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An Insilco Approach on Trachyspermum Ammi to Discover a Drug that Reduces the Burden of Gallstone and Gallbladder Tumor

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Abstract
Nowadays, after pandemic circumstances people from developing countries and in particular Indians have faith towards herbal medicine to treat chronic disorders. Previous literature stated that Indians may be genetically influenced to develop gallstones in their liver due to a single gene mutation, potentially leading to liver malfunctions and stone formation. In Tamil Nadu, gallstones containing bilirubin, cholesterol, and calcium are prevalent. Initially asymptomatic, they can lead to cancer and mortality. South Indians believe herbal medicine can treat gallstones without side effects. Based on the above, we made an attempt to find the bioactive compounds from *Trachyspermum ammi* (*Ajwain* seeds) using ethanol, petroleum ether and water for extraction. Gas chromatography-mass spectroscopy was used to analyze the plant's ethanol extract (GC-MS). Out of the thirty compounds that were chosen based on retention time, five aromatic functionally active compounds underwent docking studies using the bicyclo [3.1.0] of the tumor suppressor protein's SMAD4 receptor. hexan-2-ol, oleyl alcohol, trifluoroacetate, 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, thymol, phosphinoline, 1,2,3,4-tetrahydro-.The *Insilco* studies with protein-drug interaction significantly interact with 10 different proteins as a cascade process for biological changes. The study concluded that the extract exhibits anti-inflammatory, antimicrobial, antioxidant, and anticancer properties, specifically for gallbladder.

Key words:
Ajwain, Thymol, γ-Terpinene, Tetradecanoic Acid, N-Hexadecanoic Acid, Caryophyllene, Oleyl Alcohol Trifluoroacetate,

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Introduction

Indian's burden of gall stone and gall bladder cancer is increasing with north Indians more affected than south Indians. A single gene mutation in the liver can potentially cause liver malfunctions and the formation of stones. In Tamil Nadu, the most common types of stones are those containing bilirubin, cholesterol, and calcium, with 26%, 7%, and 8% respectively. Gallstones initially appear asymptomatic but can lead to cancer and increased mortality rates. South Indians believe herbal medicine can treat gallstone without side effects, and the World Health Organization supports natural resource research [1]. The phytochemical screening of plants is a preliminary step towards verification, which could lead to the development of new herbal medicine sources. [2] These therapeutic plants contain various chemical components such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, and rotenoids. The annual herbaceous and aromatic plant known as *ajwain (Trachyspermum ammi)* is a member of the Apicacae family. It can reach a height of 60 to 90 cm, with feathery leaves and red and white flowers. Small, egg-shaped seeds that vary in color from brown to gray were chosen in an effort to develop a novel treatment for gallstone disorders [3]. *Ajwain* seeds have a strong, dry, spicy flavor with a hint of bitterness. It is also known as Omam in Tamil and Malayalam, Bishop's weed in English, *Ajwain* in Hindi, Vamu in Telugu, Dipyaka, Yamini, Yaminiki, and Yaviniki in Sanskrit [4]. Because *Ajwain* has comparable tasting chemicals, primarily thymol, it tastes similar to thyme. Because the seeds of *Ajwain* include various bioactive components that have pharmacological or physiological effects, they have a high potential to reduce cramps, gas, and any other abdominal discomfort [5]. Well-known for its medicinal and pharmacological properties, *ajwain* is an herb with many Ayurvedic uses and health advantages [6]. *Ajwain* seeds offer numerous benefits including anti-flatulence, antiarthritic, antirheumatic, antispasmodic, aphrodisiac, asthma, bronchitis, cardialgia, carminative, expectorant, indigestion, migraine, polyuria, toothache, and common cold. [7] *Ajwain* seed oil has numerous therapeutic properties, including antibacterial, antifungal, fungitoxic, insecticidal, antimicrobial, anti-spoilage, antioxidant, ant-filarial, nematocidal, and anti-inflammatory effect [8,9]. In this present study, bioactive compounds form *Ajwain* seeds (*Trachyspermum ammi*) were isolated and identified the active constituents present in seeds using GC-MS, and also to find out binding affinity of the drug with tumor suppressor protein such as SMAD4 to check the feasibility to consider as a drug to reduce the burden of gall stone.

Materials and Methods

Plant materials

Ajwain seeds (*Trachyspermum ammi* L*)* acquired from Chennai, Tamil Nadu, India. The specimen was examined by Dr. Senthilkumar Umapathy, Department of Botany, MCC, Tambaram, Chennai-60059.

Preparation of different extracts

Grained and powdered *Ajwain* seeds were cleaned and dried. Weighing out 100g of powder, it was soaked for 72 hours in three different solvents (water, petroleum ether, and ethanol) with intermediate soaking. The filtrate was then filtered through Wattman grade-1 filter paper and extracted using soxhlet. After the extracts were transferred to a china dish and kept on a sand bath at the ideal temperature, they were concentrated by placing them on a boiling water bath. [10,11] The yields of the various extracts were recorded for future research and included ethanolic (3 gm), petroleum ether (2 gm), and aqueous (5 gm) extracts.

