Original Article

ISOLATION AND CHARACTERIZATION OF 13-CYCLOPENTANE TRIDEANOIC ACID FROM CYANTHILLIUM CINERIUM (L.) H. ROB. AND IT'S IN-SILICO BASED ANTI-DIABETIC EFFECT

Mohamud Maitheen[1]. A, Prabha. S.B[2]
[1,2]Department of Microbiology, School of life sciences, VISTAS, Pallavaram,Chennai, India
Email: maitheen@hotmail.com
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Abstract

Naturally available medicinal plants are found to possess enormous biological properties based on their bioactive constituents present in that plant. Cyanthillium cinereum (L.) H. Rob belongs to Asteraceae family has been reported as anti-microbial, antioxidant, anti- inflammatory properties. This study was aimed to explore the isolation and characterization of bioactive components present in the extracts of Cyanthillium cinereum and its anti-diabetic activities based on in-silico approaches. The methanolic extract of Cyanthillium cinereum was further proceeded to gel chromatography to isolate the active compound and its structure of obtained compound was elucidated by UV-VIS spectrometry, FT-IR spectrum, LC-MS, GC-MS and H1 & C13 NMR studies were carried. Based on these studies, the isolated compound was identified as "13- Cyclopentane Trideanoic acid" for the first time from this plant. The molecular formula for 13- Cyclopentane Trideanoic acid is C18H34O2 and molecular weight is 282.46 g/mol. Further, the ADMET and PASS analysis was carried out for 13-Cyclopentane Trideanoic acid, the results further reveals that this compound showed promising Drug likeliness properties and anti-diabetic activity scores. Thereby, based on these in-silico analysis, we intent to dock this compound with GLUT1, the binding affinity -165.71 showed that 13- Cyclopentane Trideanoic acid was interacting with GLUT1 at their active sites. Taken together, the isolated compound 13-Cyclopentane Trideanoic acid from Cyanthillium cinereum possess antidiabetic effects. In future, the anti-diabetic property of this compound may be explored in other models.

Keywords: Methanol; Chromatography; Spectral analysis; Docking; ADMET; PASS.

INTRODUCTION

Cyanthillium cinereum (L) H. Rob, common cosmopolitan weed spread all over geographical distribution of India. The other synonyms are Vernonia cinereum (Linn.) L, and locally known as little ironweed, belongs to the family Asteraceae (Sulaiman, et al., 2022). Due to its vast traditional practice globally, Cyanthillium cinereum is known as Sahadevi, due its complete curing effects of all kinds of diseases and also listed in the book of Ayurveda (Dharani et al., 2022). This plant exerts a wide array of bioactive principles with potential medicinal properties. Cyanthillium cinereum contains various phytochemicals includes cardiac glycosides, phenols, flavonoids, steroids, tannins, catechins, saponins and phlobatannins. Based on our previously reported work, the whole plant extract of Cyanthillium cinereum showed the presence of alkaloid, carbohydrates, saponin and phenolic compounds, hexane extraction showed the presence of phenolic compounds, whereas aqueous extract revealed the presence of carbohydrates, glycosides, saponins, protein and amino acids (Maitheen et al., 2022). The biological activities of this medicinal plants such anti-pyretic (Iwalewa et al., 2003), anti-bacterial, anti-fungal (Headley et al., 2020), anti-oxidant (Maitheen et al., 2022), anti-inflammatory (Saraphanchotiwitthaya & Sripalakit, 2015; Luetragoon et al., 2021), anticancer (Suja & Varkey, 2019), antispasmodic (Nguyen et al., 2021).

Type 2 Diabetes mellitus and its secondary complications has been recognized globally as a cause of mortality in both developed and developing countries (Khan et al., 2020). Type 2 diabetes mellitus is characterized by the partial or complete resistant towards the function of insulin, thus leads in the progression of hyperglycemia (Halim & Halim, 2019). Hence, this could cause deleterious effects on all the organs, therefore treatment options should be an integrative approach to overcome the complication

of type 2 diabetes mellitus. Current therapeutic scenario reported that anti-diabetic drug should be capable to overcome this complete insulin resistance via regulation of pancreatic β cells in the pancreas and involves the homeostasis of glucose in the body. To date, Ayurveda, is a well-known integrative approach towards the cure of all aliments in human kind. Currently, supplementation of oral anti-hyperglycemic drugs against type II diabetes could cause adverse effects, therefore combination therapy along with phytochemicals would be a better holistic therapeutics options against diabetes mellitus (Beidokhti & Jäger, 2017; Guo et al., 2020).

