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Screening of Novel MAPK Inhibitor from Ethanolic Extract of Stem of *Leucas aspera* (Thumbai) using GCMS and *In Silico* Analysis

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KEYWORDS

Inflammation, Inflammatory bowel disease, MAP kinase, Leucasaspera, autodock.

A B S T R A C T

Leucasaspera (Family: Lamiaceae) commonly known as "Thumbai" is generally used as an insecticide and whole plant decoctions are traditionally taken for cough and cold and to treat rheumatism, inflammation and bacterial infections. The extracts of whole plants have been reported to have antiulcer, antimicrobial, hepatoprotective, antinociceptive, antioxidant, cytotoxic and prostaglandin inhibitory activities. The mitogen activated protein kinases (MAPK) have been implicated in an ever-increasingly diverse array of pathways, including inflammatory signalling cascades. Inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, are characterized by the perpetual production of inflammatory mediators. The transduction pathway behind this over-production has highlighted the potential mediating role for the MAPKs and their related signaling components. The ethanolic extracts of *Leucasaspera* stems were investigated for their action in significant anti-inflammatory action of acute and chronic inflammation. The bioactive compounds of *Leucasaspera* includes 13, Docenyloxy, 2-(9-octadecenyloxy), pentadecanoicacid, cyclopropaneoctanic acid, 5,19-Cyclo-5a-androst-6-ene-3,17-dione. The emergence of bioinformatics has provided a platform to explore diseases at the molecular level using computational techniques. In the present study, MAPK (Mitogen Activated Protein Kinase) drug targets were docked against bioactive compounds using AutoDock software. The highest binding energy was obtained using docking analysis and the protein–ligand interaction was studied and possible binding sites were predicted. The results suggested that the molecular target modulated by the bioactive constituents were useful indicators and may act as potent drugs for inflammatory bowel disease (IBD).

Introduction

Leucasaspera (Family: Lamiaceae) commonly known as 'Thumbai is distributed throughout India from the Himalayas down to Ceylon. *Leucasaspera*is also an antipyretic, it is a herb that has the ability to help reduce fever. In some forms of traditional medicine, the stem formed by crushing the Samoolam, also known as the plant's flowers, seeds, roots, berries, bark or leaves, can be inhaled to help treat nasal congestion, coughing, cold, headache and fever. In addition the juices of the flower can be extracted and used to help treat sinusitis, as well as headaches. The plant is generally used as an insecticide, and whole plant decoctions are traditionally taken for coughs and colds and to treat rheumatism, inflammation, and bacterial infections. A literature survey revealed that the extracts of whole plants have been reported to have antiulcer, antimicrobial, hepatoprotective, antinociceptive, antioxidant, cytotoxic, and prostaglandin inhibitory activities (Phytochemical and Biological Investigation of two Bangladeshi Medicinal plants, *Leucasaspera* and *Curcuma zedoria* A dissertation submitted for the partial fulfillment of the course of Pharmaceutical Research (PHRM 404) of the Department of Pharmacy, East West University for the Degree of Bachelor of Pharmacy).

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is an umbrella term for several chronic inflammatory conditions affecting the gastrointestinal tract (GI), the two most common entities being Crohn's disease (CD) and ulcerative colitis (UC). The detailed aetiology for these enigmatic diseases is not currently fully understood, and while the histology and prognosis for the patients differ between CD and UC, they share similarities in the proposed underlying causes to the development of these conditions. Currently, three broad areas are proposed to be involved in the development of IBD.

(i) genetic susceptibility including mutations of key proteins facilitating or enhancing the access of microbes to the epithelium and underlying layers.

(ii) luminal antigenic drive (e.g. microbes inhabiting the GI tract or food allergens)

(iii) environmental triggers, including stress and smoking. Extensive genetic profiling has succeeded in identifying several genes or genetic loci, which have been associated with CD, UC or both conditions. Many of these genes or genetic loci have been found to be linked to the signalling pathways regulating both the innate and adaptive immune system.

