50}) of phenyl alanine and clupadonic acids was tabulated in table 2.

α-glucosidase inhibition activity: The α-glucosidase activity was carried for determining the percentage inhibition. The phenylalanine from MeOH and clupadonic acid MeOH-DCM extracts showed positive inhibition against α-glucosidase.

The clupadonic acid showed 78.72% activity for the concentration of 100ug/ml with the $IC_{50\ value}$ of 17µg/ml whereas the phenylalanine has the IC_{50} value of about 25µg/ml compared with standard acarbose (Fig. 5b); also the inhibition concentrations (IC_{50}) of phenyl alanine and clupadonic acids were tabulated in table 2.

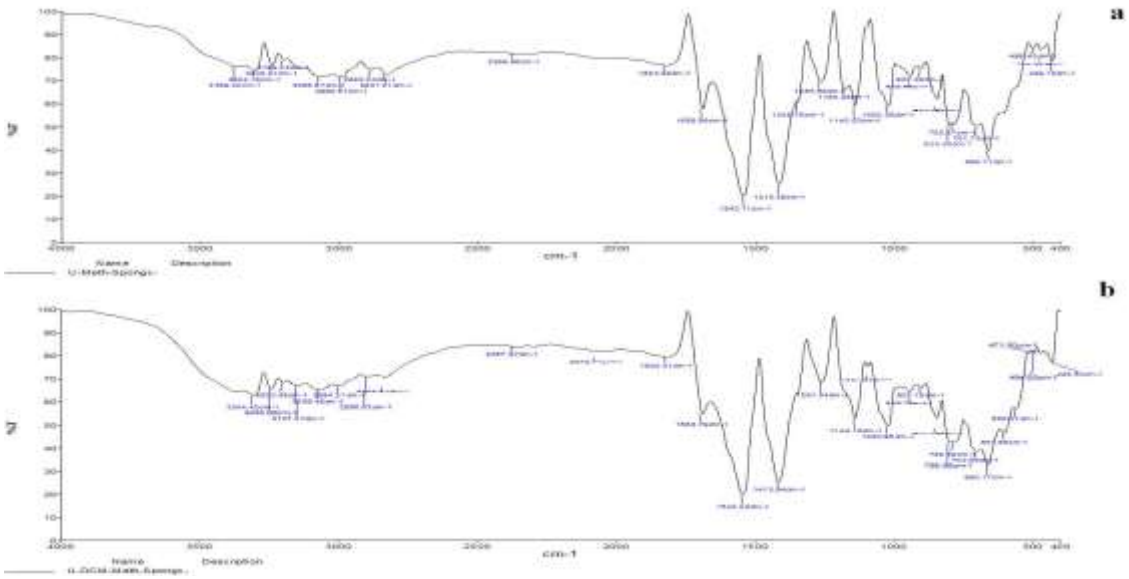

Figure 4: a and b showing the FTIR Spectrum for phenylalanine and clupadonic acid