Recently, the utilization of phytocomponents to cure aliments proved to be safer, cost effective, availability and moreover with less adverse effects as compared to synthetic anti-diabetic drugs. These phytocomponents were rich in antioxidant which could protect the β- cells from reactive oxygen species (ROS) and thereby involved in the inhibition of type II diabetes mediated ROS generation (Matough et al., 2012). Previous reports have suggested that the plants with phenolic may has anti-oxidant effects due to its highly scavenging effects and may prevent Type II diabetes (Pandey & Rizvi, 2009). The objective of this study is to isolate a compound from the extract of Cyanthillium cinereum and its biological activities were analyzed using in-silico tools.

Materials and Methods

Extraction of Cynthallium cinereum:

The extraction of the whole plant extract of Cyanthillium cinereum was carried out (Maitheen et al., 2022). Briefly, 100g of powdered Cyanthillium cinereum, 3L of Methanol was added and it was extracted by using Soxhlet apparatus through continuous extraction method. After this, the plant extract was filtered through whatmann No.1 filter paper and the extract were concentrated using rotary evaporator at 60□C. The concentrated extract was reconstituted in 20ml of methanol for further use.

Isolation of Compound M1 by Column Chromatography and structural elucidation:

The extract was loaded onto the column chromatography and it was fractionated from non-polar to polar gradient solvents to isolate the bioactive compound. Each fraction was analyzed for thin layer Chromatography to confirm the isolated compound with different ratio of ethyl acetate and methanol solvent system and the spots were visualized under UV light. The UV-Vis spectra of the compound were analyzed using UV-3600 Plus shimadzu. The presence of functional group of the isolated compound was identified by FTIR (IR Tracer-shimadzu). The molecular weight of the compound M1 was identified by GC-MS (Agilent 6890) and LC-MS (Agilent 5965) analysis were carried out based on the method described by Keskes et al., 2017. Further, the chemical structure of the compound M1 was further confirmed by H1 and C13 NMR spectral studies.

In-silico analysis of 13-cyclopentane trideanoic acid

Ligand Preparation

The 2D structure and canonical smiles of 13-cyclopentane trideanoic acid has been downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

In silico prediction ADMET properties

ADMET properties of 13-cyclopentane trideanoic acid was predicted using online freeware tool: SwissADME tools (http://www.swissadme.ch/index.php#top).

PASS analysis

Prediction of activity spectra for substances (PASS) tool was used to predict the anti-diabetic properties of 13- Cyclopentane trideanoic acid. The canonical smiles were uploaded and the activity scores were filtered based on pa and pi values for anti-diabetic effects (Mathew et al., 2013).

Molecular docking

Molecular docking was carried out based on the ADME properties and PASS of 13- cyclopentane trideanoic acid, based on the drug likeliness, toxicity score and PASS analysis scores for anti-diabetic activity, the molecular interactions of 13-

Cyclopentane trideanoic acid with GLUT1 protein was carried out.

Preparation of protein

The 3D structure of GLUT1 (PDB id: 4PYP) was downloaded from RSCB Protein Database (https://www.rcsb.org/) as .pdb format. The active site of the protein was predicted using CASTp server (http://sts.bioe.uic.edu/castp/index.html?2pk9).

Preparation of Ligands

The ligand 13- cyclopentane trideanoic acid was prepared by using online Openbabel tool (http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html)and downloaded as .pdb format.

Molecular Docking

The interaction between GLUT1 protein and 13- cyclopentane trideanoic acid was carried out using patch dock online server (https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php). The molecular interaction was visualized using Discovery studio tools (https://discover.3ds.com/discovery-studio-visualizer-download).

Results and Discussion

Extraction and isolation of compound M1 by Column chromatography

Previous studies from our laboratory has shown that ethyl acetate extract of Cyanthillium cinereum showed the presence of terpenoids, tannins, flavonoids and steroids and the structure of the elucidated compound was confirmed by various spectroscopic methods (UV, IR, H1 NMR, C13 NMR, LC-MS and GC-MS). In accordance with these results, previous data also revealed that organic extract of leaves of Cyanthillium cinereum showed the presence of various phytochemical constituents (Varsha et al., 2015). Further, previous studies from our laboratory had showed that methanol and hexane extract have highest potential of free radical scavenging activity (Maitheen et al., 2022). In addition, earlier reports have shown that organic extracts of Cyanthillium cinereum has an anti-fungal activity by inhibiting the growth of Candida strains isolated from urine samples (Fanou et al., 2022). Hemolysis of RBC (Red blood cells) is caused due to the attack of free radical onto the membrane poly unsaturated fatty acids. Ethanolic extract of Cynthallium cinereum decreases the hemolysis by protecting erythrocytes from oxidative damage (Guha et al., 2011). The ethyl acetate extract of Cyanthillium cinereum was loaded onto the column chromatography and the bioactive compounds were fractionated and confirmed by Thin Layer chromatography as represented in Figure (1) and it was showing the Rf value of 0.85 using ethyl acetate and methanol as a mobile phase in the ratio of 8:2.