Phytochemical Screening

Biochemical tests were used to analyze the phytochemicals in a diluted 10 mg/10 ml mixture of petroleum ether, water, and ethanol extract. [12, 13, 14] Many phytochemicals, including carbohydrates, tannins, flavonoids, phenols, coumarins, terpenoids, triterpenoids, quinones, glycosides, cardiac glycosides, [15] saponins, [16] flavonoids, [17] alkaloids, and anthraquinones, were found using these techniques.

GC-MS analysis

Complex organic compounds, [16] drugs, unidentified samples, fire investigations, and environmental analysis are among the bioactive compounds in the ethanoic extract that can be identified using GC-MS spectroscopy. In an inert atmosphere, helium is pumped at 1 ml/min while sample is injected into the column at 10°C/min rate. For chromatographic conditions, an ionization voltage of 70eV and an ion mass range of 50–600 units were employed, along with a programmed oven temperature between 50 and 250°C. Following a comparison of the GC output results with the NIST database to determine the results of isolated compounds, the output results were then run through the NIST mass spectra library. The interpretation of mass spectroscopy and gas chromatography is displayed in the GC-MS final output.

Toxicity prediction of compounds isolated from Ajwain seeds

Herbal remedies have long been used to treat different illnesses since the beginning of human history. Since *Ajwain* seeds are a traditionally used medicinal plant, the effects of its extracts on lethality (LD50) and sub-acute toxicity were assessed. However, the safety of certain medicinal plants is unknown. Bicyclo is one of the isolates [3.1.0]. The following substances were examined for toxicity: hexan-2-ol, 2-methyl-5-(1-meth)-, 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, thymol, oleyl alcohol, trifluoroacetate, and phosphinoline, 1,2,3,4-tetrahydro-.

Molecular Docking Analysis (Auto Dock Vina)

By utilizing Auto Dock Tools (ADT) to conduct molecular docking studies in order to investigate the molecular targets and mechanisms of *Ajwain* in the treatment of gallbladder disorders. It is a free Auto Dock vina database graphic user interface (GUI) [19]. To dock compounds against the protein's active site, a standard protocol was employed in conjunction with autodocking. A central grid box of 14.527 Å, 56.689 Å, and -5.122 Å was created using 58, 58, and 40 points that directed x, y, and z directions. The grid point spacing was 0.375 Å. PyMOL and Auto Dock tools were used for post-docking studies. Create nine distinct conformations for every ligand in order to rank them according to their binding energies in the auto docking scoring functions. To analyze, pick the conformations with the lowest binding energy and the best prospects. Exploring the molecular targets and mechanisms of *Ajwain* in the treatment of gallbladder disorders based on molecular docking studies using Auto Dock Tools (ADT). It is a free graphic user interface (GUI) for Auto Dock vina database [19]. Autodocking was used with a standard protocol to dock compounds against the proteins active site. Grid box constructed using 58, 58, & 40 points, directing x, y, & z directions, with a grid point spacing 0.375 Å and a central grid box of 14.527 Å, 56.689 Å, & −5.122 Å. Post-docking studies were performed with Auto Dock tools and PyMOL. Autodocking scoring functions for each ligand produced nine different conformations, which were ranked according to their binding energies. Select the most favorable conformations and lowest binding energy to analyze interactions between target receptor & ligands by software (Discovery Studio Visualizer).

Ligands preparation

2D structures of all compounds were drawn using software (Chemdraw 16.0) and converted to 3D structures using software (Chem3D 16.0) for analysis, each molecule's three-dimensional coordinates when they are loaded into Chem3D to minimize energy use. Preparation of macromolecules: For docking, a protein target that was recovered from the RCSB Protein Data Bank (PDB ID: 6VYB) is used [19, 20]. Water molecules and all bound ligands were eliminated from the receptor's active site. String: Research on protein-protein interactions is crucial for finding mutant proteins. This aids in the disease's diagnosis.

String Server 11.5 was used to examine this data. [21].

Results and Discussion

Phytochemical screening of *Ajwain* seeds shown in Table 1. Ethanolic extract consist of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, phyto steroids, and phlobatannins. Petroleum ether extract consists of tannins, saponins, flavonoids, alkaloids, quinones, coumarins, phyto steroids, and phlobatannins. Aqueous extract consists of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, and coumarins. From this result it was found that seeds of *Ajwain* comprise enriched number of phytochemicals includes carbohydrates, tannins, saponins, flavonoids, cardiac-glycosides, terpenoids, triterpenoids, phenols, phyto-steroids & anthraquinones, that may be clinically reported against various diseases and disorders occurring in humans. Early reports say that *Ajwain* seeds composed chemical constituents such as carbohydrates, proteins, crude fat, crude fiber, glycosides, tannins, saponins and flavones [6]. Phytochemicals are biologically active, naturally occurring chemical compounds that provide health benefits to humans.[22]. This study motivated us to identify, isolate, characterize and investigate novel biological compounds from the ethanolic extract by GC-MS for *Ajwain* seeds efficacy.

