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PHYTOCHEMICAL SCREENING, GC-MS CHARACTERIZATION AND MOLECULAR DOCKING STUDIES OF ROOT EXTRACTS OF *Datura metel*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Datura metel (Solanaceae) which is commonly known as thorn's apple, Indian apple or devil's trumpet Isan annual herb of temperate zones which is distributed all over the world. *D. metel* belongs to the family Solanaceae. Different researchers conducted researches and studies on *D. metel* because of its various traditional and pharmacological uses, including hepatoprotective, antiviral, antibacterial, anti-asthmatic, analgesic, antipyretic, nephroprotective, anticancer, and antifungal effects, *Datura metel* has been identified as a species of pharmaceutical importance.

The objective of this study is to identify the Bioactive compounds by Phytochemical screening, identification of chemical composition of compounds through GC-MS and Molecular Docking Studies of hexane and ethanol Root Extracts of *Datura metel* against Inflammation.

In this study the docking results reveals that the ligands are well able to bind with the protein Mitogen Activated Kinase 2 (MK2) complexed with compound. The compounds discovered in the *Datura metel* root that were discovered using GC-MS data have been described as anti-inflammatory medicines with a range of targets, including Mitogen Activated Kinase 2. With the help of *In silico* docking studies, it was able to get a molecular understanding of the inhibitory mechanisms of newly identified drugs against an anti-inflammatory therapeutic target.

Keywords: *Datura metel*; mitogen activated kinase; GC-MS; molecular docking; anti-inflammatory target.

1. INTRODUCTION

"Nature has been showed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Medicinal plants are employed for the ailment of several microbial and non-microbial diseases due to their valuable effects in health care. The affordability, reliability, availability and low toxicity of medicinal plants in therapeutics made them popular and acceptable by all religions and implementation in health care all over the world" (Akharaiyi, 2011).

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"Plants have been producing a diverse range of bioactive molecules, making them rich sources of different types of medicines. Higher plants, sources of medicinal compounds have continued to play a dominant role in maintenance of human health since ancient times" [1]. "Over 50% of all modern clinical drugs are of natural origin. Natural products play an important role in drug development programs in the pharmaceutical industry" (Baker et al.,1995).

Datura, often touted as "Thorn Apple", though considered as one of the deadliest plant species, for its super toxic components, when consumed raw is also surprisingly a powerhouse of medical components if purified properly [2-6]. Be it the leaves, fruits, flowers, stem or roots, *Datura* has been traditionally used in both folklore medications and alternative therapies [7,8]. However, due to the strong hallucinogenic properties of the plant, *Datura* is often used to relieve asthmatic symptoms and reduce the pain during the surgery and bone setting procedures. Though a strong narcotic plant, *Datura* offers umpteen health benefits and is extensively used for alleviating pain, enhancing heart functions, improving fertility, inducing sleep, easing childbirth and promoting hair and skin health [9-12].

Datura metel with local tamil name "Oomatham", is an erect shrub with spreading branches. It is also known as Devil's Trumphet. "A perennial herbaceous plant, belonging to the Solanaceae family can reach a height of 1.5m. Leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous [13-16]. Flowers are large, solitary, and trumpet-shaped with a sweet fragrance usually appreciated in the mornings and evenings, with a wide range of colors, ranging from white to yellow and light to dark purple. The flowers are hermaphrodite and are pollinated by insects. The fruit is in the form of a capsule covered with short spines. *Datura* can tolerate average soil but prefers soil which is rich and moist or even very alkaline soil but hardly survives under shade [17-19]. It prefers a warm temperature and is distributed in warmer regions of the world" (Drake et al., 1996). "Datura probably is of American origin and widely cultivated in all tropical and subtropical regions for its beautiful flowers" [20]. "*D. metel* can also be found in East Asia or India, and is used in traditional herbal medicine. In Traditional Chinese Medicine, the flowers of *D. metel* are known as baimantuoluo and used for skin inflammation and Psoriasis" [21]. "In Ayurvedic medicine, seeds of *D. metel* are used to treat Skin rashes, Ulcers, Bronchitis, Jaundice and Diabetes" [22]. "In Brazil, seeds are used for tea making which would serve as a sedative and flowers are dried and smoked as cigarettes" [23]. The dried leaves, flowers and roots were used as narcotic, antispasmodic, antitussive, bronchodilator, anti-asthmatic and as hallucinogenic. The plant was also used in diarrhea, skin diseases, epilepsy, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin ulcers, wounds and burns [24-26]. In ayurveda, the plant was considered bitter, acrid, astringent, germicide, anodyne, antiseptic, antiphlogistic, narcotic and sedative.