The mitogen activated protein kinases (MAPK) have been implicated in an ever-increasingly diverse array of pathways, including inflammatory signaling cascades. Inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, are characterized by the perpetual production of inflammatory mediators. The transduction pathway behind this over-production has highlighted the potential mediating role for the MAPKs and their related signaling components.

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is universally used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the ligands. Hence, docking plays a significant role in the rational drug design.

Molecular docking studies indicate how two or more molecular structures interact with each other for example, drug and enzyme or receptor of protein, fit together. Molecular docking software's are mainly used in drug research. The most important application of docking software is virtual screening. Virtual screening selects the most interesting and promising molecules from an existing database for advanced research. This places demands on the used computational method which must be fast and reliable (Yang *et al*., 2011; Ai *et al*., 2011; Amuthalakshmi, 2013).

Materials and Methods

Collection of plant materials

The fresh stems of *Leucasaspera* plant were collected from the region of Chennai, Tamilnadu. The collected plant was carefully examined, identified, weighed, air dried in the shade and powdered. 20gms of stem powder was extracted with 200ml of ethanol by using the above apparatus for 24hrs. The extract was then filtered and stored in air tight containers.

GC-MS Compound Identification

In the present study the analysis of Bio-active compounds from *Leucasaspera* stem extract was carried out through GC-MS analysis. The Ethanolic extract of *Leucasaspera* stem was employed for GC-MS analysis. In this investigation only five chemical constituents have been identified.

Preparation of protein

Uniprot

UniProt is a comprehensive, high-quality and free online database of protein sequence and functional information, mainly derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from the research literature. The UniProt/Swissprot Knowledgebase UniProtKB is the central access point for extensive curated protein information, including function, classification, and cross-reference (Uniprot C, "The Universal Protein Resource UniProt in 2010". Nucleic Acids Res, 38 Database issue: D142–D148, (2009) (http://www.uniprot.org/**)**

PDB

The PDB is a repository for 3-D structural data of proteins and nucleic acids. These data, typically obtained by X-ray crystallography or NMR Spectroscopy, are submitted by biologists and biochemists from around the world, are released into the public domain, and can be accessed for free. The mission of the PDB is to maintain a single Protein Data Bank Archive of macromolecular structural data that is freely and publicly available to the global community (www.rcsb.org/pdb).

Pfam

The Pfam database contains information about protein domains and families. Pfam-A is the manually curated portion of the database that contains over 10,000 entries. For each entry a protein sequence alignment and a hidden Markov model is stored. These hidden Markov models can be used to search sequence databases with the HMMER package written by Sean Eddy. Because the entries in Pfam-A do not cover all known proteins, an automatically generated supplement is provided called Pfam-B. Pfam-B contains a large number of small families derived from clusters produced by an algorithm called ADDA.
(http://pfam.sanger.ac.uk)

http://www.modelling.leeds.ac.uk/qsitefinder/

ACD Chem Sketch

Advanced chemistry development's ACD/chem sketch is a chemically intelligent drawing interface that allows you to draw almost any chemical structure including organics, organometallics, polymers, and markush structures. Use it to produce professional looking structures and diagrams for reports and publications (www.acdlabs.com/../chemsketch/).

OPEN BABEL

Open Babel is a chemical toolbox designed to speak the many languages of chemical data. It's an open, collaborative project allowing anyone to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas (http://openbabel.org/).

Pass online

PASS Online predicts over 3500 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc. Prediction is based on the analysis of structure activity-relationships for more than 250,000 biologically active substances including drugs, drug-candidates, leads and toxic compounds.

Autodock

Auto Dock is a suite of automated docking tools. The software is used for modelling flexible small molecules such as drug molecules and its binding to receptor proteins of known three dimensional structures. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools are used to prepare, run and analyze the docking simulations, in addition to modeling studies (http://autodock. scripps.edu/resources/tools).