Table 2 shows the list of bioactive compounds isolated and identified from the ethanolic extract of *Ajwain* seeds based on RT, molecular formula, MW, and concentration (%). Table 3 shows the clinical significance of the isolates. The isolated compounds were confirmed by comparison with a mass spectral library of NIST. Each compound has a unique character and is collected from PUBCHEM and PUBMED [23]. The most important applications of isolated compounds were emphasized here. The GC-MS chromatogram results in figure 1 and 2 show that 30 chemical components such as bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methyl (RT at 06.512), (+)-4-carene (RT at 07.175), benzene, 1-methyl-2-(1-methylether (RT at 07.335), β-phellandrene (RT at 07.406), γ-terpinene (RT at 07.912), bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methl) (RT at 09.069, 09.577, 09.710, 09.936), 3-cyclohexen-1-ol, 4-methyl-1-(1-methyl) (RT at 10.247), thymol (RT at 12.018), 2-methyl-5-(propan-2-ylidene) cyclohexane-1 (RT at 12.192), eugenol (RT at 12.719), copaene (RT at 12.953), caryophyllene (RT at 13.605), p-cymene-2,5-diol (RT at 13.997), tetradecanoic acid (RT at 17.690), 1-(7-methoxy-2H-1,3-benzodioxol-5-yl) propane (RT at 17.909), 2,6,10-trimethyl,14-ethylene-14-pentadecene (RT at 18.462), neophytadiene (RT at 18.934), 3-benzylsulfonyl-2,6,6-trimethylbicyclo (3.1.1) (RT at 19.354), *n*-hexadecanoic acid (RT at 19.972), hexadecanoic acid, ethyl ester (RT at 20.114), 9,12-octadecadienoic acid (Z, Z)-, methyl ester (RT at 21.156), 9-octadecenoic acid (z)-, methyl (RT at 21.206), 2-hexadecen-1-ol, 3,7,11,15-tetrame (RT at 21.364), trans, trans-9,12-octadecadienoic acid, propyl (RT at 21.832), 1-heptanol, 2,4-dimethyl-, (2s,4r) -(-) (RT at 21.893), oleyl alcohol, trifluoroacetate (RT at 22.031), phosphinoline, 1,2,3,4-tetrahydro- (RT at 22.537), octatriacontyl trifluoroacetate (RT at 23.209), oleyl alcohol, trifluoroacetate (RT at 23.351), 3-cyclopentylpropionic acid, 2-dimethylamino ester (RT at 24.449), and 9-octadecenoic acid (Z)-, 2,3-dihydroxypropy (RT at 26.872) show elevated peaks.

An earlier report says that *Ajwani* seeds are not only used in cookery but also in medicine, cosmetic, food and flavor industries [6]. They are effective against cough, wheezing, pleurisy and other respiratory problems, and extracts were used to treat infected eyes and ears drops. The spice was also used to treat tremors, palsy, paralysis, and various neurological disorders. The herb possesses strong carminative and stimulant properties. They are highly valued as a gastrointestinal medicine and as an antiseptic [8,9]. *Ajwain* seeds are still used to treat gastrointestinal disorders like vomiting, nausea, reflux, abdominal cramps, and loss of appetite. They were considered as an anthelmintic, aphrodisiac, galactagogue and diuretic agent [24]. *Ajwain* seeds are used in the food and flavor industry as bakery products and as a preservative in beverages, pan mixtures, confectionaries, pulses, and pickles. Aroma of *Ajwain* have found their place in the perfumery and cosmetic industry, oils of *Ajwain* are an essential ingredient in ointments and lotions [25, 26]. Thymoquinone exhibits anticancer activity by acting as a potent antimutagenic and immunomodulatory agent. It has also been reported to increase the activities of catalase, glutathione peroxidase and superoxide dismutase, which are beneficial against various types of cancer [27]. The above literature well justifies the potential of *Ajwain*. Hence this study prompted to identify the class of toxicity and the decrease in LD_{50} (mg/kg) value indicates the toxic status of the selected compounds.

The table 4 shows the results of toxicity studies of selected compounds identified from GC-MS such as Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-meth)-, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, Thymol, Oleyl alcohol, trifluoroacetate, and Phosphinoline, 1,2,3,4-tetrahydro- were found to be non-toxic. All the compounds showed a class level of 4 to 6, indicating that they were at a non-toxic condition. These compounds were further screened by Insilco analysis and their docking probes were inspected with SMAD4 protein of gallbladder cancer figure 3. The table 5 shows the results of binding affinity of selected isolates bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methyl)-, 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, thymol, oleyl alcohol, trifluoroacetate, and phosphinoline, 1,2,3,4-tetrahydro-with the SMAD4 protein of gallbladder cancer was observed with the interaction of GLY and GLY. The binding affinities were found to be (-2.0), (-2.3), (-3.8), (-1.6) and (-2.6) Kcal/mol. The figure 4 shows that SMAD4 significantly interacts with 10 different proteins such as SMAD2, SMAD3, SKIL, SKI, TGFBR1, CTNNB1, JUN, CEBPB, EP300, and FOXH1 networks. These results of protein-protein interaction studies suggest that when the compound interacts with SMAD4, it is also induced to interact with the other protein as a cascade process for biological changes.

