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BIOACTIVE COMPOUNDS IDENTIFICATION IN *Abutilon Indicum* THROUGH GC-MS ANALYSIS AND MOLECULAR DOCKING STUDY

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ABSTRACT

One of the most important medicinal plants of India, *Abutilon Indicum,* is used for inflammatory joint diseases, ulcers, and scorpion bites, besides being used as-antioxidant, laxative, diuretic, analgesic, and antimicrobial agent. The plant leaves' ethanol extract was analyzed through GC-MS; the secondary metabolites were identified, and the lead molecules were evaluated by molecular docking with liver protein. From the GC-MS analysis, about 20 compounds were identified. Among them, the highest area of about 65% with a retention time (RT) of 10.082 min is for diethyl phthalate, followed by 18% of methyl salicylate. The best docking pose shows that the ligands show several interactions, including hydrogen bonding π-anion and π-alkyl and alkyl interactions, and the docking score is better for the predominant compound diethyl phthalate than methyl salicylate.

Keywords: *Abutilon Indicum*, GC-MS, Docking, Liver Protein, Methyl Salicylate, Diethyl Phthalate.

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INTRODUCTION

The Malvaceae plant *Abutilon Indicum* sometimes referred to as "Thuthi," is found in the hottest regions of India.[1,2] According to reports[3,4], traditional healers utilize the leaves as a folk treatment for inflammatory joint diseases. In India, reports on psychosomatic medications used to treat scorpion bites have also been made from the *Abutilon Indicum* plant. Many components of the *A.indicum* plant, including leaves, stems, roots, flowers, bark, and seeds, are used for diversified activities such as anti-inflammatory, antioxidant, diuretic, laxative, anti-ulcer, immunomodulatory, hypoglycemic, antimicrobial, infertility, and analgesic treatments in traditional medical systems[5-12]. It's used in Siddha medical method treatments for ulcers, jaundice, piles, and leprosy as well. The study on rats using an aqueous extract of *A. indicum* reported hepatoprotective efficacy in contradiction to hepatotoxicities produced by carbon tetrachloride and paracetamol.[13] Because of their cytotoxic properties, plant secondary metabolites have been employed in the treatment of tumors. The chloroform extract of *A. indicum* leaf contains several cytotoxic substances, including methyl caffeate, syringic acid, stigmasterol, quercetin, and cholesterol.[15,16] The hydromethanolic extract of *A. indicum* leaf has yielded another substance called phytol, which registered ROS-mediated apoptosis.[17]Additionally, *A. indicum* has been used to treat vaginal infections, diarrhea, gonorrhea, bladder inflammation, ulcers, and fast healing of wounds.[18] It can also be used as an enema.[19] Based on the medicinal properties of *Abutilon Indicum,* which was selected as an important material for the identification of secondary metabolites through GC-MS analysis and identified bioactive molecules focused for protein binding by molecular docking studies.

EXPERIMENTAL

Plant Collection and Extraction

The whole plant (leaf, root, and stem) of *A. indicum* was collected from a nearby village at Nagapattinam, Tamil Nadu. The collected leaf was dried in the shade and then made into powder. Then the dried 500 g of plant powder was dissolved in 1 L ethanol, then the ethanolic portion was concentrated by a rotary evaporator. Thus, the obtained crude ethanol extract was focused on the possible identification of the compounds by GC-MS analysis.

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Detection Method

Gas Chromatography-Mass Spectrometry Analysis of A. Indicum

The plant extract was diluted in *n*-hexane (10 µL in 300 µL) before injecting 10 µL of solution into a split-mode gas chromatographic instrument. The analysis was performed using an Agilent Technologies 7890 gas chromatograph with a flame ionization detector, split-split less injector, and HP-5 column (30 m×0.32 mm, film thickness 0.25 µm). Helium (1 mL/min, 210 °C) was used as a carrier gas. The column temperature was configured to range from 40 to 260 °C at the rate of 4 ºC/min, with the injector and detector set to 250 and 280 °C, respectively. The percentage composition was determined using peak areas rather than correction factors. Mass spectra were recorded in EI mode (70 eV) between *m/z* 40 and 450.