PyMOL

PyMOL is an open-source tool to visualize molecules available from (z. It runs on Windows, Linux and MacOS equally well. PyMOL has excellent capabilities in creating high-quality images from 3D structures, it has well developed functions for manipulating structures and some basic functions to analyze their chemical properties. The possibilities to write scripts and plugins as well as to incorporate PyMOL in custom software are vast and superior to most other programs. PyMOL has been written mostly in the Python language (www.python.org).

Docking studies

Databases and tools

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. Computer – Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and data bases. The structure of 2ojg was retrieved from PDB database. Using Chemsketch the structures of the compounds were generated by their SMILES notation obtained from uniprot and

the structural analogues of these compound were sketched. The docking analysis was carried by AUTO DOCK docking software. For our study we used bioinformatics tools, biological databases like PubMed, pubchem, uniprot, PDB (Protein Data Bank) and software's like auto dock and PYMOL.

Results and Discussion

Ethanolic extract of *Leucasaspera*

The Ethanolic extract of *Leucasaspera* stem was employed for GC-MS analysis. In this investigation only six chemical constituents have been identified (Table-1). They are 13-Docosenoic acid, 2-(9-octadecenyloxy), Cyclopropaneoctanoic acid, Pentadecanoic acid, 5,19-Cyclo-5a-androst-6-ene-3. The peak levels of different chemical compounds are shown in Fig 2.

Autodock

Auto Dock is a suite of automated docking tools. The software is used for modelling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structure. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools is used to prepare, run and analyze the docking simulations, in addition to modeling studies. Auto Dock is the most cited docking software because it is very fast, it provides high quality predictions of ligand conformations and good correlations between inhibition constants and experimental ones.

Sequence Retrieval: Mapk

The protein sequence for the P28482 was obtained from the protein sequence database of UniProt (http://www.uniprot.org/ uniprot/). The source organism is Homo sapiens.

Structure Retrieval

The three-dimensional structure of MAPK was available in the PDB database.The PDB id is 2OJG. The 3D structure was visualized using the Rasmol Tool (shown in Fig. 4).

MAPK have the specific domain region. The protein kinase domain is a structurally conservedprotein domain containing the catalytic function of protein kinases.

Preparation of Ligands

GCMS analysis of *Leucasaspera* bioactive compounds are selected. The two-dimensional structures of the ligands were generated using the ACD/Chem Sketch tool. This software contains tools for 2D cleaning, 3D optimization, and viewing. These data are saved as a molecular format file (MDL MOL format). The molecular format converter tool (Open Babel) is used to convert this file into the PDB format and is used during docking analysis. The structure and molecular formula of inhibitors were shown below.

Docking Analysis of Mapk against Bioactive Compounds

The inhibitors docked with MAPK receptor using Autodock software (Version 4.2) . The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation.

Table.1 (Bio Activity Chemicals Identified in LEUCAS ASPERA (Ethanol Extract) GC-MS -Peak Report –TIC

No	R.Time	Name of the chemical compound	M.F	M.W	Peak Area	Area %
1	22.62	13-Docosenoic acid, methyl ester (Z).	$C_{22}H_{42}O_2$	338.56768 g/mol	5006992	18.9%
2	19.47	2-(9-Octadecenyloxy)	$C_{21}H_{42}O_3$	342.55638 g/mol	3692544	7.3%
3	19.8	Cyclopropaneoctanoic acid,2-((2-pentylcyclopropyl)methyl)-methyl ester.	$C_{20}H_{35}O_2$	308.49864 g/mol	3594464	6.7%
4	17.18	Pentadecanoic acid, 14-methyl, methyl ester.	$C_{15}H_{30}O_2$	242.3975 g/mol	4181456	32.3%
5	17.83	15, 19-Cyclo-5a-androst-6-ene-3, 17-dione	$C_{18}H_{22}O_3$	284.40 g/mol	3429568	8.3%