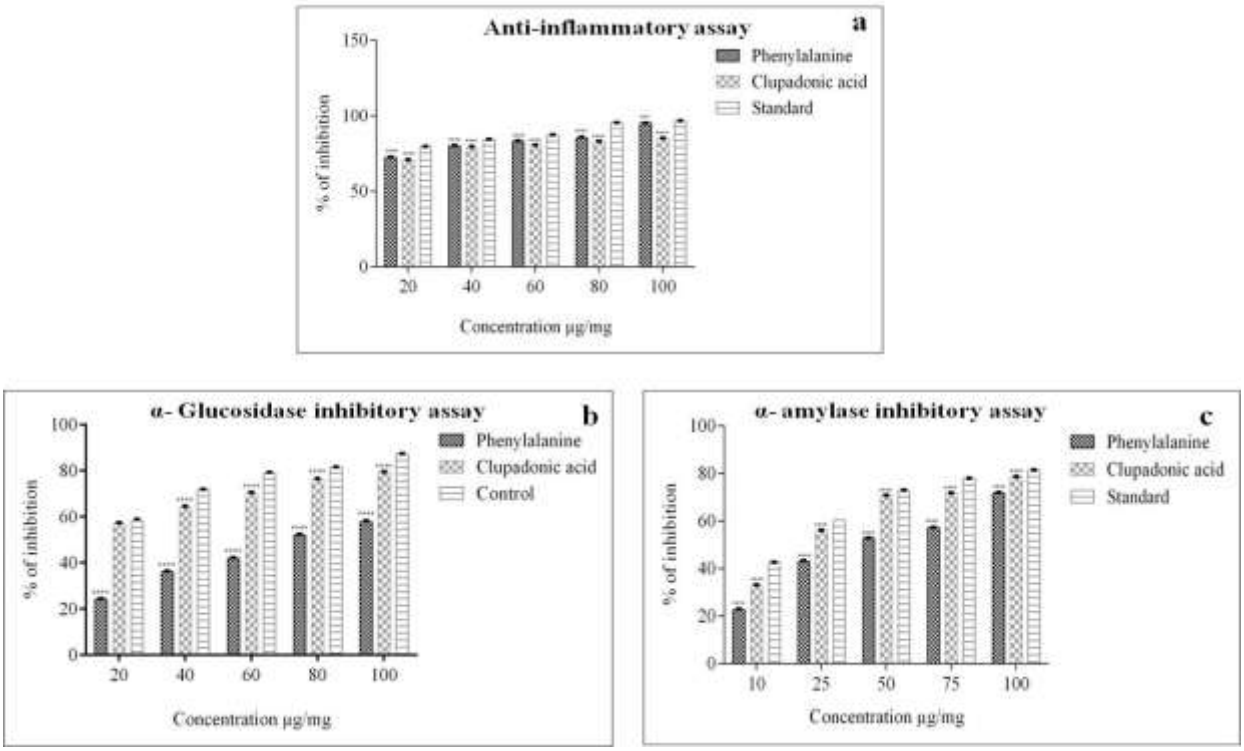

Figure 5: (a) showing the Protein denaturation assay for phenyl alanine and clupadonic acid; (b) α-glucosidase inhibitory activity for phenylalanine and clupadonic acid and (c) α-amylase inhibitory activity phenyl alanine and clupadonic acid

α-amylase activity result: Phenylalanine from MeOH and clupadonic acid from MeOH-DCM extracts of the sponges was tested against α-amylase carbohydrate hydrolyzing enzyme shown in fig. 5c. The clupadonic acid from MeOH-DCM sponge extract showed potent α-amylase inhibitory activity than the methanol extract of species. Inhibition concentration (IC_{50}) of phenyl alanine and clupadonic acids were tabulated in table 2.

Molecular docking results: The interaction between phenylalanine and the three receptors, IXU7, 1SMD and 4Y14 has been illustrated in fig. 6a, b and c respectively. Phenylalanine interacts with the 1XU7 receptor forming a hydrogen bond with arginine (Arg) 66 on the B chain of the receptor. The bond length and the binding energy were found to be 2.389 A° and -7.10 kcal/mol respectively. It formed a similar bond with A chain of 1SMD protein at tyrosine (Tyr) 2 position with a bond length of 2.172 A° and binding energy of -7.78. The 4Y14 receptor did not show any hydrogen bonding with the phenylalanine. Similarly, the clupanodonic acid docked with a bond length of 2.054 A° at the Arg 66 position (Fig. 7a, b and c). Its binding energy was calculated to be -11.59 kcal/mol. However, it showed no interaction with the 1SMD receptor (Fig.7).

Moreover, clupanodonic acid showed four strong interactions with a hydrogen bonding at Glycine (GLY) 220, Isoleucine (ILE) 219, Serine (SER) 216 and Alanine (ALA) 217 and bond lengths 1.769, 2.192, 2.332 and 2.167 A° respectively. The binding energy for this interaction was -10.89 kcal/mol.

Table 1
The yield percentage of extracts

S.N.	Extracts	Dried sample weight (g)	Yield percentage (%)
1	MeOH	20 g	5.45 %
2	MeOH + DCM	20 g	11.55 %

Note: MeOH- Methanol; MeOH-DCM- Methanol and Dichloromethane

Table 2
Shows the Inhibition Concentration (IC_{50}) values of phenyl alanine and clupadonic acid towards anti-diabetic and anti-inflammatory activity

Metabolites	Anti-inflammatory (Protein denaturation assay) IC_{50} – µg/ml	Anti-diabetic IC_{50} - µg/ml	
		α- amylase inhibitory assay	α - glucosidase inhibitory assay
Phenyl alanine	14	72	45
Clupadonic acid	17	18	22