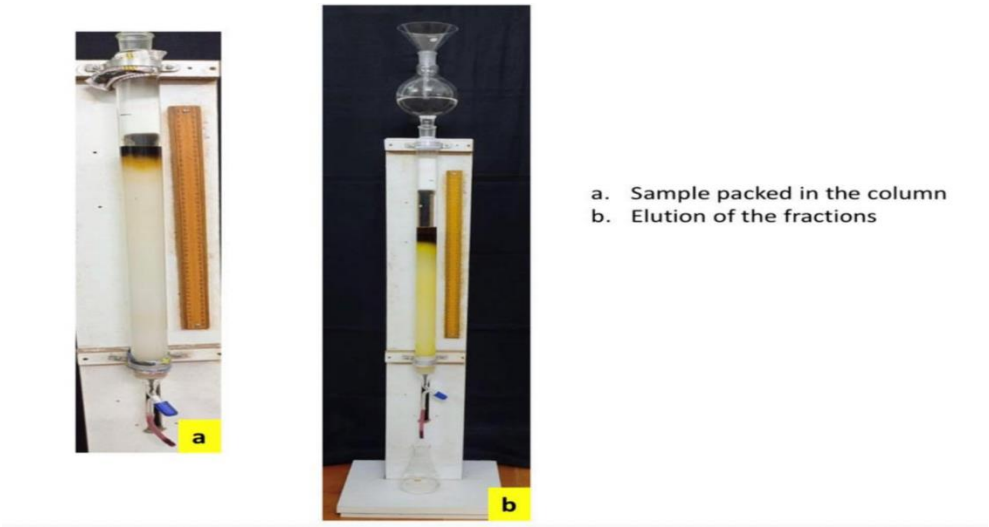


Figure 1 (a) : Compound isolation by Column chromatography

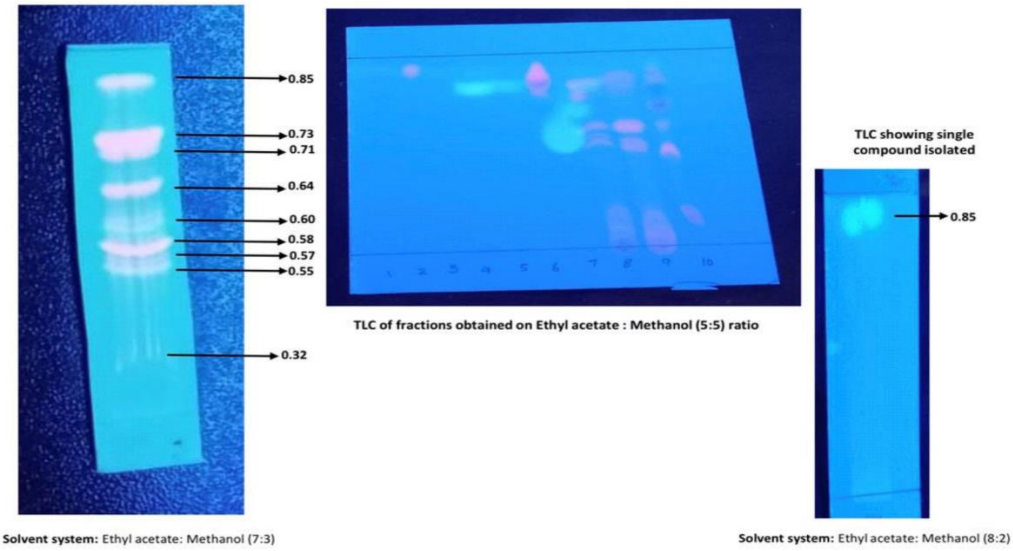


Figure 1 (b) : Separation of compounds by Thin layer chromatography

Structural characterization of compound M1

The purified compound M1 was yellow in color, waxy in nature and readily soluble in methanol. Based on the previous results, the TLC/HPTLC analysis of the aqueous and alcoholic extracts of the Cynthallium cinereum showed the presence of various phytochemicals based on the Rf value (Roy et al., 2019). Figure (2) represents the UV-Vis spectra anlayis of the compound M1. FTIR spectrum showed the peak at the absorption regions for the compound M1 were 1027.31, 1170.52, 1245.45, 1380.08, 1462.75, 1640.77, 1739.24 (ester peak), 2853.99, 2925.75(-CH peak), 3427.14ppm (figure 3). Further, NMR studies revealed that, -CH2 group was found to be lying from 2.09 to 2.39 ppm in 1H NMR. In 13C NMR, the -CH group lies between 18.20-23.69ppm (Figure 4). Molecular weight of the compound M1 was analyzed using GC-MS (Figure 5) and LC-MS (Figure 6), based on these analyses the molecular weight of the compound was found to be 296 m/z which is exhibited by a sharp peak at 296.0 and the molecular formula is C18H34O2. Finally, based on these spectral results and earlier literatures, the isolated compound M1 was identified as 13-cyclopentane trideanoic acid. To support our study, tow novel compounds sesquiterpenes lactones were isolated from the stem extract of Cyanthillium cinereum and its structural identification was carried out (Kuo et al., 2003). Another study showed the essential oil extraction from the roots and flower of Cyanthillium cinereum showed presence novel compound trans-β-bergamotene derivatives based on LC-MS and GC-MS analysis (Boué et al., 2019).