Table.2 Preparation of Ligands

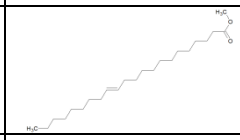
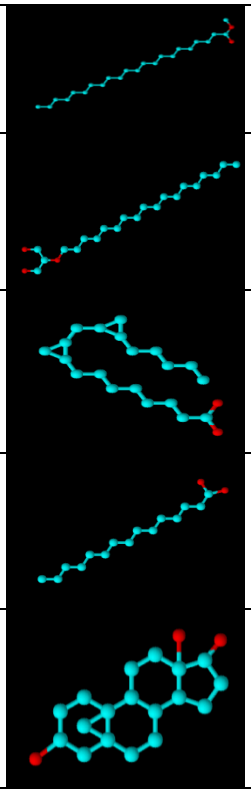
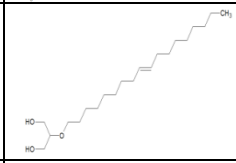
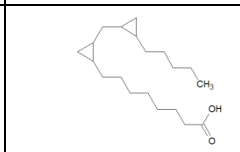
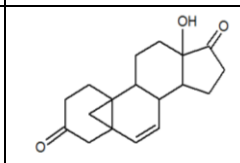
COMPOUND NAME	2D STRUCTURE	3D STRUCTURE	H-Bond DONOR	H-Bond ACCEPTOR	Rotatable Bond Count	XLogP 3
13-docosenoic acid			1	2	19	8.7
2-(9-Octadecenyloxy)			2	3	19	6.7
Cyclopropaneoctanoic acid			1	2	14	7.7
Pentadecanoic acid, 14-methyl, methyl ester..			1	2	13	5.8
15, 19-Cyclo-5a-androst-6-ene-3, 17-dione			2	0	0	2.01

Table.3 Molecular Interactions between 13-DOCOSENOIC ACID and the MAPK

MAPK		13-Docosenoic acid	Distance (Å)
Residue	**Atom**		
ARG65	NH2	O	2.4
THR188	OG1	H	4.0
GLN103	OE1	O	2.6

Table.4 Molecular Interaction between Cyclopropaneoctanoic acid and the MAPK

MAPK		Cyclopropaneoctanoic acid	Distance (Å)
Residue	**Atom**		
ARG 65	NH1	O	3.2
ARG 65	CZ	H	3.O

Table.5 Molecular Interaction between Pentadecanic Acid andtheMAPK

MAPK		pentadecanic acid	Distance (Å)
Residue	**Atom**		
ARG65	NH	O	2.5
TYR188	CE2	H	5.8
TYR34	OG1	O	4.4

Table.6 Molecular Interaction between 5,19-cyclo-5a-androst-6-ene-3, 17-dione and the MAPK

MAPK		5, 19-cyclo-5a-androst-6-ene-3, 17-dione	Distance (Å)
Residue	**Atom**		
GLN103	NH2	O	3.2
LEU154	CD2	O	3.6
ASP109	OD1	O	2.6

Fig.1 Leucasaspera

- Kingdom: Plantae
- Class: Dicotyledonae
- Subclass: Eudicots
- Order: Tubiflorae
- Family: Labiatae
- Genus: *Leucas*

Fig.2 Inflammatory Bowel Disease

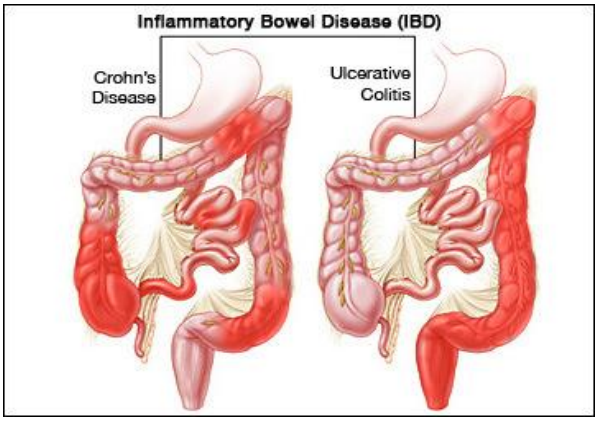


Fig.3 Bio Activity Chemical Identified in Leucaaspera (Ethonol Extract) GC-MS-Peak Report-TIC