1.1 Docking

When two molecules are bonded together to form a stable complex, a computer method called molecular docking attempts to predict the preferential orientation of a ligand to its macromolecular target (receptor). Even while each docking method works a little bit differently, they all have the same ligand and receptor, sampling, and scoring features. Sampling entails positioning the ligand in terms of conformation and orientation within the boundaries of the receptor-site interaction [27-31]. A scoring function ranks the ligands according to their conformation, orientation, and translation choices. A successful docking experiment requires precise prediction of the ligand structure (pose prediction) or its propensity for binding (affinity prediction). The main areas where existing techniques vary are ligand placement in the "combining" site, conformational space exploration, and score or binding estimation. Both the orientation of the side chains in the binding site and the fold of the protein backbone in that area are necessary for the interaction with the ligand. The fact that docking is frequently carried out while maintaining the protein surface stiff precludes evaluation of the impacts of induced-fit within the binding site, which is one of the most important constraints of docking [32-35].

2. MATERIALS AND METHODS

2.1 Collection of Root Sample

Fresh roots of *Datura metel* were detached from the whole plant by hand plucking during February 2021 from Arakkonam, Ranipet district, Tamil nadu, India. The plant identification was confirmed with the botanical descriptions given in the literatures (Ali Esmail, 2015, Kawo. A. H et al., 2009). The collected roots were washed using tap water to remove the dust and other adhering materials. Then the cleaned roots were placed in clean and dust free place under shades for air drying at normal room temperature and pressure about two to three weeks.

2.2 Preparation of Root Extract

The air-dried root samples of *Datura metel* were cut into small pieces. This sample was used for extraction process by cold percolation method and sequential extraction method using solvents with increasing polarity (hexane and ethanol). 30 g of root sample

were immersed in 300 mL of hexane solvent in a clean conical flask and the mouth of flask was covered with an aluminum foil. This set up was kept undisturbed for 72 hours. After 72 hours, the leave residues were filtered from the solvent using whatman No1 filter paper and then air dried for further processing. The same root sample were transferred to 300 mL of ethanol solvent and kept for 72 hours. The filtered extracts were concentrated under high pressure and temperature around 50 -60 ˚ C and 60-70˚ C for hexane and ethanol by using rotary evaporator.

Fig. 1. Whole plant of *Datura metel*

Fig. 2. Dried root sample

2.3 Analysis for Phytochemicals

Phytochemical analysis of various root extracts of *Datura metel* were tested at Entomological Research Institute, Loyola College, Chennai. Phytochemical screening of plant extracts was carried out by employing standard procedures (Sahira banu et al., *2015*, Brindha. P et al., 1981).

Fig. 3. Hexane extract of *Datura metel*

Fig. 4. Ethanol extract of *Datura metel*

2.3.1 Test for alkaloids

The substance or extract is treated with 5% acetic acid. This solution is stirred well and filtered. The aqueous solution is divided into two parts. To one part of solution, add 2 drops of Drandoff's reagent and the appearance of reddish orange precipitate indicates the presence of alkaloids. To another part of solution, add 2 drops of Mayer's reagent, Buffy white colour precipitate indicates the presence of alkaloids.

2.3.2 Test for flavonoids

The substance or extract is dissolved in ethanol or methanol or water. Add magnesium turnings and 2 drops of concentrated Hydrochloric acid. Gently warm if necessary. Appearance of reddish pink or orange red color indicates the presence of flavonoids.

2.3.3 Test for steroid

The substance or extract is treated with 2 mL of chloroform solution and add few drops of concentrated Sulphuric acid. Appearance of layered red column indicates the presence of steroids.

2.3.4 Test for triterpenoids

A pinch of substance or extract is taken in a dry test tube. To that add a little bit of tin foil and 1 mL of thionyl chloride. Gently warm test tube if necessary. Appearance of reddish pink, violet or purple color indicates the presence of triterpenoids.

2.3.5 Test for glycoside- sugar

A pinch of substance or extract is taken in a watch glass. Add equal quantity of Anthrone. This mixture is dissolved in alcohol and then dried on a water bath. Add 1 drop of concentrated Sulphuric acid and rub with a glass rod. Gently heat if necessary. Appearance of dark green to purple colour indicates the presence of glycoside- sugar.

