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Available online at: <http://www.iajps.com> Research Article**MOLECULAR DOCKING ANALYSIS OF BIOACTIVE
COMPOUNDS FROM AN ENDANGERED PLANT *MAPPIA
FOETIDA* AGAINST ANTI-CANCER PROTEIN 1G83.****Pooja. R*, and Y. L. Ramachandra**Department of Biotechnology & Bioinformatics,
Kuvempu University, Jnanasahyadri, Shankaraghatta,
Shivamogga Dist., Karnataka, India-577 451.**Article Received:** June 2020 **Accepted:** July 2020 **Published:** August 2020**Abstract:**

M. foetida belongs to the family Icacinaceae and it is used for the treatment of cancer disease. It contains many secondary metabolites such as alkaloids, phenols, tannins, flavonoids, amino acids and saponins. Thin layer chromatography has been used for secondary metabolite separation which shows the diversity in plant extracts. This proves that all secondary metabolites having medicinal properties. From GCMS analysis 70 compounds have been found, in which 7 compounds have followed Lipinski rule which has anticancer property verified through in silico analysis.

Keywords: TLC, Secondary metabolites, Phenolic compounds, GCMS (Gas Chromatography-Mass Spectrometry), *Mappia foetida*, Docking analysis.

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INTRODUCTION:

The important medicinal properties of *M. foetida* are chemically active which produce a physiological action on human body. Even though recent medicine has well developed, people used medicinal plants for their need and care. From ancient time, Ayurveda is said to be holistic, curable and health of life. Ayurveda is quite advanced and codified gadget of existence and fitness science is based on its very own particular and unique ideas and essential ideas [1]. These traditional methods still follow in basic needs in some countries [2]. Medicinal properties of *M. foetida* are determined in chemical substances that produce a particular physiological movement on the human frame [3].

Plant based pills offer exquisite contribution to trendy therapeutics; for example, for the treatment of neck cancer, leukaemia in children, non Hodgkins lymphomas and Hodgkins; *Catharanthus rosesus* [4] has been used, in which Vinblastine is isolated. During childhood, Vincristine is recommended for heavy lymphocytic leukaemia, Hodgkins, cervical and breast cancer and lymphosarcoma [5].

M. foetida is also known as *Nothapodytes foetida* in earlier days and it is used in the treatment of cancer diseases. Plant drugs which are used to cure skin diseases, mental illness, cancer, hypertension, jaundice, diabetes and tuberculosis. Many medicinal plants play an important role for the development of therapeutic agents. Production of plant drugs are used in modern medicines for cureness.

Common names of *M. foetida* are Durvasanemara, Ghanera, Kalagaura, Kalgur. This plant is found in

some parts of the Western Ghats of South Maharashtra, Central and South India.

M. foetida, 3-8m tall, small tree, wrinkled bark, thickness about 5mm, smooth grey. Branches are corky, leaf scars are prominent and they are slightly angled [6]. Leaves are arranged alternatively, egg shaped broadly, long 1-25 cm, wide 4-12cm. Sometimes leaf base is unequal. Flowers are creamy yellow, about 5 mm across, bisexual, foul smelling. Petals are inside hairy. About 2 x 1 cm, purplish black, oblong to ellipsoid fruits are present. They are ripe when they are purplish black [7].

Camptothecin is present in *M. foetida* plant and it is mainly used for cancer disease. Alkaloids which are present inside are said to be effective as anticancer drug in 21st century [8]. The cellular goal of camptothecin is DNA topoisomerase I and numerous analogues have been synthesized as ability healing agents. Cellular goal of CPT, DNA topoisomerase I and some of analogues synthesized as ability healing agents [9]. It inhibits the *in vitro* replication of HIV and it effects in remission of breast, uterine and lung cancer [10,11,12]. Some water soluble substances of CPT i.e Topotecan and irinotecan are used in the treatment of ovarian and colorectal cancer and also CPT has been discovered in Chinese deciduous tree, *Camptotheca acuminata* [13,14,15]. The cytotoxic effect of Camptothecin on *Plasmodium falciparum* proves to be an interesting goal for new drug improvement in antimalarial activity [16].

MATERIALS AND METHODS:

M. foetida plant collected from Western Ghats of Mookambika Wildlife Sanctuary, Shivamogga district, Karnataka, India.

**Study Site:**

The study area is situated at Mookambika Wildlife Sanctuary of Western Ghats, in Shivamogga district of Karnataka. It lies between 13° 42' and 13° 59' north range and 74° 39' to 74° 50' east longitude. The sanctuary consists of 274 km² (106 sq. miles) notified during the year 1974, by the Government of Karnataka vide notification AFD.48.FWL.74 dated 17 June 1974 [17].

Surface sterilization of plant material:

Freshly collected plant material is washed thoroughly under running tap water followed by sterile distilled water to remove the adhered debris. Stem and leaves were surface sterilized under aseptic condition in sequential steps by immersing in mercuric chloride (1mg/1ml) for 10 min and 70 % ethanol for another min followed by washing finally with distilled water.

Implants Inoculation:

Once the sterilization done, plant parts i.e leaves and twigs were cut into very small pieces approximately $0.5 \times 0.5 \text{ cm}^2$ and kept on PDA (Potato Dextrose Agar) around 5-6 pieces and kept for incubation for the growth of endophytes.

Identification and Mass production of Endophytic fungi:

Endophytic fungi have been identified using slides from fungi cultured and were stained with Lacto phenol cotton blue stain and observed under phase contrast and bright field microscope. Identification is done, based on morphological characteristics i.e surface texture, margin character, aerial mycelium, mechanism of spore production, growth pattern using standard manuals [18]. The identified fungi were sub cultured in PDA slants for further use and stored in refrigerated conditions. Endophytic fungi were cultured on broth (PDB- Potato Dextrose Broth) for mass cultivation. The inoculated flasks were incubated at room temperature ($26 \pm 2^\circ\text{C}$) for 8-15 days and allowed to grow the fungal mats.