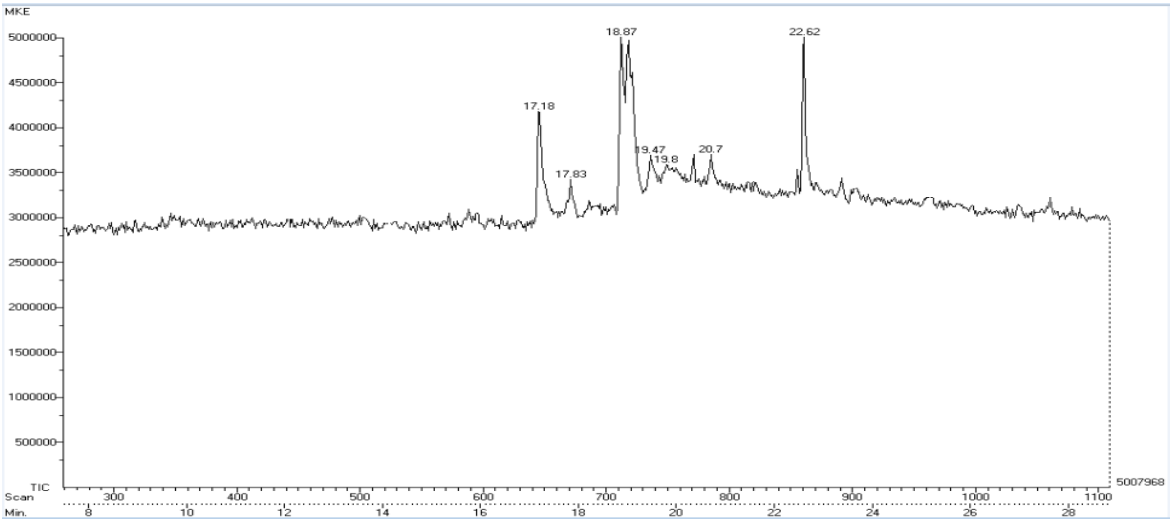
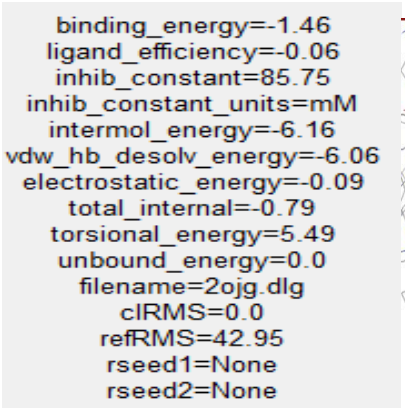
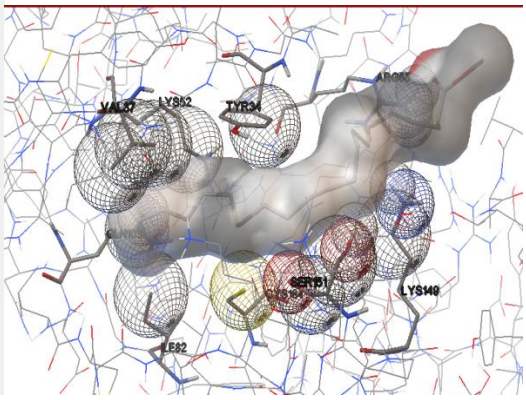