2.3.6 Test for phenol

A small quantity of substance or extract is dissolved in 1 mL of methanol or ethanol or water. Add few drops of neutral ferric chloride. Appearance of colors like green, blue green, brown or red indicates the presence of phenol.

2.3.7 Test for tannins

The substance or extract dissolved in water or alcohol is treated with 1 mL of basic lead acetate in water. Buffy white precipitate indicates the presence of tannins.

2.3.8 Test for saponins

The substance or extract is dissolved in water or aqueous alcohol is shaken vigorously for few seconds and allowed to stand permanent lather indicated the presence of saponin.

2.3.9 Test for coumarin

The substance or extract is dissolved in alcohol and then treated with few drops of alcoholic sodium hydroxide or potassium hydroxide. Appearance of dark green colour indicates the presence of coumarin.

2.3.10 Test for anthraquinones

The substance or extract is treated with few drops of magnesium acetate solution. Appearance of pink color indicates the presence of anthraquinones.

2.4 GC-MS (Gas Chromatography-Mass Spectrometry) Analysis of the Root Extract of *datura metel*

"The sequential extract of Hexane and Ethanol were subjected to Gas chromatography-mass spectrometry analysis. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The injector temperature was set at 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min−1; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230°C; ion source temperature 230°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library" [36].

2.5 Docking Analysis

2.5.1 Protein preparation

The 2D structure of proteins was obtained from Protein Data Bank (PDB) (*www.rcsb.org*). The base structure of the protein was prepared by removing all the heteroatoms attached to the actual structure. The binding pocket proteins (MK2) was optimized by excluding the proteins coordinates of the heteroatom molecule. Swiss-pdb viewer (spdbv) was used to visualize the 2D structure of the protein and its energy was minimized for further docking studies.

2.5.2 *In-silico* generation of ligands

The available ligand structures were downloaded from PubChem. The openbabel server was used to convert the sdf files of the ligands to pdb format providing details on the coordinates of the ligand.

2.5.3 Molecular docking studies

AutoDock 4.2 was used to predict how substrates or drug candidates, bind to a receptor of a known 2D structure. It was used to create gpf-grid parameter file and dpf-dock parameter file, for adding polar hydrogen, Kollman charges and converting to ad4 type atoms. These were added to the receptor for the preparation of protein in docking simulation. AutoDock requires precalculated grid maps. This grid must surround the region of interest (active site) in the macromolecule. The grid center was changed, which

covered all the amino acid residues in the considered active pocket. Docking software autoDock 4.2 program supplied with auto grid 4.2 and autoDock 4.2 was used to produce grid maps. The grid logarithmic files and docking locking logarithmic files were prepared by the software Cygwin. The Lamarckian genetic algorithm (lga) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for each compound. The dlg file showed docked structure at different runs with different binding energy (b.e). The poses of the docked structure at the run with the least binding energy was finally selected. The Lamarckian genetic algorithm (lga) was chosen for the search for the best conformers. The highest-ranked ligands were compared with the known experimental structure using the standard cartesian root-mean-square deviation (rmsd) measure (between similar atoms in the pose and experimental structure). The pose for the ligand-receptor complex was analyzed three-dimensionally for hydrogen-bond based interactions at the active site of Dihydroorotate dehydrogenase protein using the software UCSF chimera. The selected ligand was highlighted. The drug-likeness, ADME properties and toxicity of the selected ligands were further analyzed. In the case of ADME toxic structures, a computer-assisted search for alternate drug candidates was also evaluated.

2.5.4 AutoDock

AutoDock is a suite of c programs used to simulate interactions between small flexible ligands and macromolecules of known structure (Morris et al, 1998). Docking is achieved through a search of conformational space using a Lamarckian genetic algorithm coupled with energy assessments using a method based on the amber force field. The combination of these two functions produces a family of molecular coordinates detailing possible docked ligand conformations which can then be used as a starting point for theoretical ligand design and study. Confidence in the docked conformation is represented by an energy value based on both quantum and molecular mechanical modelling of atomic forces. The genetic algorithm used in autoDock defines a ligand's "chromosome" as having seven standard genes accounting for the ligand's cartesian coordinates, and four variables specifying its orientation [37-39]. Once the genes have been defined the genetic algorithm starts by creating a population of random individuals confined within a user-specified box also containing the protein. For each individual the three translation genes (x,y,z) are given a random value between the minimum and maximum of the search area, the four genes describing the orientation given a random quaternion consisting of a unit vector and rotation angle, whilst the torsion angle genes (if any) are given random values between -180º and 180º. These gene values are then converted into a corresponding phenotype that enables the assessment of each individual's "fitness" measured by interactions both within the ligand and between the ligand and the protein. This assessment is followed by a selection procedure that decides which individuals will be allowed to progress into the next refinement round. AutoDock also performs a limited local search based on the energy phenotype of each resulting chromosome, followed by reverse-transcription of the optimized phenotype back into the genome in much the same way as hypothesized by the discredited Lamarckian evolutionary theory.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The phytochemical screening of revealed the presence of important pharmacological bioactive substances as well as medicinal and nutritional potentials in the roots. The results showed the presence of Alkanoids, Flavonoids, steroids, Triterpenoids, Sugars, Phenolic compounds and Saponins in various extracts.