Table 1: Phytochemical screening of *Ajwain* seeds.

S.No	Phytochemical	Eth-ext	Pet-ext	Aqu-ext
1.	Carbohydrates	+	-	+
2.	Tannins	+	+	+
3.	Saponins	+	+	+
4.	Flavonoids	+	+	+
5.	Alkaloids	+	+	+
6.	Quinones	+	+	+
7.	Glycosides	+	-	+
8.	Cardiac glycosides	+	-	+
9.	Terpenoids	+	-	+
10.	Triterpenoids	+	-	+
11.	Phenols	+	-	+
12.	Coumarins	+	+	+
13.	Phyto steroids	+	+	-
14.	Phlobatannins	+	+	-
15.	Anthraquinones	-	-	-

(+) = Presence, (-) = Absence, (Eth-ext) = Ethanolic extract, (Pet-ext) = Petroleum ether extract, (Aqu-ext) = Aqueous extract)

Table 2: RT, Area %, Height % and m.wt. of isolates from the alcoholic extract of *Ajwain* by GC-MS.

Isolates	R.Time	Area %	Height %	m.wt. g/mol	Molecular formula
Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene	06.512	00.22	0.66	136.23	$C_{10}H_{16}$
(+)-4-carene	07.175	00.17	0.39	136.23	$C_{10}H_{16}$
Benzene, 1-methyl-2-(1-methyl ethyl)	07.335	02.57	5.51	134.22	$C_{10}H_{14}$
β-phellandrene	07.406	00.51	0.62	136.23	$C_{10}H_{16}$
γ-terpinene	07.912	04.64	6.17	136.23	$C_{10}H_{16}$
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl)	09.069, 09.577, 09.710, 09.936	00.83, 00.08, 00.19, 00.08	0.47, 0.13, 0.13, 0.14	154.25	$C_{10}H_{18}O$
3-Cyclohexen-1-ol, 4-methyl-1-(1-methyl)	10.247	00.59	0.29	196.29	$C_{12}H_{20}O_2$
Thymol	12.018	23.76	8.56	150.22	$C_{10}H_{14}O$
2-Methyl-5-(propan-2-ylidene)cyclohexane-1,	12.192	00.59	0.98	170.25	$C_{10}H_{18}O_2$
Eugenol	12.719	00.98	1.70	164.20	$C_{10}H_{12}O_2$
Copaene	12.953	00.47	0.77	204.35	$C_{15}H_{24}$
Caryophyllene	13.605	00.40	0.78	204.35	$C_{15}H_{24}$
p-Cymene-2,5-diol	13.997	00.67	0.68	166.22	$C_{10}H_{14}O_2$
Tetradecanoic acid	17.690	00.37	0.55	228.37	$C_{14}H_{28}O_2$
1-(7-Methoxy-2H-1,3-benzodioxol-5-yl) propane	17.909	00.72	0.99	226.23	$C_{11}H_{14}O_5$
2,6,10-trimethyl,14-ethylene-14-pentadecene	18.462	01.18	1.89	278.30	$C_{20}H_{38}$
Neophytadiene	18.934	00.34	0.74	278.50	$C_{20}H_{38}$
3-Benzylsulfonyl-2,6,6-trimethylbicyclo (3.1.1)	19.354	00.36	0.68	292.43	$C_{17}H_{24}O_2S$
n-Hexadecanoic acid	19.972	04.04	3.36	256.42	$C_{16}H_{32}O_2$
Hexadecanoic acid, ethyl ester	20.114	02.48	4.27	284.48	$C_{18}H_{36}O_2$
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	21.156	00.50	0.93	294.47	$C_{19}H_{34}O_2$
9-Octadecenoic acid (z)-, methyl ester	21.206	00.57	1.39	296.48	$C_{19}H_{36}O_2$
2-hexadecen-1-ol, 3,7,11,15-tetra methyl	21.364	00.51	0.65	296.50	$C_{20}H_{40}O$
Trans,trans-9,12-Octadecadienoic acid, propyl ester	21.832	06.14	6.73	322.50	$C_{21}H_{38}O_2$
1-heptanol, 2,4-dimethyl-, (2s,4r)-(-)	21.893	02.49	6.53	144.26	$C_9H_{20}O$
Oleyl alcohol, trifluoroacetate	22.031	14.83	7.56	364.30	$C_{20}H_{35}F_3O_2$
Phosphinoline, 1,2,3,4-tetrahydro-	22.537	02.08	1.33	200.22	$C_{13}H_{13}P$
Octatriacontyl trifluoroacetate	23.209	00.68	0.94	647.00	$C_{40}H_{77}F_3O_2$
3-Cyclopentylpropionic acid, 2-dimethylamino ester	24.449	01.11	0.97	213.32	$C_{12}H_{23}NO_2$
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropy ester	26.872	02.57	2.73	356.53	$C_{21}H_{40}O_4$

Table 3: Clinical significance some of the imperative isolated and identified active principal compounds from the ethanolic extract of *Ajwain* by GC-MS.