Fig.4 3D Visualization Using Rasmol Domain Analysis

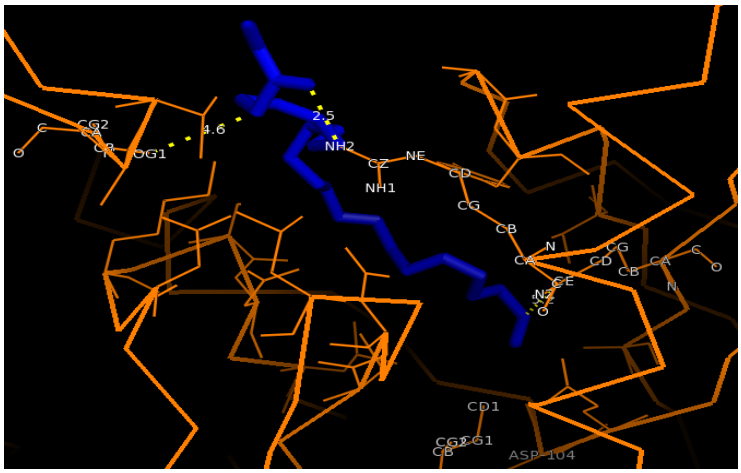
Fig.5 13-DOCOSENOIC ACID AND MAPK (a)Binding energy score (b) Interaction between MAPK and13-DOCOSENOIC ACID was visualized using Auto dock (c) Hydrogen bond interaction between MAPK and13-DOCOSENOIC ACID was visualized using Pymol



(a)

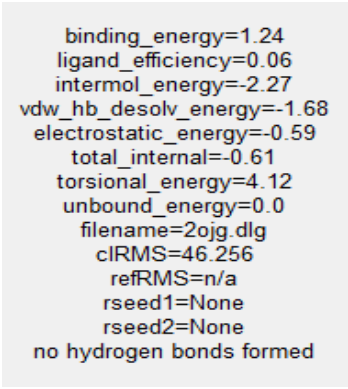


(b)

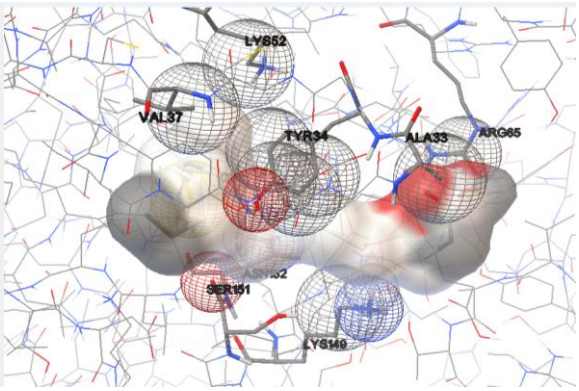


(c)

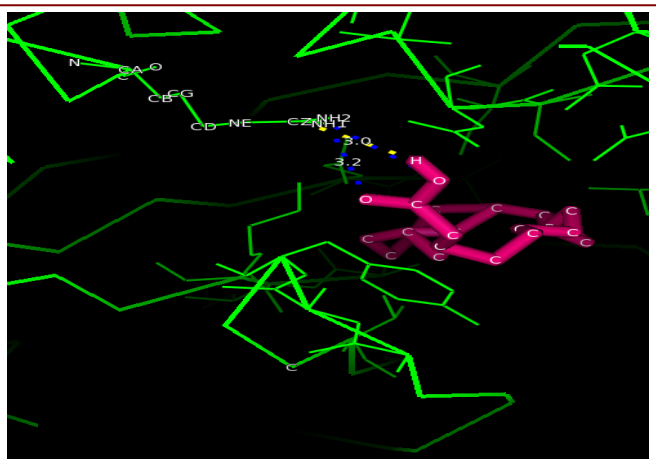
Fig.6 CYCLOPROPANEOCTANOIC ACID AND MAPK (a)Binding energy score (b) Interaction between MAPK andCYCLOPROPANEOCTANOIC ACID was visualized using Auto dock (c) Hydrogen bond interaction between MAPK andCYCLOPROPANEOCTANOIC ACID was visualized using Pymol



(a)



(b)