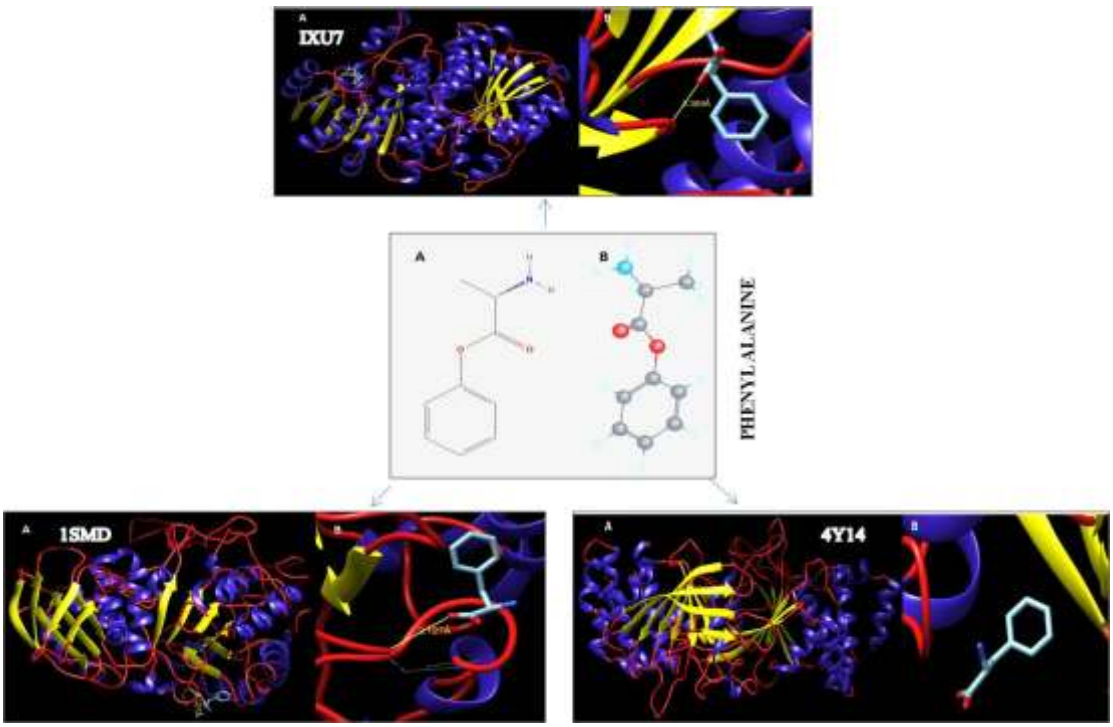

Fig. 6: Showing the 2D and 3D structure of Phenylalanine; (a) Interaction with 1XU7; (b) Interactions with 1SMD and (c) Interactions with 4Y14.