Table 1. Phytochemical analysis of root extracts of *Datura metel*

S. No	Name of the compound	Hexane extract	Ethanol extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Steroids	+	+
4	Triterpenoids	+	+
5	Sugars	+	+
6	Phenolic compounds	+	+
7	Tannins	–	–
8	Saponins	+	+
9	Coumarin	–	–
10	Anthraquinones	–	–

3.2 GCMS

The peaks are marked with retention time in the GC-MS chromatogram of the ethanol and hexane extract of the roots of *Datura metel*. Their retention time

(RT) and amount of their presence as indicated by listed in Tables 2 and 3. The qualitative analysis of the extract showed the presence of eleven compounds in ethanolic extract and nine compounds in hexane extract.

3.2.1 *Datura metel* ethanol extract

Qualitative Report

File: C:\TurboMass\2021.PRO\Data\DME-(21ES-0290).raw
Acquired: 23-Mar-21 04:38:29 AM
Printed: 26-Mar-21 12:24 PM
Description:
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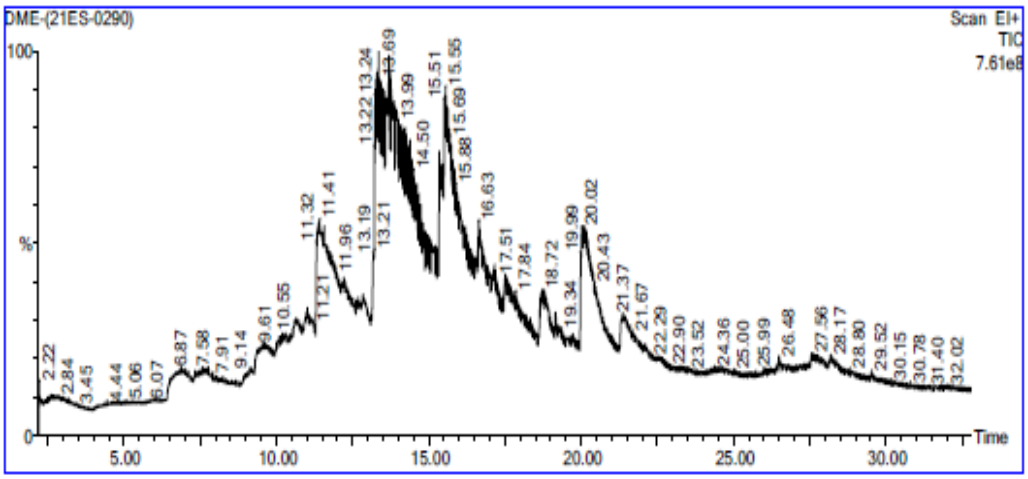
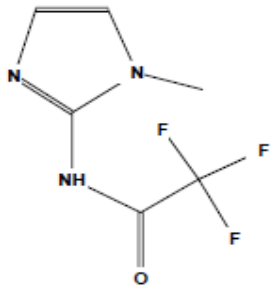
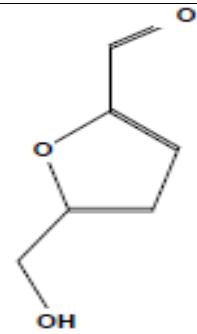
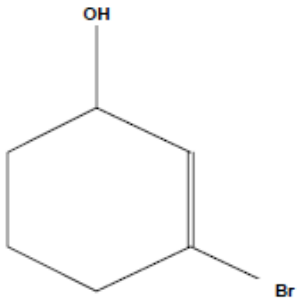
Page 1 of 1
Sample ID: DME-(21ES-0290)
Vial Number: 55