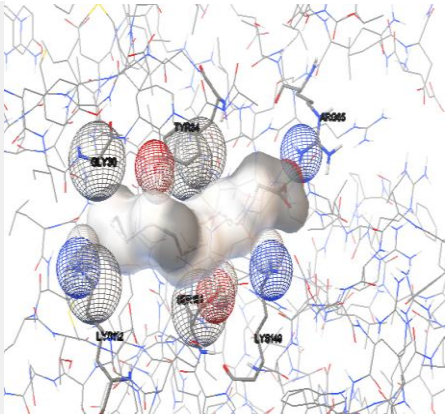


(c)

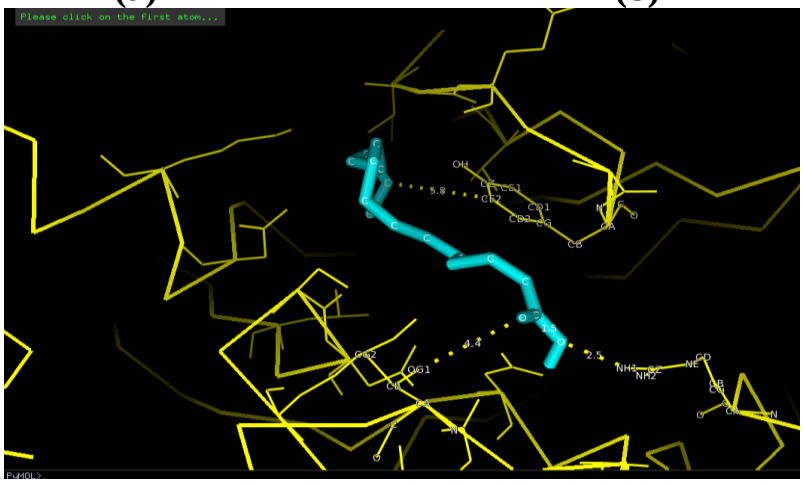
Fig.7 PENTADECANIC ACID AND MAPK (a)Binding energy score (b) Interaction between MAPK andPENTADECANIC ACIDwas visualized using Auto dock (c) Hydrogen bond interaction between MAPK andPENTADECANIC ACIDwas visualized using Pymol

binding_energy=-1.02
ligand_efficiency=-0.06
inhib_constant=178.41
inhib_constant_units=mM
intermol_energy=-4.44
vdw_hb_desolv_energy=-3.99
electrostatic_energy=-0.45
total_internal=-0.42
torsional_energy=3.84
unbound_energy=0.0
filename=2ojg.dlg
cIRMS=48.846
refRMS=n/a
rseed1=None
rseed2=None

(a)