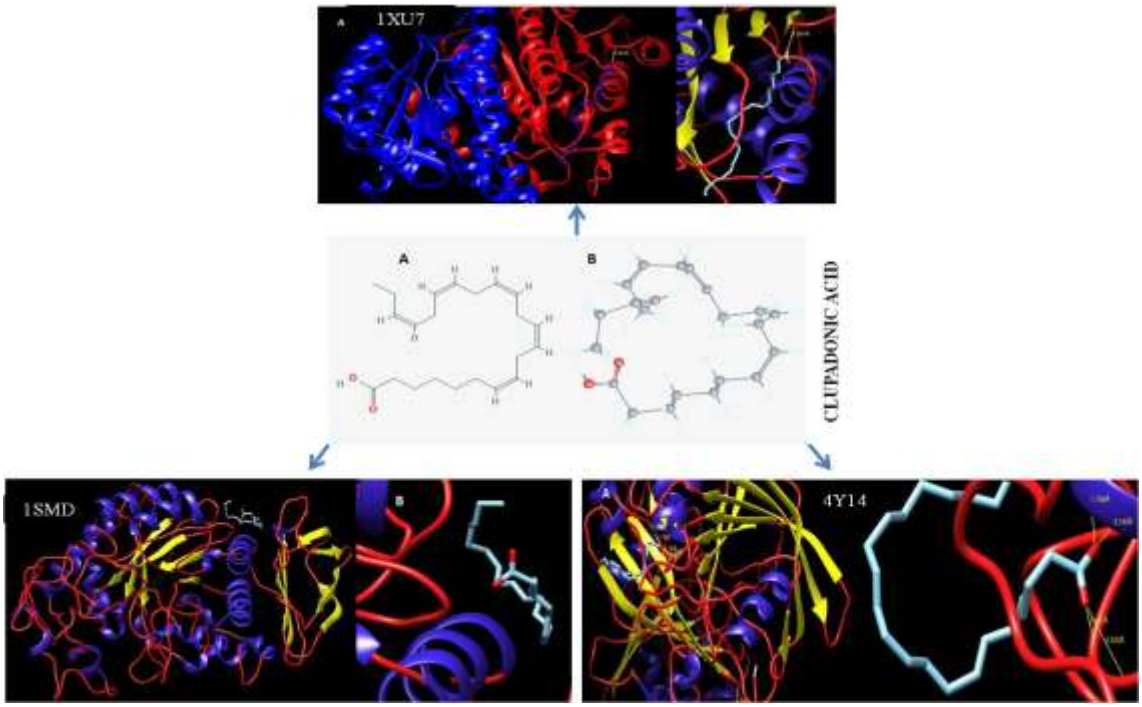

Fig. 7: Showing the 2D and 3D structure of Clupanodonic acid; (a) Interaction with 1XU7; (b) Interaction with 1SMDand (c) Interaction with 4Y14

Discussion

The yield of MeOH/DCM extract was higher than that of MeOH extract. A study reported[1] a yield of 9.44% (85 g of yield from the initial dry weight of 0.9 kg) for *H. erecta* from Sharm el-Sheikh, Red Sea, Egypt. Their result was higher than the MeOH extract but lower than the MeOH/DCM yield obtained in our study. Thus, the influence of solvents and extraction procedures could be key characteristics in molecular isolation and identification. The compound phenylalanine, an aromatic amino acid which was isolated from the MeOH and MeOH/DCM extract of *H. erecta*, had a molecular formula of $C_9H_{11}NO_2$ deduced by ESI-MS at *m/z*165.19 [M^+] with an addition of oxygen molecule it shows the characteristic nature of tyrosine. The same set of mass fragmentation pattern was reported[4].

Docosapentaenoic acid or clupanodonic acid (353Da) of molecular formula ($C_{22}H_{34}O_2$) having the formation of sodium adduct in its molecule as $(M+Na)^+$ designates any straight chain 22:5 fatty acid that is a straight long-chain n-3 polyunsaturated fatty acid that is intermediary between eicosapentaenoic acid and docosahexaenoic acid in the n-3 synthesis pathway. DPA is an element of our traditional diet through fish and lean beef. In recent years, DPA has received growing attention as an essential bioactive fatty acid in light of its potential beneficial health effects which include anti-inflammatory actions and some evidence supporting the role of DPA in reducing inflammation especially in cancer models[16].

Additionally, a recent study looking at the anti-tumorigenic effects of n-3 fatty acids in colorectal cancer found anti-proliferative and pro-apoptotic effects for all 3 fatty acids, with DPA demonstrating the strongest effects in both *in vitro* and *in vivo* models of colorectal cancer[21].

Doxepin a phenolic derivative was obtained from the methanol and methanol-dichloromethane extract of *H. erecta* of mass having 279Da ($C_{19}H_{21}NO$) which was reported earlier in the extracts of *Callyspongia diffusa* [7]. Cyclotrisiloxane, hexamethyl comes under the compound group of alkanes and has been detected from methanol-dichloromethane extracts of *H. erecta* of the mass range having 222Da of molecular formula ($C_6H_{18}O_3Si_3$) exhibiting significant pharmacological effects such as antimicrobial, anti-inflammatory, antioxidant etc[7].

Another research has proved the presence of cyclotrisiloxane through GC-MS from the ethanol extracts of *Salacia oblonga* wall[2]. Denaturation of proteins is a well-documented reason behind inflammation. The anti-inflammatory drugs have shown dose-dependent capability to thermally induced protein denaturation while Bovine albumin is warm, it will get denaturated and specific antigens with too sensitive response known with sicknesses, for instance, bodily fluid infection, nephritis, arthritis, joint inflammation and systemic lupus erythematosus.

Thus, the assay applied for the discovery of those drugs can stabilize the protein from denaturation process[18]. As a part of the investigation, ability of methanol extract of *H. erecta* to inhibit protein denaturation was calculated as 95.57% which is equivalent to the standard drug aspirin (96.67%) at a concentration of 100 µg/ml. The anti-inflammatory activity was greater in methanol extract when compared with another extract. Herein, the ethanolic crude extract of

Callyspongia crassa had anti-inflammatory activity as 61.47% in protein denaturation[15]. Another study[31] found that bioassay-guided fractionation of the anti-inflammation activity of the Red Sea sponges, *Scalarispongia aqabaensis* and *Callyspongia siphonella* of chloroform fraction of methanol extracts.