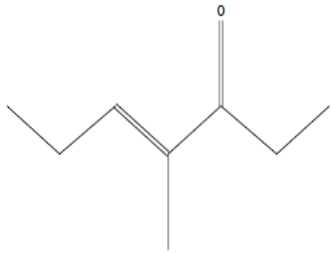
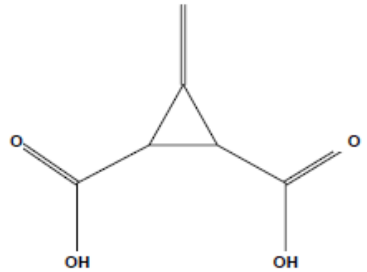
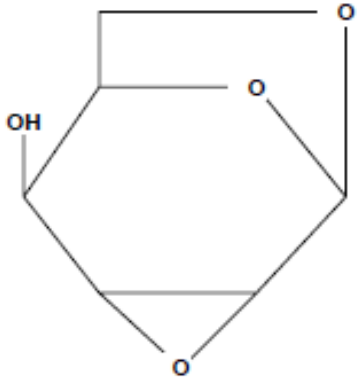


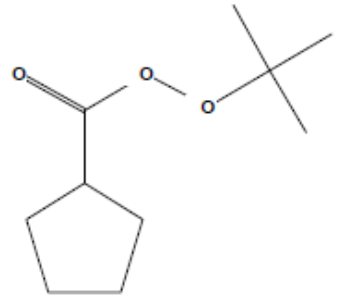
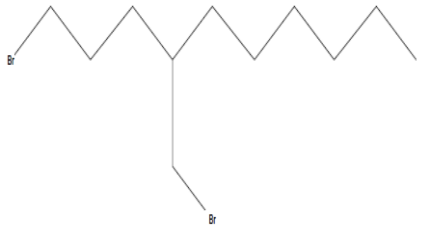
#	RT	Scan	Height	Area	Area %	Norm %
1	9.611	1482	43,198,456	17,854,012.0	4.747	17.62
2	13.353	2230	181,174,288	7,415,427.5	1.972	7.32
3	13.503	2260	140,848,176	6,926,263.0	1.841	6.84
4	13.693	2298	225,406,704	19,094,888.0	5.077	18.84
5	13.728	2305	193,493,680	22,585,864.0	6.005	22.29
6	13.893	2338	138,824,272	14,577,246.0	3.876	14.39
7	14.088	2377	116,731,208	9,104,769.0	2.421	8.99
8	14.523	2464	84,964,560	7,195,916.0	1.913	7.10
9	15.354	2630	246,097,840	33,376,356.0	8.874	32.94
10	15.549	2669	358,335,872	87,136,392.0	23.167	85.99
11	15.869	2733	155,871,664	13,763,141.0	3.659	13.58
12	15.989	2757	100,335,312	9,232,977.0	2.455	9.11
13	16.649	2889	102,604,016	11,037,844.0	2.935	10.89
14	20.096	3578	239,816,432	101,328,416.0	26.940	100.00
15	21.366	3832	69,962,696	15,498,395.0	4.121	15.30

Graph 1. Qualitative report for ethanol extract

Table 2. The chemical compostion of ethanol extract of roots of *Datura metel*

NO.	RT	Name of the compound	Molecular weight	Molecular formula	Structure
1	9.611	IMIDAZOLE, 2-TRIFLUOROACETAMINO-1-METHYL	193	C6H6ON3F3	
2	13.353	2-FURANCARBOXALDEHYE, 5-(HYDROXYMETHYL)	126	C6H6O3	
3	13.728	2-CYCLOHEXEN-1-OL, 3-BROMO	176	C6H9OBr	