Sno	Isolates	Clinical Significance
1	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene	Antioxidant, anti-inflammatory, antiseptic, anti-depressant, (targeted to treat arthritis and fibromyalgia), induce mental alertness, clarity, and overall cognitive functions [28].
2	(+)-4-carene	Cosmetics, pharmaceuticals and food preservation [29].
3	Benzene, 1-methyl-2-(1-methyl ethyl)	Flavoring agents [30].
4	β-phellandrene	Fragrances [31] and antioxidant activity [32].
5	γ-terpinene	Anti-inflammatory, antioxidant, hepatoprotective and antifungal activity [33].
6	Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methyl)	Larvicidal activity [34].
7	3-Cyclohexen-1-ol, 4-methyl-1-(1-methyl)	Larvicidal activity [34].
8	Thymol	Anticancer, [35] antioxidant,[35,36] antifungal, [36] antimicrobial activity [37].
9	2-Methyl-5-(propan-2-ylidene)cyclohexane-1,	Pesticidal activity [38].
10	Eugenol	Antioxidant and antiinflammatory activity [39].
11	Copaene	Flavor in food industry, and aromatherapy (cosmatice), and acts as an insect attractant, [40]. Antioxidant and cytotoxic activity [41].
12	Caryophyllene	Antibacterial activity, antifungal, cytotoxic activity against hepatoma cancer cells. [42].
13	p-Cymene-2,5-diol	Insecticide and pesticide [43,44].
14	Tetradecanoic acid	Antioxidant, lubricant, hypercholesterolemic, cancer-preventive, cosmetic.
15	1-(7-Methoxy-2H-1,3-benzodioxol-5-yl) propane	Insecticides [34].
16	2,6,10-trimethyl,14-ethylene-14-pentadecene	Antiproliferative activity [45].
17	Neophytadiene	Antipyretic, anti-inflammatory, antimicrobial and antioxidant activity [46].
18	3-Benzylsulfonyl-2,6,6-trimethylbicyclo(3.1.1)	Antioxident [47].
19	n-Hexadecanoic acid	Antioxident, pesticide, flavor, 5-α-eductaseinhibitor, antifibrinolytic, hemolytic, lubricant, nematicide, antialopecic, hypocholesterolemic, antiinflammatory, anti-bacterial [45].
20	Hexadecanoic acid, ethyl ester	Antioxidant, hemolytic, hypocholesterolemic, flavor, nematicide, anti-androgenic [48].
21	9,12-Octadecadienoic acid (Z,Z)-, methyl este	Anti-inflammatory and antiarthritic [49].
22	9-Octadecenoic acid (z)-, methyl ester	Antimicrobial activity and anticancer activity[50].
23	2-hexadecen-1-ol, 3,7,11,15-tetra methyl	Antimicrobial and anti-inflammatory activity [51].
24	Trans,trans-9,12-Octadecadienoic acid, propyl ester	Responsible for providing allelopathic effect [52].

25	1-heptanol, 2,4-dimethyl-, (2s,4r)-(-)	-
26	Oleyl alcohol, trifluoroacetate	Alcohol dehydrogenase inhibitor helps the digestive process [53].
27	Phosphinoline, 1,2,3,4-tetrahydro-	-
28	Octatriacontyl trifluoroacetate	Insecticide [54].
28	3-Cyclopentylpropionic acid, 2-dimethylamino ester	Analgesic, antiinflammatory activity [55].
30	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropy ester	Antimicrobial and anticancerous activity [56].

Table 4: The toxicity prediction of compounds in *Ajwain* using ProTox-II tools

Isolates	**Predicted LD_{50} (mg/Kg)**	**Predicted toxicity class**	**Water partition coefficient (logP)**	**Molecular refractivity**	**Toxicity**
Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methyl)-,	5000	6	54.62	0	Non-toxicity
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,	1016	4	48.80	20.23	Non-toxicity
Thymol	640	4	48.01	20.23	Non-toxicity
Oleyl alcohol, trifluoroacetate	1343	6	99.26	26.23	Non-toxicity
Phosphinoline, 1,2,3,4-tetrahydro-	6700	6	43.87	0	Non-toxicity

Table 5: Amino acid interaction between the *Ajwain* compounds and SMAD4 receptor (gall bladder tumour suppressor gene) and their binding affinity.