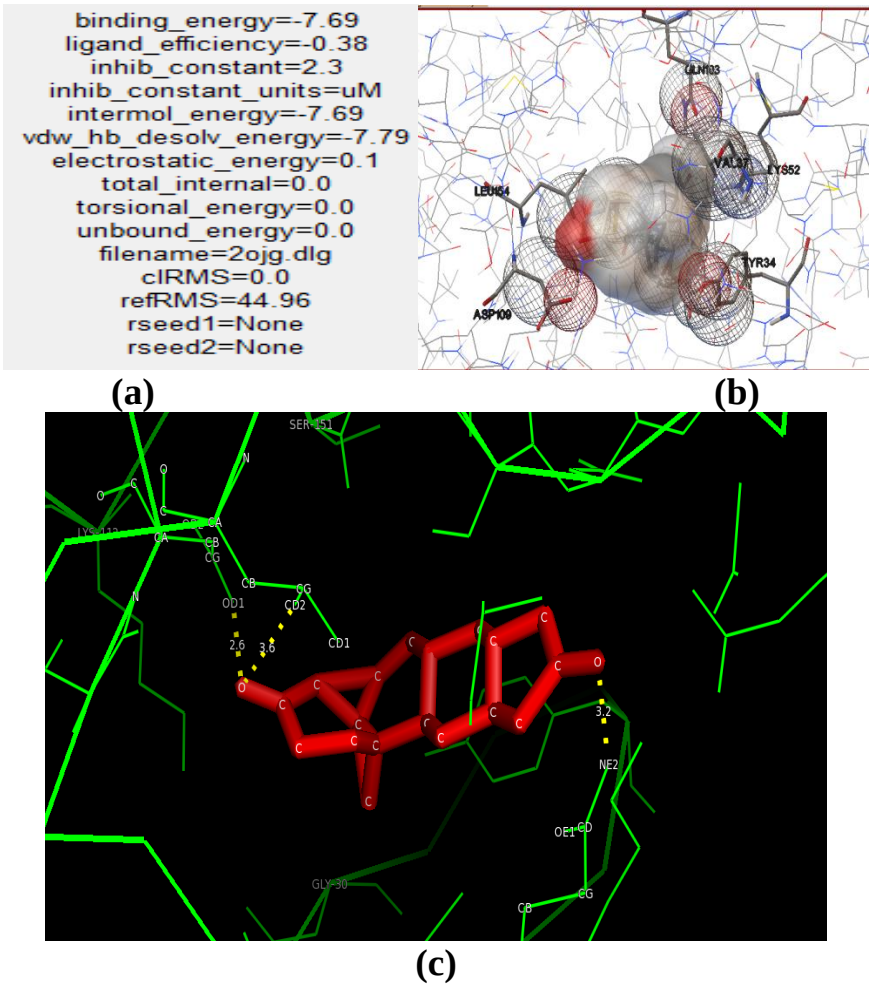


(b)



(c)

Fig.8 5, 19-CYCLO-5A-ANDROST-6-ENE-3, 17-DIONE AND MAPK (a)Binding energy score (b) Interaction between MAPK and5, 19-CYCLO-5A-ANDROST-6-ENE-3, 17-DIONEwas visualized using Auto dock (c) Hydrogen bond interaction between MAPK and5, 19-CYCLO-5A-ANDROST-6-ENE-3, 17-DIONEwas visualized using Pymol



Autodock results were analyzed to study the interactions and the binding energy of the docked structure. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were visualized using the Acceryls Visualizer discovery studio tool.

A bond is formed between two atoms by overlapping the atomic orbitals. This overlap of atomic orbitals to form molecular orbitals occurs only at certain distances between the atom. When the amino acid residues of the active site is closer, then the interactions is much higher than the other sites.

Conclusions

Phytochemical analysis in ethonolic extract of *Leucasaspera* stem by using GC-MS revealed presence of 13,docenyloxy, pantadecanicacid, cyclopropaneoctanic acid, 5,19-cyclo-5a-androst-6-ene-3,17-dione.
Bioactive compounds from *Leucasaspera* stem has anti-inflammatory activity and *insilico* docking analysis was carried out against drug target MAPK using Autodock 4.2 software. The result shows that

compounds from *Leucasaspera*stem has good binding energy with least docking score and the bioactive compound. Based on Binding energy and Hydrogen bond formed and Inhibitory constantvalue the docking results were analyzed. Cyclopropaneoctanic acid forms two hydrogen bond interaction in the docking energy of 1.24 Kcal/Mol and 13-Docenyloxy, pentadecanicacid, 5,19-and Cyclo-5a-androst-6-ene-3,17-dione forms three hydrogen bond interaction with the docking energy of -1.46, -1.02, and -7.69 Kcal/Mol, respectively. The work is significant in emphasizing the potent inhibitory effects of bioactive compounds on MAPK activity and may offer therapeutic advantages in thetreatment and prevention of Bowel's disease.

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Molecular docking studies of B-RAF expression inhibitors identified from *Strychnospotatorum*(Thethankottai)

Phytol - A chemical constituent of *Leucasaspera* through HPTLC, U.G. Basarkar Assistant Professor, P.G. Dept. of Botany, G.E. Society's, HPT Arts RYK Science College, Nasik-422 005, Maharashtra, India. Chemical compounds investigation of *lucas aspera* leaves – a potential folklore medicinal plant.

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