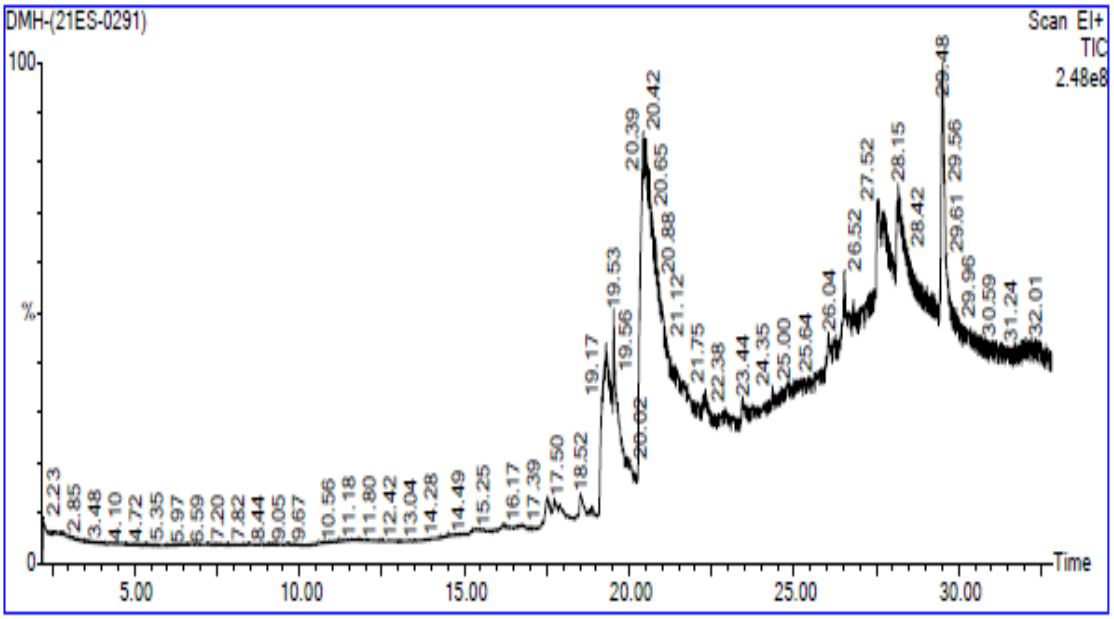
NO.	RT	Name of the compound	Molecular weight	Molecular formula	Structure
4	13.893	4-HEPTEN-3-ONE, 4-METHYL	126	C8H14O	
5	14.523	3-METHYLENECYCLOPROPANE-TRANS-1,2-DICARBOXYLIC ACID	142	C6H6O4	
6	15.354	2,3-ANHYDRO-D-GALACTOSAN	144	C6H8O4	

NO.	RT	Name of the compound	Molecular weight	Molecular formula	Structure
7	15.549	T-BUTYL CYCLOPENTANEPEROXYCARBOXYLATE	186	C10H18O3	
9	16.649	1-BROMO-4-BROMOMETHYLDECANE	312	C11H22Br2	
10	20.096	1,19-EICOSADIENE	213	C12H23O2N	
11	21.366	Z, Z-3,11-OCTADECADIEN-1-OL ACETATE	308	C20H36O2	

3.2.2 *Datura metel* hexane extract

Qualitative Report

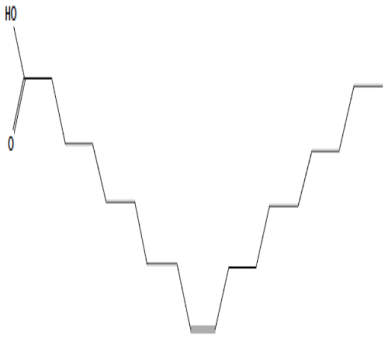
File: C:\TurboMass\2021.PRO\Data\DMH-(21ES-0291).raw
Acquired: 23-Mar-21 05:16:20 AM Printed: 26-Mar-21 12:22 PM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP Page 1 of 1
Sample ID: DMH-(21ES-0291) Vial Number: 56

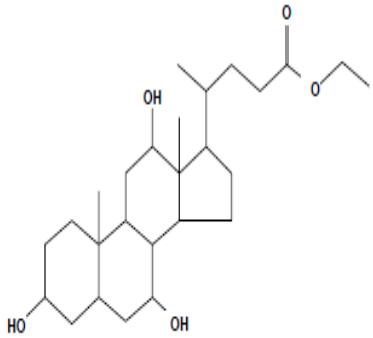
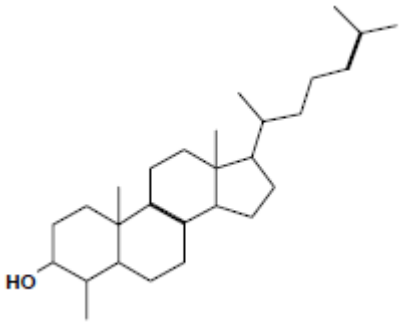
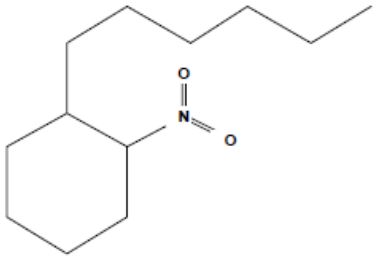