SNo	**Receptor**	**Ligand**	**Binding affinity (Kcal/mol)**	**Amino acid interaction**
1	SMAD4	Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methyl)-,	-2.0	GLY
2	SMAD4	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,	-2.3	GLY
3	SMAD4	Thymol,	-3.8	GLY and GLU
4	SMAD4	Oleyl alcohol, trifluoroacetate, and	-1.6	-
5	SMAD4	Phosphinoline, 1,2,3,4-tetrahydro-	-2.6	-

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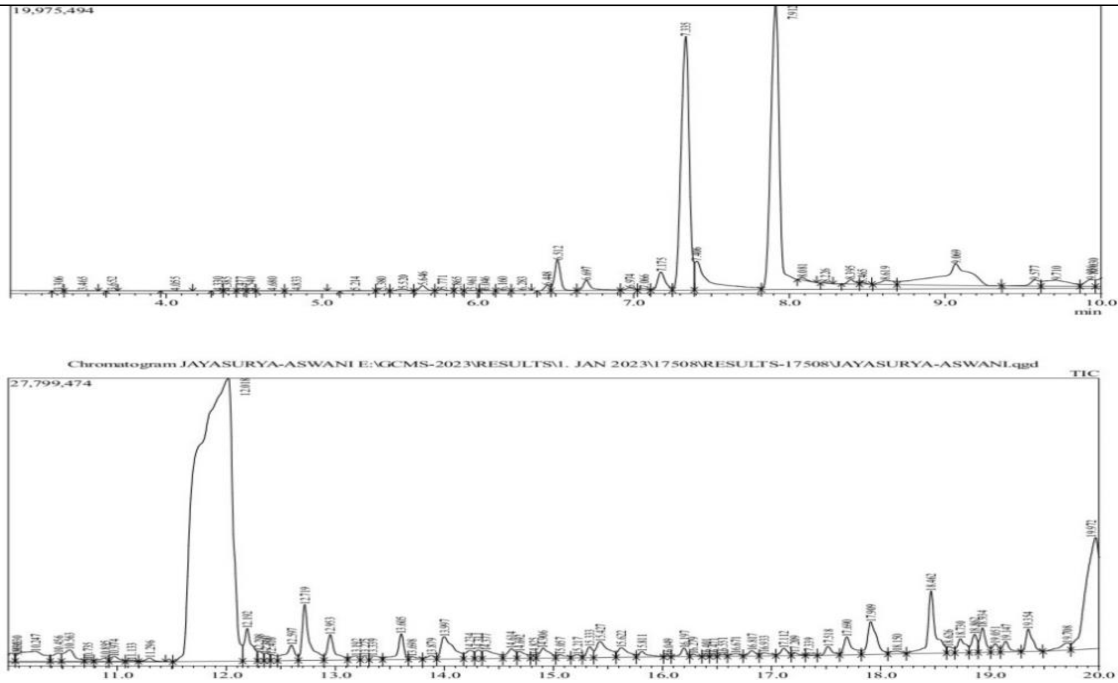

Figure 1

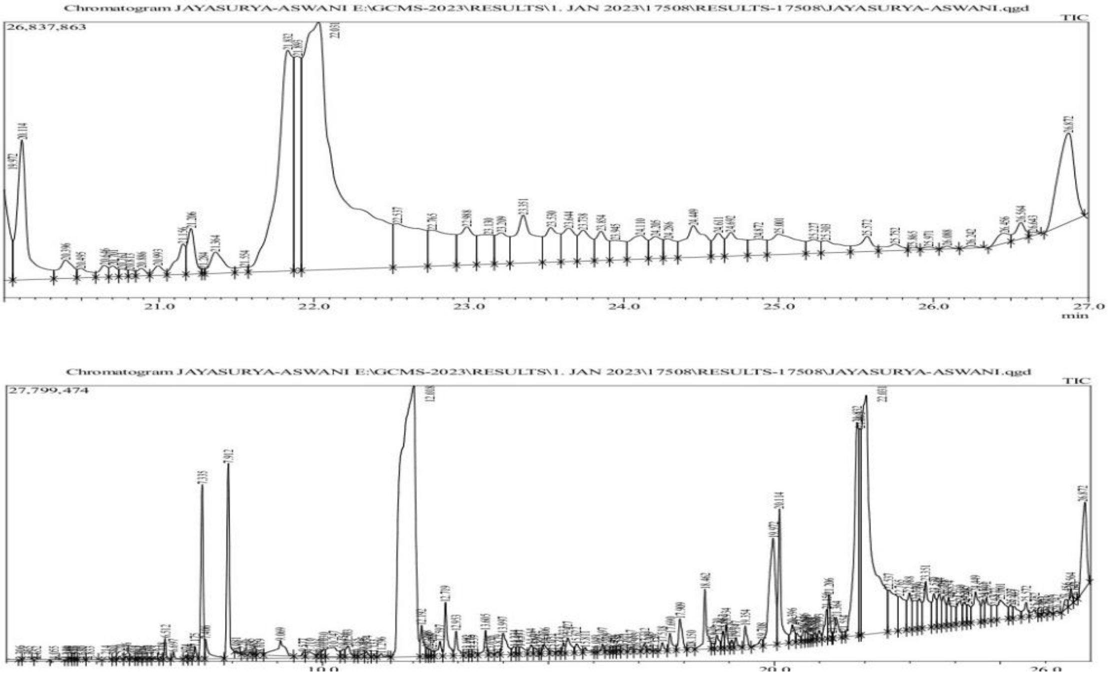

Figure 2

Figure 1 and 2, shows the individual and total ionic chromatogram (GC/MS) of ethanolic extracts from the seeds of *Ajwain* obtained by using Elite-1 fused silica (stationary phase) in a capillary column and He carrier gas (mobile phase) at 70eV.