Molecular Docking

The docking analysis was performed using AutoDock vina academic software.[20] The chemical structure of the active ligands, 1,2-benzenedicarboxylic acid diethyl ester ($C_{12}H_{14}O_4$) and methyl 2-hydroxybenzoate ($C_8H_8O_3$), and other important compounds were drawn using ChemDraw15.0 (www.CambridgeSoft.com). The three-dimensional structures of the target protein, human Vitamin K-dependent protein Z (PDB ID: 3H5C), were obtained from the website (https://www.rcsb.org). The ligand was docked to the target protein complexes, with the molecule acting as a rigid body and the ligand as flexible. The search was extended to include the entire receptor protein for blind docking using AutoDockVina. The results were evaluated by ranking the various complexes according to the estimated binding energy.

RESULTS AND DISCUSSION

The important components of the ethanol extract of *A. indicum* were identified by calculating their Kovats retention index (RI) and comparing their mass spectra to reference compounds from the National Institute of Standards and Technology mass spectral data.[21]Twenty chemical compounds were identified through GC-MS analysis of the ethanol extract of *A. indicum* leaves(Table-1, Fig.-1).

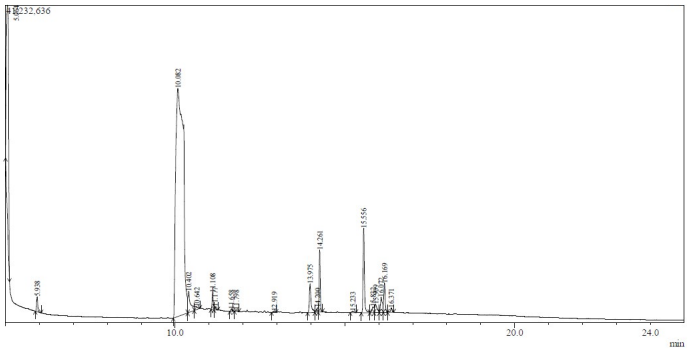


Fig.-1: GC-MS Chromatogramof *Abutilon Indicum*Ethanol Extract

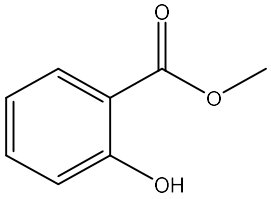
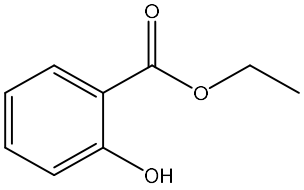
Table-1: GC-MS Analysis of Ethanol Extractof *Abutilon Indicum*

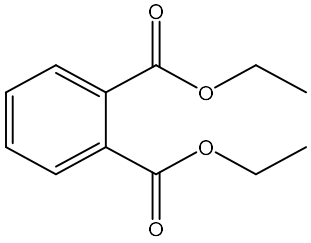
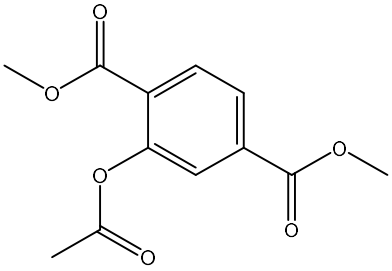
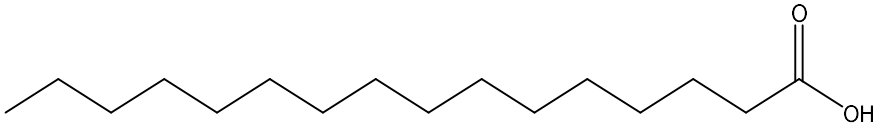
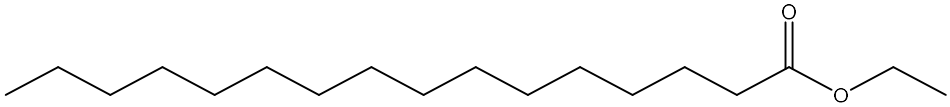
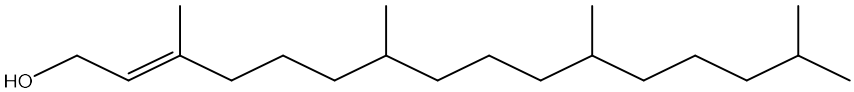
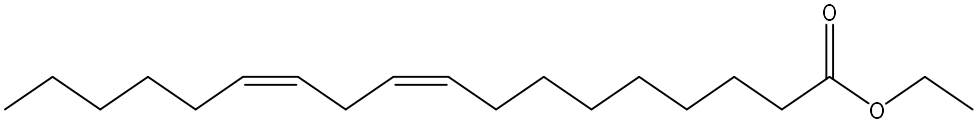
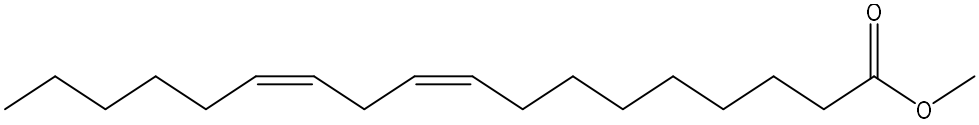
Peak #	Retention Time	Area	Area %	Height	Height %	AH	Name of the compound
1	5.064	126533898	17.98	27592944	28.38	4.59	Benzoic acid, 2-hydroxy, methyl ester (or) Methyl salicylate (or) Methyl 2-hydroxybenzoate.
2	5.938	4704136	0.67	1950963	2.01	2.41	Benzoic acid, 2-hydroxy, ethyl ester (or) Ethyl salicylate (or) Ethyl 2-hydroxybenzoate.
3	10.082	455006958	64.64	29663512	30.51	15.34	1,2-Benzenedicarboxylic acid diethyl ester (or) Diethyl phthalate
4	10.402	13588298	1.93	2748200	2.83	4.94	1,4-Benzenedicarboxylic acid, 2-(acetyloxy)-, dimethyl ester (or) Dimethyl 2-acetoxyterephthalate
5	10.642	2585123	0.37	317211	0.33	8.15	Undecanoic acid (or) Undecylic acid