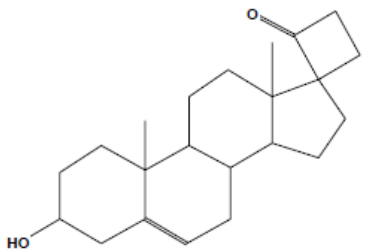
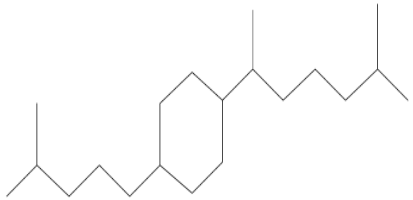
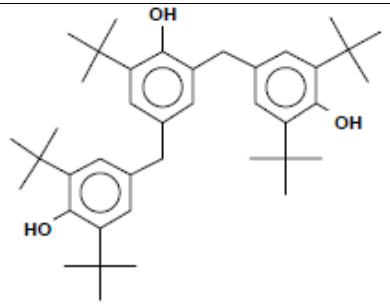
#	RT	Scan	Height	Area	Area %	Norm %
1	19.295	3418	50,724,308	11,438,051.0	6.700	12.42
2	20.421	3643	167,820,320	92,110,280.0	53.954	100.00
3	21.266	3812	34,539,468	9,914,852.0	5.808	10.76
4	26.518	4862	35,793,128	4,752,188.0	2.784	5.16
5	27.563	5071	54,652,112	7,513,369.5	4.401	8.16
6	27.704	5099	48,101,564	8,548,819.0	5.008	9.28
7	27.884	5135	31,162,110	4,791,784.0	2.807	5.20
8	28.149	5188	58,916,516	15,343,976.0	8.988	16.66
9	29.504	5459	129,657,800	16,306,933.0	9.552	17.70

Graph 2. Qualitative report for hexane extract

Table 3. The chemical compostion of hexane extract of roots of *Datura metel*

NO.	RT	Name of the compound	Molecular weight	Molecular formula	Structure
1	19.295	EICOSANOIC ACID	312	C20H40O2	
2	20.421	OLEIC ACID	282	C18H34O2	
3	21.266	1-OCTADECYNE	250	C18H34	

NO.	RT	Name of the compound	Molecular weight	Molecular formula	Structure
4	26.518	ETHYL ISO-ALLOCHOLATE	436	C26H44O5	
5	27.563	CHOLESTA-8,24-DIEN-3-OL, 4-METHYL-, (3.BETA.,4.ALPHA.)	398	C28H46O	
6	27.704	1-HEXYL-2-NITROCYCLOHEXANE	213	C12H23O2N	

NO.	RT	Name of the compound	Molecular weight	Molecular formula	Structure
7	27.884	SPIRO[ANDROST-5-ENE-17,1'-CYCLOBUTAN]-2'-ONE, 3-HYDROXY-, (3.BETA.,17.BETA.)	328	C22H32O2	
8	28.149	CYCLOHEXANE, 1-(1,5-DIMETHYLHEXYL)-4-(4-METHYLPENTYL)	280	C20H40	
9	29.504	2-TERT-BUTYL-4,6-BIS(3,5-DI-TERT-BUTYL-4-HYDROXYBENZYL)PHENOL	586	C40H58O3	

3.3 Docking

The docking results reveals that the ligands are well able to bind with the protein Mitogen Activated Kinase 2 (MK2) complexed with compound. The Table 4 shows the Minimum Binding Energy and Exasutiveness of the target when binds with ligand.

Table 4. Docking results for MK2 complexed with compound 76

Compound name	Protein	Minimum binding energy (kcal/mol)	Exasutiveness
ETHYL ISO-ALLOCHOLATE	MK2 Complexed with Compound 76	-7.1	9
2-CYCLOHEXEN-1-OL, 3-BROMO	MK2 Complexed with Compound 76	-4.5	9
2-FURANCARBOXALDEHYE, 5-(HYDROXYMETHYL)	MK2 Complexed with Compound 76	-4.5	9