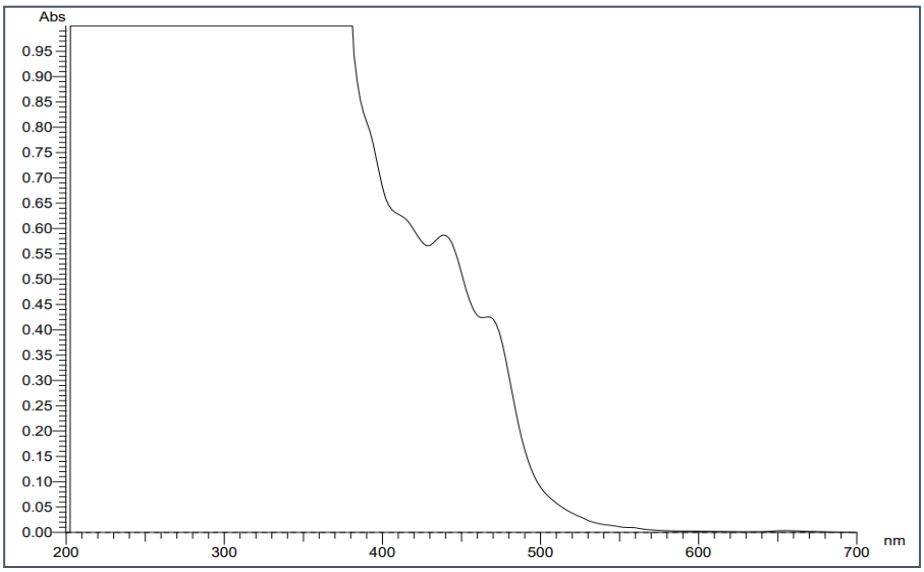


Figure 2: UV- Visible spectra of 13-cyclopentane trideanoic acid

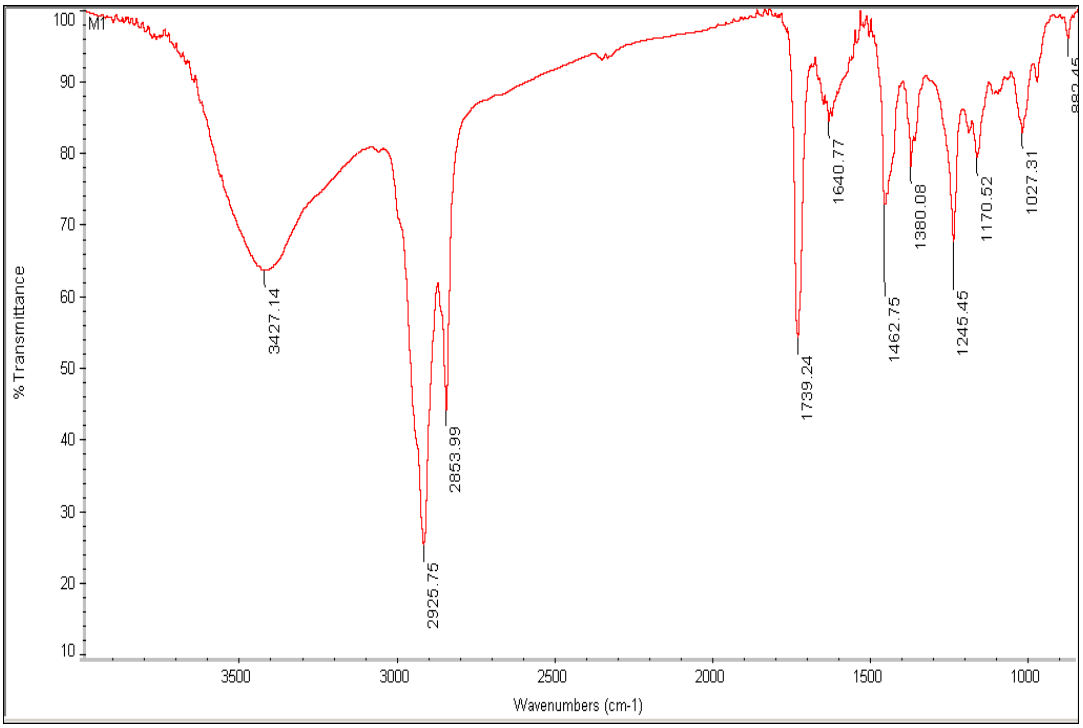


Figure 3: FTIR analysis of 13-cyclopentane trideanoic acid