6	11.108	5066840	0.72	2174580	2.24	2.33	1,2-Benzenedicarboxylic acid, diethyl ester (or) Ethyl phthalate
7	11.177	1033562	0.15	399217	0.41	2.59	α-Copaene-11-ol
8	11.658	630150	0.09	269567	0.28	2.34	1,2-Benzenedicarboxylic acid, diethyl ester (or) Ethyl phthalate
9	11.798	682335	0.10	211876	0.22	3.22	Tetradecanoic acid (or)Myristic acid
10	12.919	582503	0.08	134192	0.14	4.34	Ethyl iso-allocholate
11	13.975	13032659	1.85	3749037	3.86	3.48	Hexadecanoic acid (or) Palmitic acid
12	14.200	1667987	0.24	576043	0.59	2.90	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) 9-cis-octadecenyl 9-cis-hexadecenoate
13	14.261	18152429	2.58	8085881	8.32	2.24	Hexadecanoic acid, ethyl ester (or) Ethyl palmitate
14	15.233	790905	0.11	184018	0.19	4.30	Propyl hexadecanoate
15	15.556	30262188	4.30	10997266	11.31	2.75	Phytol (or) 3,7,11,15-tetramethyl-2-hexadecen-1-ol
16	15.832	3304650	0.47	746120	0.77	4.43	(Z)6-Pentadecen-1-ol
17	15.909	5296511	0.75	1065013	1.10	4.97	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (or) Methyl linolenate
18	16.072	8508952	1.21	1988057	2.04	4.28	Ethyl linoleate (or) Linoleic acid ethyl ester
19	16.169	10624397	1.51	3835743	3.94	2.77	Methyl linoleate
20	16.371	1834529	0.26	551470	0.57	3.33	Octadecanoic acid, ethyl ester (or) Ethyl sterate

These substances were recognized using MS (Mass Spectrometry) coupled with GC (Gas Chromatography). Table-1 displays the identified chemicals together with their retention time (RT), peak height, and concentration (peak area %), whereas the chemical structure of the important (abundant wise) compounds is demonstrated in Table-2. The chromatogram of the extract is reproduced in Fig.-1, whereas Fig.-2 shows the lead compounds' docking images. Among the 20 compounds, the highest amount area % was 64.64, with a retention time (RT) of 10.082 mins (Peak 3) for the diethyl phthalate, and the second highest amount area % was 17.98, with RT of 5.064 mins (Peak 1) for the methyl salicylate. Reports available on the bioactivity of methyl salicylate and even diethyl phthalate; however, we docked these molecules with liver protein to showcase its efficacy.