Hence, the polar solvent extracts possess more potent activity than the non-polar extract. The MeOH-DCM extract showed 78.72% activity with the IC_{50} 17µg/ml in the present study. In this connection, Saludes et al[27] reported that the α-glucosidase inhibitor, 1,4-dideoxy-1,4-imino-D-arabinitol, isolated from two marine sponges collected in Western Australia, was accountable for the α-glucosidase inhibitory activity in different sponge extracts collected over a wide geographic area by LC-MS.

A therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be done by retarding the absorption of glucose through the inhibition of the saccharide hydrolyzing enzymes α- amylase, α-glucosidase and β-galactosidase within the digestive tract. Inhibition of those enzymes delays carbohydrate digestion and extend carbohydrate digestion time, inflicting a reduction in the rate of glucose absorption and therefore blunting the post-prandial plasma glucose rise[25]. Thus, α-Amylase inhibitory activity may be potentially useful in control of obesity and diabetes. The present studies suggested that the inhibition of α-amylase by methanol extract was observed to be as dose-dependent, exhibiting the most significant reducing activity as 58.31% at a concentration (100µg/mL). Furthermore, methanol exhibited the reducing activity of 79.15% at the same concentration.

Another study[28] evaluated the amylase inhibitory effect of different extracts from four marine sponges and only one extract showed highest activity at 1mg/ml concentration. The anti-amylase inhibitory activity may be due to the ability of phenolic compounds to interact with and/or inhibit proteins enzymes. Many natural resources have been investigated with respect to the anti-diabetic and suppression of glucose. Consequently, the inhibition percent was notably allied with the increase in concentration of inhibitors. The actuality that α-glucosidase and α-amylase showed different inhibition kinetics, seemed to be owing to structural differences associated with the origin of the enzymes[17]. A study[19] described the anti-diabetic, anti-inflammatory, anti-tumor and anti-proliferative upshot of many species due to their constituents of mono, sesquiterpenes, phenolic compounds and flavonoids such as cinnamic acid, caffeic acid and rosmarinic acid.

The *insilico* studies for the targeted molecules phenylalanine and clupanodonic acid were performed for the T2DM. The three receptors selectors for this study were 1XU7, 1SMD and 4Y14. The 1XU7 is an NADPH dependent enzyme receptor of 11β-HSD1 *viz*. 11β-hydroxysteroid dehydrogenase type I) or "cortisone reductase". It is highly expressed in metabolic tissues like liver, adipose tissue and the CNS. In these tissues, HSD11B1 lowers cortisone to the active hormone cortisol that activates glucocorticoid receptors. 11βHSD1 inhibition is an important target for the treatment of glucocorticoid-associated diseases, especially of Type 2 DM[3,9]. The 1SMD represents the apo form of the human salivary α-amylase enzyme[23]. The protein-tyrosine phosphatase 1B (PTP1B) [4Y14] is a negative regulator of the insulin signaling pathway and is considered a promising potential therapeutic target, in particular for treatment of Type 2 DM.

Hydrogen bond interaction and the length of the bond play an important role in inhibition. The clupanodonic acid exhibited higher binding energy and shorter wavelength than the phenyalanine. Among the three receptors, the clupanodonic acid showed higher interaction with 4Y14 receptor. Clupanodonic acid or docosapentaenoic acid (DPA) belongs to the class of Free fatty acids (FFA) and is abundantly from meat of marine organisms[14]. The FFA proved to inhibit better the PTP1B activity[29]. This statement is in agreement with the inhibition activity of the clupanodonic acid against the PTP1B 4Y14 receptor.

Conclusion

In the previous literature, marine sponges have provided many novel secondary metabolites that possess varied chemical structures with potent anti-diabetic and anti-inflammatory activities. In the present study, four known compounds were isolated from the sponge *H. erecta* and their mass identification was done using spectroscopic analysis. These metabolites exhibited good anti-diabetic and anti-inflammatory activity. Moreover, the phenylalanine and clupadonic acid were analyzed through *in silico* studies with three types of receptors related to the T2DM for knowing their docking potential and molecular interaction.

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