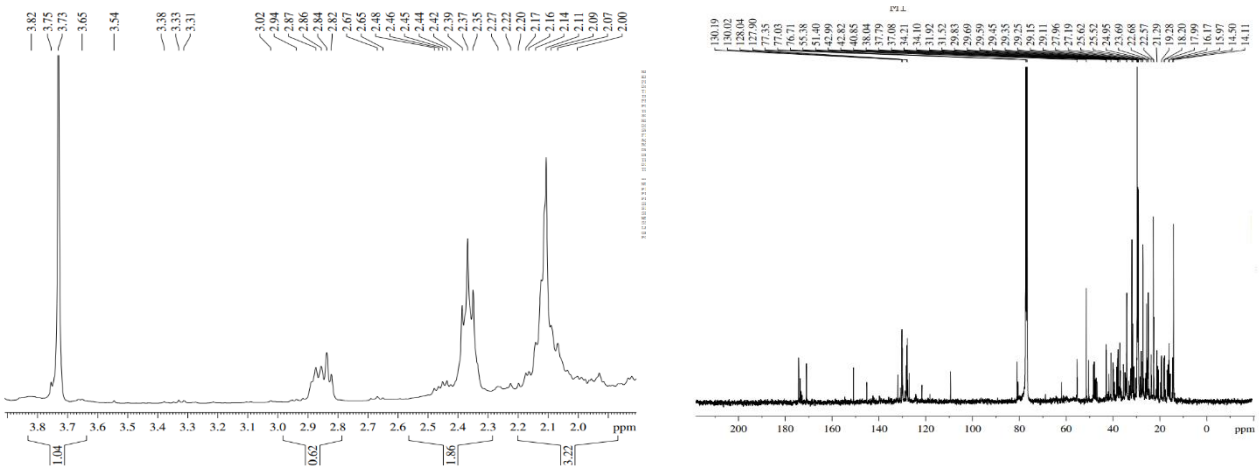


Figure 4 : NMR analysis of 13-cyclopentane trideanoic acid

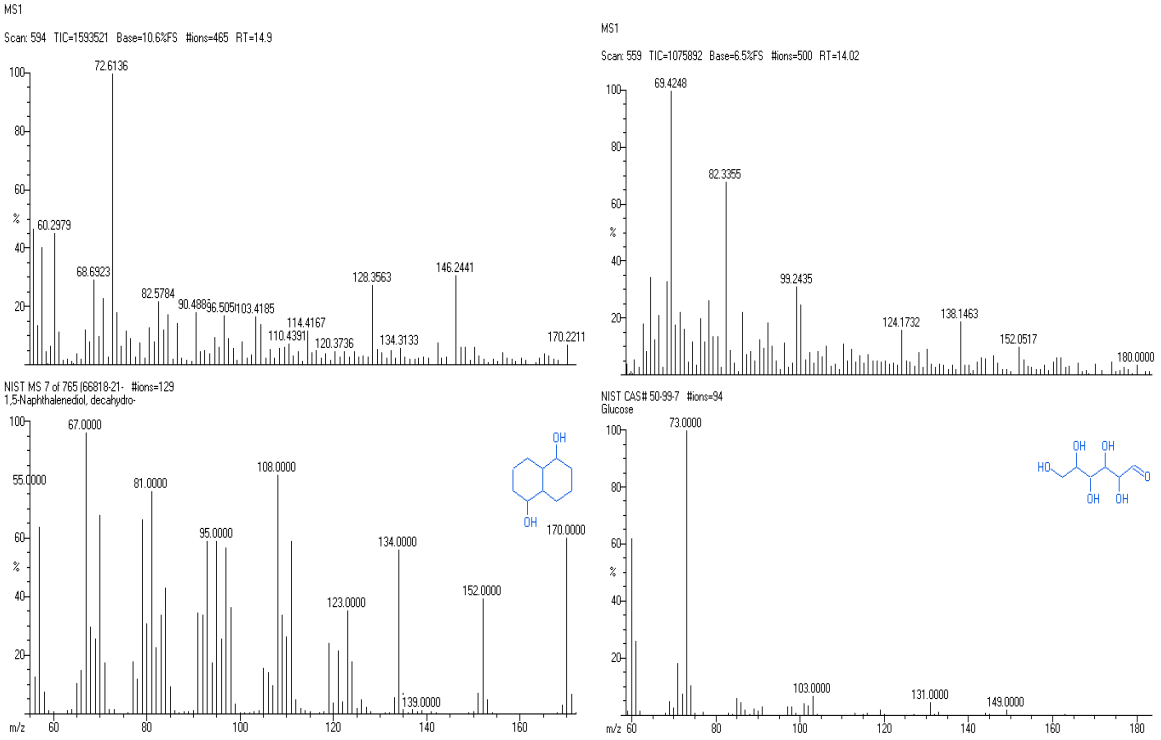


Figure 5 : GC-MS analysis of 13-cyclopentane trideanoic acid