Table-2: Chemical Structures of The Important Compounds

Peak	Structure/Name
Peak 1	 Methyl salicylate
Peak 2	 Ethyl salicylate

Peak 3	 Diethyl phthalate
Peak 4	 Dimethyl 2-acetoxyterephthalate
Peak 11	 Hexadecanoic acid
Peak 13	 Ethyl palmitate
Peak 15	 Phytol
Peak 18	 Ethyl linoleate
Peak 19	 Methyl linoleate

The molecular docking study was utilized to explore the binding interaction of the title compound with the active sites of human vitamin K-dependent protein Z (PDB ID: 3H5C) through the AutodockVina program. A cluster analysis based on root mean square deviation values for the beginning geometry was then done, and the lowest energy conformation of the more populated cluster was chosen as the most reliable solution. The docking data are summarized in Table-3, whereas the best docking poses are shown in Figs.-2 and 3 for better understanding.

It is seen that the methyl 2-hydroxybenzoate (methyl salicylate) ligand shows several interactions, including hydrogen bonding π-anion and π-alkyl and alkyl interaction. However, the conventional hydrogen bonding interactions (O-H..O) with GLY409:O-UNK1:H and A: SER59-UNK1 were observed with 4.17 and 4.14 Å bond distances, respectively. Further, the π-anion interactions with A: GLU301-UNK1 and π-alkyl interactions with A: TYR404-UNK1:C were shown within the estimated value of 4.6 Å (5.50 and 4.90 Å, respectively) The alkyl hydrophobic interactions was predicated with A: MET402-UNK1:C (5.43 Å), A: VAL300-UNK1:C

(5.56 Å), and A: LEU411-UNK1:C (4.48 Å) residues. In a nutshell, these interactions contribute to the stabilization of the ligand-protein complex and could compose a critical protagonist in the necessary attraction between methyl 2-hydroxybenzoate and the target protein. Similarly, the various interactions between diethyl phthalate and the target protein are summarized in the table for better comparison and understanding of the prominent two molecules with the liver protein. The docking score proves the importance of diethyl phthalate; its binding energy of -6.6 kcal/mol is higher than the binding energy of methyl salicylate -5.7 kcal/mol.

Table-3a: Interactions of Methyl Salicylate and Amino Acid Residues of Vitamin K-Dependent Protein Z (PDB ID: 3H5C)

Ligand	Binding Energy (kcal/mol)	Ligand atoms (ring)	Docked amino acid residue (bond length)
Methyl salicylate (Peak 1)	-5.7	Conventional hydrogen bond interactions: O-H O π-Anion interactions: O π π-Alkyl interactions: O-H Alkyl hydrophobic interaction: O-H O-H O-H	 GLY409:O-UNK1:H(4.17 Å) A:SER59-UNK1(4.14 Å) A:GLU301-UNK1(5.50 Å) A:TYR404-UNK1:C(4.90 Å) A:MET402-UNK1:C(5.43 Å) A:VAL300-UNK1:C(5.56 Å) A:LEU411-UNK1:C(4.48 Å)

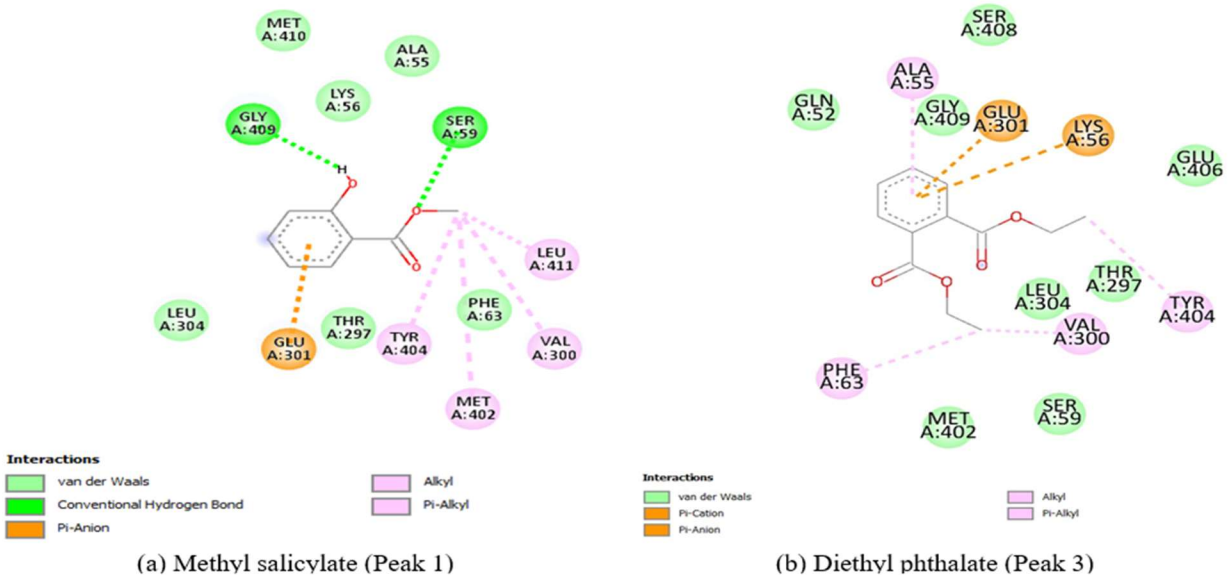
Table-3b: Interactions of Diethyl Phthalate and Amino Acid Residues of Vitamin K-Dependent Protein Z (PDB ID: 3H5C)