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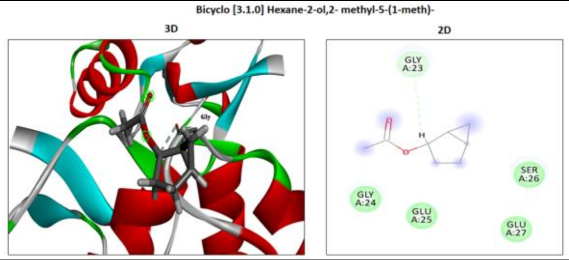

Figure 3(a). 3D and 2D structural interaction of bicyclo [3.1.0] hexane-2-ol,2-metyl-5-(1-methyl)- with SMAD4 protein of gall bladder cancer

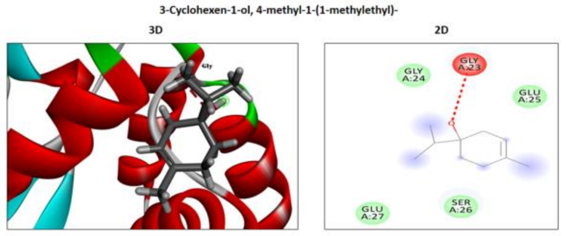

Figure 3(b). 3D and 2D structural interaction of 3-cyclohexen-1-ol, 4-methyl-1-(1-m with SMAD4 protein of gall bladder cancer.

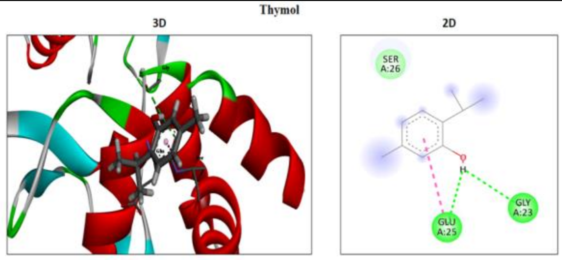

Figure 3(c). 3D and 2D structural interaction of thymol with SMAD4 protein of gall bladder cancer.

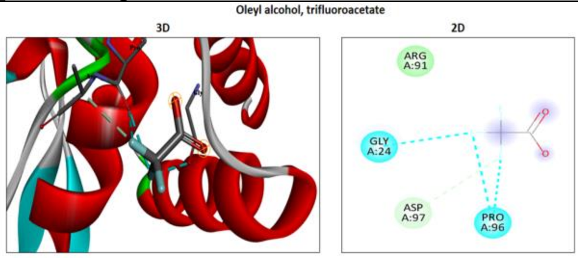

Figure 3(d). 3D and 2D structural interaction of oleyl alcohol, trifluoroacetate with SMAD4 protein of gall bladder cancer.

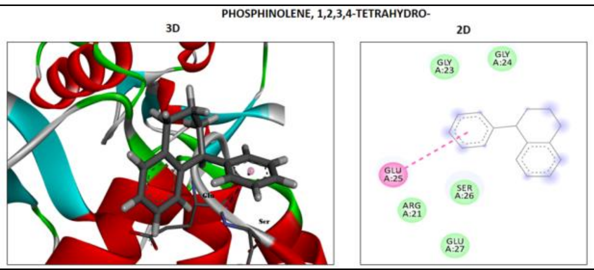

Figure 3(e). 3D and 2D structural interaction of phosphinoline, 1,2,3,4-tetrahydro- with SMAD4 protein of gall bladder cancer.

Figure 3: Molecular docking of components isolated from *Ajwain* against SMAD4 protein of gallbladder cancer