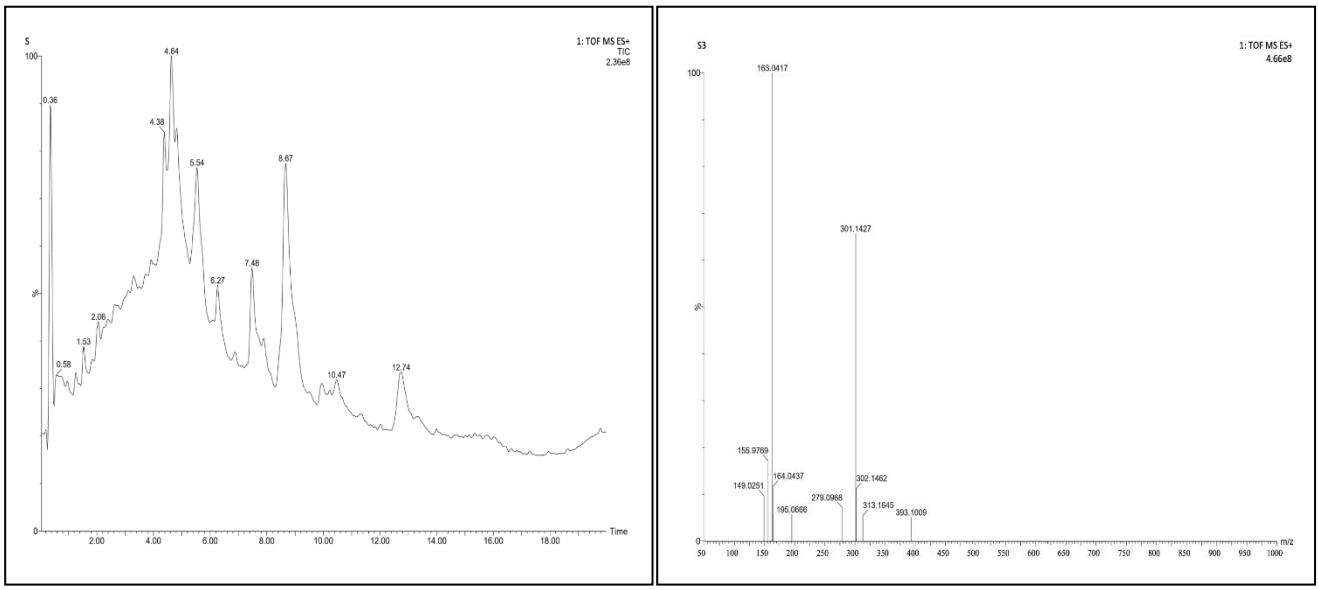


Figure 6 (a) : LC-MS analysis of 13-cyclopentane trideanoic acid

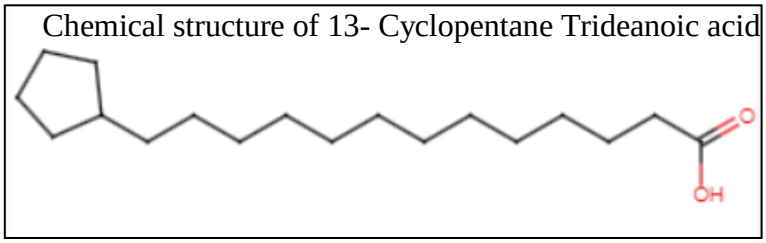
ADMET properties and PASS analysis of 13- cyclopentane trideanoic acid