Ligand	Binding Energy (kcal/mol)	Ligand atoms (ring)	Docked amino acid residue (bond length)
Diethyl phthalate (Peak 3)	-6.6	van der Waals interactions: O-H π – Cation and Anion interactions: O π π-Alkyl interactions: O-H	A:GLY409-UNK1(4.81 Å) A:GLN52:- UNK1(4.12 Å) A:SER408- UNK1(5.17 Å) A:GLU406- UNK1(5.67 Å) A:LEU304- UNK1(4.33 Å) A:THR297- UNK1(4.18 Å) A:MET402- UNK1(4.87 Å) A:SER59- UNK1(5.16Å) A:GLU301- UNK1(5.47 Å) A:LYS 56- UNK1(4.31 Å) A:ALA55- UNK1(4.67 Å) A:VAL300- UNK1(4.25 Å) A:PHE63- UNK1(4.11Å) A:TYR:404- UNK1(5.40 Å)

CONCLUSION

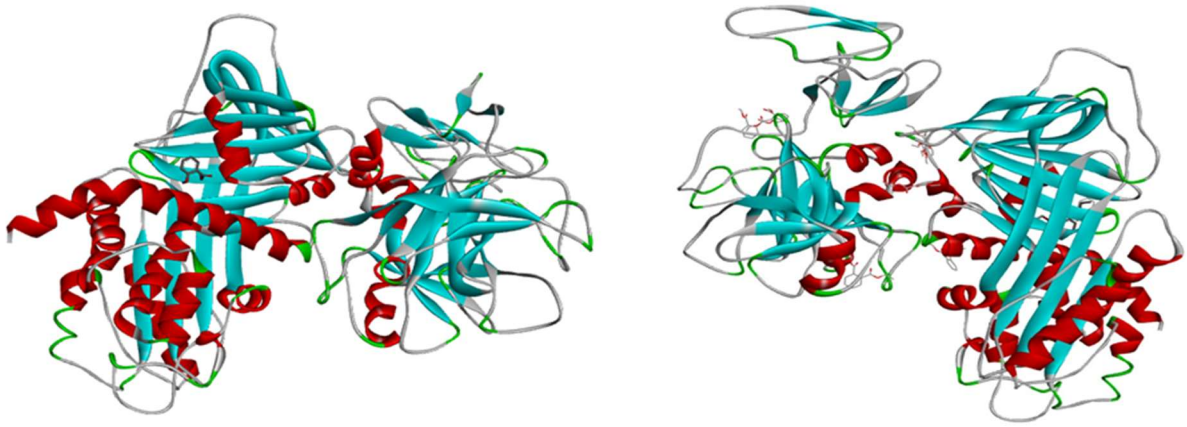
The important medicinal plant *Abutilon Indicum*'s ethanol extract was targeted GCMS analysis to identify the possible compounds to expose its biological activity. Accordingly, 20 compounds were identified; among

them, methyl salicylate (Peak 1 ~18%) and diethyl phthalate (Peak 3 ~65%) were found to be the prime compounds, for which a docking study with liver protein was examined. Of them, diethyl phthalate provided a better docking score over methyl salicylate, thus supporting the importance of diethyl phthalate in the biological actions of the *A. Indicum* plant.



(a) Methyl salicylate (Peak 1) (b) Diethyl phthalate (Peak 3)

Fig.-2: Protein-ligand interactions



(a) Methyl salicylate (Peak 1) (b) Diethyl phthalate (Peak 3)

Fig.-3: Liver Protein Molecular Docking

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication.The research profile of the authors can be verified from their ORCID IDs, given below:

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