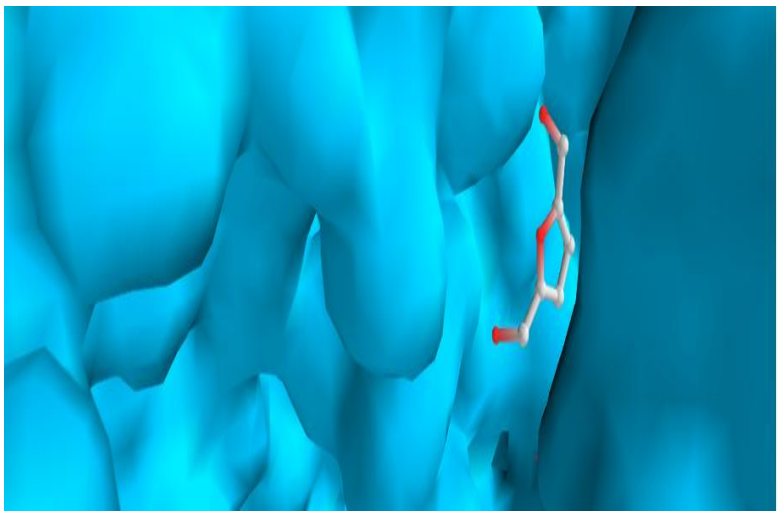


Fig. 5. Docking results of 2-FURANCARBOXALDEHYE, 5-(HYDROXYMETHYL) with MK2

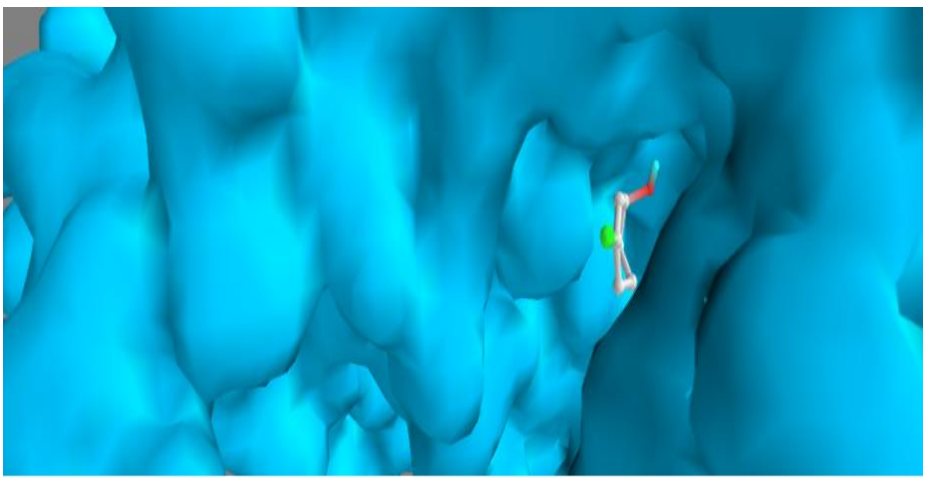


Fig. 6. Docking results of 2-CYCLOHEXEN-1-OL, 3-BROMO with MK2

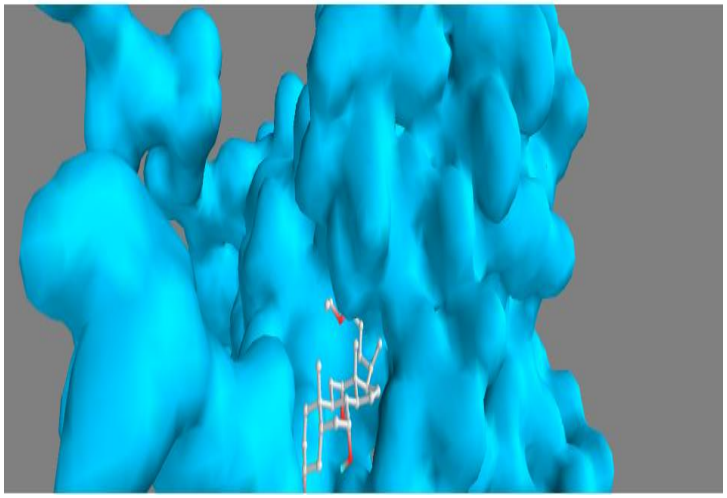


Fig. 7. Docking results of ETHYL ISO-ALLOCHOLATE with MK2

4. CONCLUSION

The outcomes of molecular docking conclusively show that particular compounds attach to their respective targets' active sites, indicating that the compounds may have the potential to reduce inflammation. According to the GC-MS analysis, *Datura metel* includes several phytoconstituents that can be employed for a variety of medicinal applications. In this study, chemicals found in the *Datura metel* root that were identified using GCMS data have been described as anti-inflammatory medications with a variety of targets, including Mitogen Activated Kinase 2. (MK2). A molecular knowledge of the inhibitory mechanisms of discovered compounds against an inflammatory therapeutic target was achieved using in silico docking studies (MK2). Furthermore, docking studies show that superior MK2 inhibitors than those currently on the market include 5-(hydroxymethyl), 2-cyclohexen-1-ol, 3-bromo, and ethyl iso-allocholate.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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