Figure 7 (a) and Table (1) represents the ADMET Prediction of 13- cyclopentane trideanoic acid. Our results predicted the Drug-like properties of 13- cyclopentane trideanoic acid are less toxic, water solubility, drug like score with no side effects. A study on the anti-cancer effects of phytochemicals present in Cyanthillium cinereum also showed drug like properties based on the Lipinski's rule (Ariya &Joseph, 2020). The PASS analysis of 13- cyclopentane trideanoic acid was carried; Table (2) represents the anti-diabetic activity scores of the compound 13- cyclopentane trideanoic acid. These results revealed that 13-cyclopentane trideanoic acid may be a potential target against diabetes mellitus.

S.No	**Table 1:Physicochemical Properties and ADMET properties of 13-cyclopentane trideanoic acid**	
1.	Formula	$C_{18}H_{34}O_2$
2.	Molecular weight	282.46 g/mol
3.	Num. heavy atoms	20
4.	Num. atom. heavy atoms	0
5.	Fraction Csp3	0.94
6.	Num. rotatable bonds	13
7.	Num. H-bond acceptors	2
8.	Num. H-bond donors	1
9.	Molar Refractivity	88.30
10.	TPSA	37.30 Å²
11.	Lipophilicity (Log $P_{o/w}$ (iLOGP))	4.04
12.	Water solubility (Log *S* (ESOL))	-5.58
13.	Druglikeliness (Lipinski)	Yes; 1 violation: MLOGP>4.15

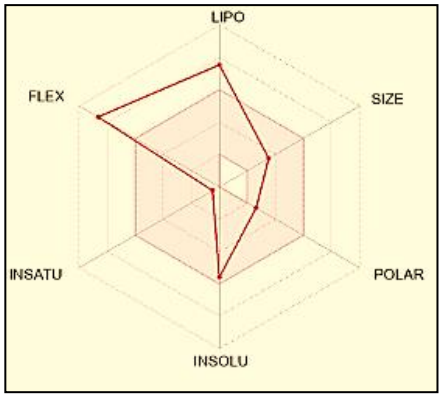
14.	**Pharmacokinetics**	
15.	GI absorption	High
16.	BBB permeant	Yes
17.	P-gp substrate	No
18.	CYP1A2 inhibitor	Yes
19.	CYP2C19 inhibitor	No
20.	CYP2C9 inhibitor	No
21.	CYP2D6 inhibitor	No
22.	CYP3A4 inhibitor	No
23.	Log K_p (skin permeation)	-2.56 cm/s