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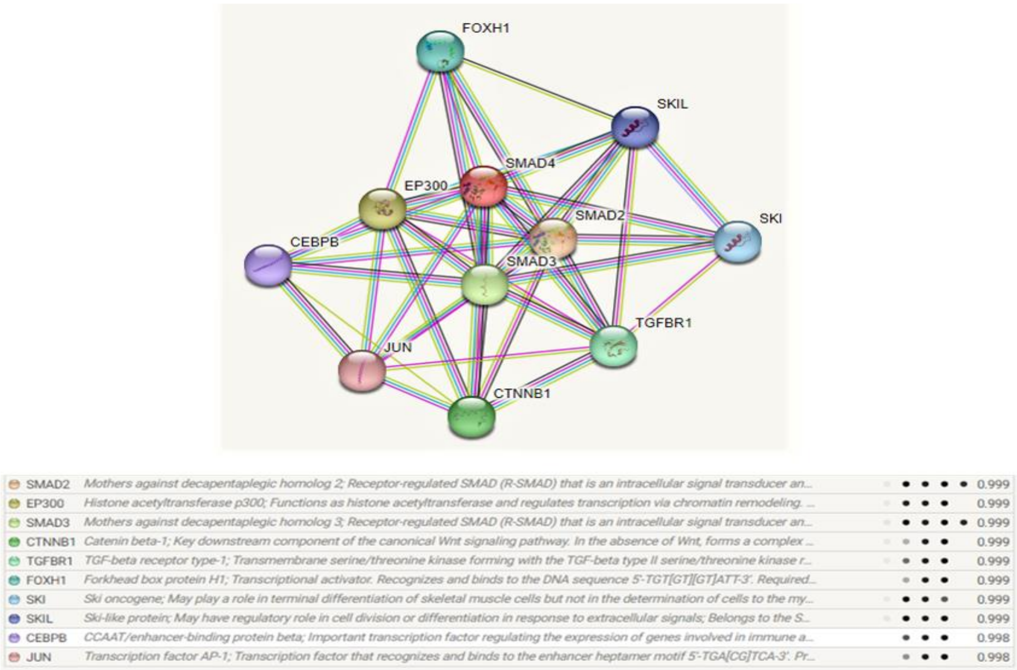

Figure 4: Interactions of SMAD4 with 10 different proteins.

Summary

In the present study, seeds of *Ajwain* have shown various secondary metabolites and plenty of active principle compounds. The GC-MS chromatogram show that 30 chemical components such as bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene, (+)-4-carene, benzene, 1-methyl-2-(1-methyl ethyl), β-phellandrene, γ-terpinene, bicyclo [3.1.0]hexan-2-ol, 2-methyl-5-(1-methl), 3-cyclohexen-1-ol, 4-methyl-1-(1-methyl), thymol, 2-methyl-5-(propan-2-ylidene)cyclohexane-1, eugenol, copaene, caryophyllene, p-cymene-2,5-diol, tetradecanoic acid, 1-(7-methoxy-2H-1,3-benzodioxol-5-yl)propane, 2,6,10-trimethyl,14-ethylene-14-pentadecene, neophytadiene, 3-benzylsulfonyl-2,6,6-trimethylbicyclo (3.1.1), *n*-hexadecanoic acid, hexadecanoic acid, ethyl ester, 9,12-octadecadienoic acid (Z,Z)-, methyl ester, 9-octadecenoic acid (z)-, methyl ester, 2-hexadecen-1-ol, 3,7,11,15-tetra methyl, trans,trans-9,12-octadecadienoic acid, propyl ester, 1-heptanol, 2,4-dimethyl-, (2s,4r)-(-), oleyl alcohol, trifluoroacetate, phosphinoline, 1,2,3,4-tetrahydro-, octatriacontyl trifluoroacetate, 3-cyclopentylpropionic acid, 2-dimethylamino ester, and 9-octadecenoic acid (Z)-, 2,3-dihydroxypropy ester, show elevated peaks. Toxicity studies of selected isolates include bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-meth)-, 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, thymol, oleyl alcohol, trifluoroacetate, and phosphinoline, 1,2,3,4-tetrahydro- were found to be non-toxic at LD_{50} (mg/kg). Furthermore, a computational approach for *Insilco* analysis to examine the docking the selected compounds with the SMAD4 protein of gallbladder cancer. Best binding affinity score (-2.0, -2.3, -3.8, -1.6, and -2.6 Kcal/mol) was observed for the selected compounds with the receptor protein. Protein-protein interaction studies shows that, SMAD4 protein significantly interacts with 10 different proteins such as SMAD2, SMAD3, SKIL, SKI, TGFBR1, CTNNB1, JUN, CEBPB, EP300, and FOXH1 as network. SMAD4 is induced to interact with the other proteins as a cascade process for biological changes. Hence *Ajwain* seeds may possess many pharmacological and therapeutic properties such as anti-inflammatory, antimicrobial activity, antioxidant, anticancer specifically for gallbladder.

Conclusion

In the present study, seeds of *Ajwain* have shown various secondary metabolites and plenty of active principle compounds. The GC-MS chromatogram shows that 30 chemical components show elevated peaks. Toxicity studies of selected compounds were found to be non-toxic at LD_{50} (mg/kg). Furthermore, Insilco docking analyzes the selected compounds with the SMAD4 protein of gallbladder cancer. Best binding affinity score was observed for the selected compounds with the receptor protein. Protein-protein interaction studies show that SMAD4 protein significantly interacts with 10 different proteins as a cascade process for biological changes. The results concluded that *Ajwain* seeds may have many pharmacological and therapeutic properties includes anti-inflammatory, antimicrobial activity, antioxidant, anticancer specifically for gallbladder disorders.

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