Chemical structure of 13- Cyclopentane Trideanoic acid



Canonical smiles : C1CCC(C1)CCCCCCCCCCCCC(=O)O

RADAR representation of 13-Cyclopentane Trideanoic acid



Boiled egg representation of 13-Cyclopentane Trideanoic acid

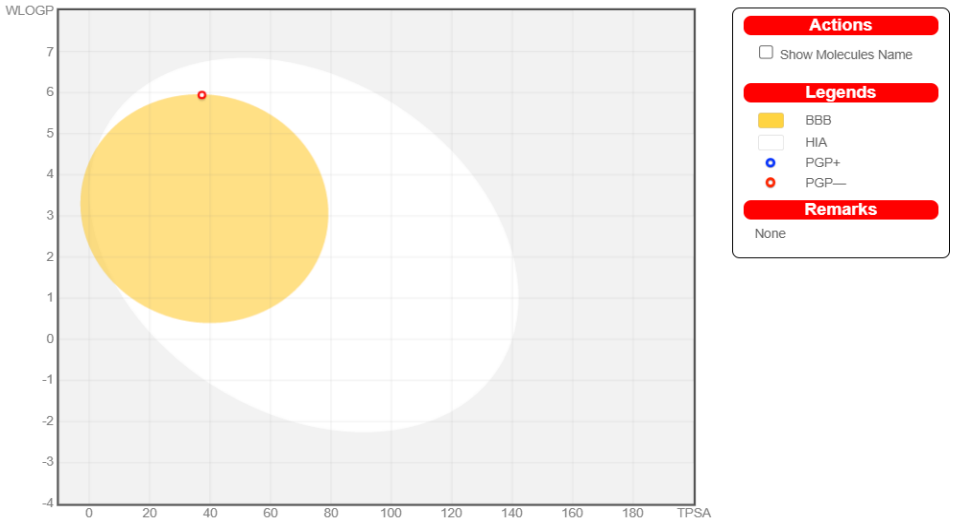


Figure 7 (a): Structure of 13-cyclopentane trideanoic acid

Table 2 : PASS analysis of 13-cyclopentane trideanoic acid

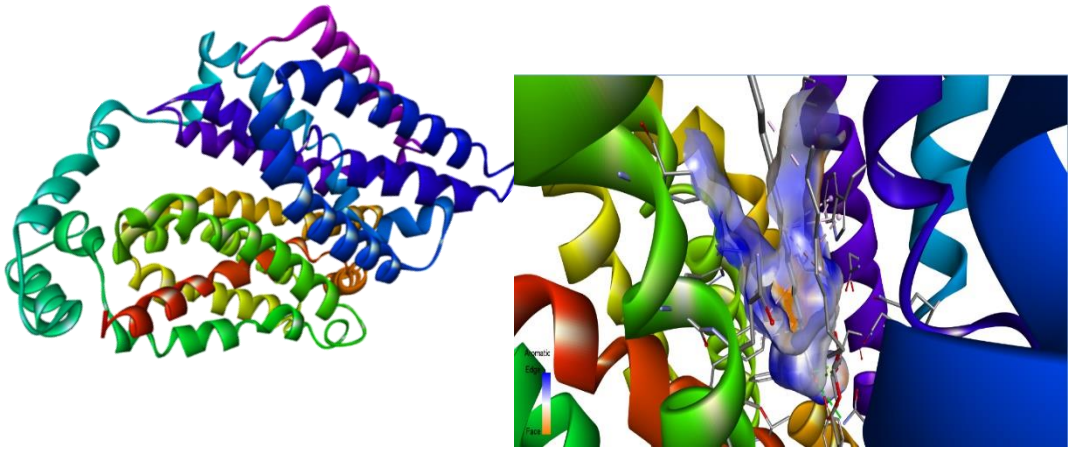
S.No	Biological activity	PASS SCORES	
		Pa	Pi
1.	Antidiabetic	0.293	0.086
2.	Antidiabetic (type 1)	0.190	0.021
3.	Antidiabetic (type 2)	0.252	0.041
4.	Antidiabetic symptomatic	0.411	0.012

Interaction of compound M1 with GLUT1 – in silico analysis

Figure 7 represents the molecular docking of 13- cyclopentane trideanoic acid with the target protein – GLUT1. Ligands could be more potent and likely to interact more significantly to receptors: GLUT1 based on the predicted docking score as depicted in Figure 7 (c). Based on the results, the 13- cyclopentane trideanoic acid interact at the active site of GLUT1 protein ASN 288, THR 30, ASN 415, TYR 292, SER 73, PHE 72, PHE26, TRP 412, HIS 160, TRP 388, BNG 601 and THR 137 have shown promising binding affinity which is expressed in terms of molecular interactions of which 13- cyclopentane trideanoic acid with GLUT1 -165.71 kcal/mol. GLUT1 belongs to the family of membrane transporters family, and this protein plays a vital role in the uptake of glucose and also in the other mono/ disaccharides

(Mueckler & Thorens, 2013). A study reports that caffeine binds at the endofacial site of GLUT1 by competing with ATP, which showed the anti-diabetic activity (Sage et al., 2015). Another study showed the anti-cancer potential of the phytochemicals of Cyanthillium cinereum against matric metallo proteins (MMPs) which was upregulated during cancer progression. The bioactive compounds of Cyanthillium cinereum are apigenin, piperine and acetyl eugenol showed strong binding affinity at the active sites of MMPs (Ariya & Joseph, 2019). Cunningham and his group researchers showed that D-Glucose and GLUT1 binidng interaction was inhibited by quercetin via hydrogen bonding at GLU 254 and LYS256 sites of GLUT1 protein (Cunningham et al., 2006).

Taken together, based on the isolation and structural studies conducted, the compound “13-cyclopentane trideanoic acid” isolated from the whole plant extract of Cyanthillium cinereum would be potent anti diabeteic compound. Therefore, further in-vitro and in-vivo studies is need to show the anti-diabetic effects of “13-cyclopentane trideanoic acid”.



3D Structure of GLUT1 Protein Molecular Docked Structure of GLUT1 with 13-cyclopentane trideanoic acid

Figure 7 (c) : Molecular Docking of 13-cyclopentane trideanoic acid with GLUT1 Protein

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