Qualitative analysis by thin layer chromatography:

TLC studies were carried out to select the solvent system capable of showing better resolution and different solvent systems of different polarities were prepared.

Capillary tubes were used while applying plant extracts on pre-coated TLC plates using suitable mobile phase. After development the plates were air dried and also observed using UV at 254nm. Values were determined using retention factor and calculated [19].

$$R_f = \frac{\text{distance travelled by solute front}}{\text{distance travelled by the solvent front}}$$

Where; R_f -Retention factor

Quantitative analysis by extraction method:

Phytochemicals of *M. foetida* were determined and quantified by standard procedures [20,21,22].

Steroids: 1ml of fungal crude extract from the endophytes is dissolved using 1ml of chloroform and 2-3ml of acetic anhydride. 1-2 drops of

concentrated sulphuric acid is added. Upper layer turns red and concentrated sulphuric acid layer shows yellow with green fluorescence. Therefore, it indicates presence of steroids [23].

Terpenoids: 2ml of fungal crude extract from the endophytes + 2ml of chloroform is added. 3ml of concentrated sulphuric acid is added to form a layer. If reddish brown colour appears, it indicates presence of terpenoids [24].

Tannins: Ferric chloride test: Fungal crude extract from the endophytes is mixed with 1% FeCl_3 solution which produces green or brownish green, blue colour [25].

Saponins: 1ml of fungal crude extract from the endophytes is mixed using 20ml distilled water and kept on stirring for 15min. If foam forms, indicates the presence of saponins [26].

Alkaloids: The extraction is made with ethanol by solvent distillation process. Then the extract is purified by various methods. For qualitative detection crude extract is used [27].

Wagner's Test:

For 3ml of fungal crude extract from the endophytes, 1ml of Wagner's solution is added.

Flavonoids: Plant extract of 2ml is added to the test tube containing 1ml of dilute ammonia and mixed well with 1ml of concentrated sulphuric acid. Formation of yellow colour indicates presence of flavonoids [28].

Triterpenoids- Salkowski test: 2ml of fungal crude extract from the endophytes is mixed with 5 drops of conc. Sulphuric acid. If greenish blue color appears, it indicates the presence of triterpenoids [28].

Carbohydrate- Benedict's test: Benedict's reagent is mixed with 2ml of fungal crude extract from the endophytes and kept for boiling in water bath and observed for reddish brown precipitate which indicates the presence of carbohydrates [28].

Molecular Docking:**Preparation of Protein:**

The 3 dimensional structure of ALOX5 (Arachidonate 5-Lipoxygenase) is PDB ID: 1G83 (Protein coding gene) is downloaded using website <https://www.rcsb.org/structure/1G83>. All the docking stages and calculations were done via AutoDock Vina.

Preparation of ligand:

Based on GCMS analysis 70 compounds have been identified, in which the seven compounds viz. oxime-, methoxy phenyl; 4H-1,2,4-Triazol-3-

amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide; Carbamic acid, N-methyl, t-butyl ester; 2-Bromo-4-chloroaniline; Carbamimidothioic acid, 1-methylethyl ester and Picolinic acid, 4-chloro-, hydrazide was selected. The SDF files of these compounds were obtained from Pubchem database and converted to PDB format using OPEN BABEL tools.

Ligand- Protein docking:

Ligand-Protein docking was executed in AutodockVina^[29], that's an interactive molecular graphics application for calculating, displaying the viable docking modes of protein and ligand pairs provided in a hierarchy based on which binding affinities.

Properties of Lead-likeness:

SWISS ADME, free tool which is used to generate medicinal properties of drug likeness and physicochemical of all these 7 compounds. Lipinski's rule^[30,31] which is also called as rule of 5 (RO5) to determine whether the chemical compound along with biological or pharmacological activities.

Toxicity:

The toxicity of the compounds was detected with admetSAR, a free online web server. This server provides the possible toxicity profile of the compounds with the values suggesting the safety.

RESULTS:

Analysis of Thin Layer Chromatography

TLC plate developed at 365 nm was analysed under UV trans-illuminator. A total of eight samples along with standard CPT (20 µl) were loaded on TLC plate as line spots from Lane 1-9. The results

confirm the presence of CPT in all test samples except seventh sample of suspension culture (exogenous type) without cells.

Calculations:

Sample 1 (standard CPT) = $3.5/4.2 = 0.83$

Sample 2 (I MFT-C) = $3.7/4.2 = 0.88$

Sample 3 (I MFT-F) = $3.6/4.2 = 0.85$

Sample 4 (I MFL-Sf) = $3.8/4.2 = 0.90$

Sample 5 (II MFT-C) = $3.2/4.2 = 0.76$

Sample 6 (II MFT-F) = $3.6/4.2 = 0.85$

Sample 7 (II MFT-A) = $3.9/4.2 = 0.92$

Sample 8 (II MFL-F) = $3.0/4.2 = 0.71$

Sample 9 (II MFL-A) = $3.6/4.2 = 0.85$

Endophytic fungal screening of secondary metabolites

Each extract of fungal species of ethanol was analysed for the presence of different secondary metabolites. Qualitative endophytic fungal screening of secondary metabolites of leaf and twig extract of *M. foetida* exhibited distinguishable results by showing the presence of many significant secondary metabolites. The ethanol extract of eight endophytic fungal species yielded the different secondary metabolites, Phenolic compounds, flavonoids, alkaloids, tannins, amino acids, cardiac glycosides and terpenoids were the prominent secondary metabolites were present in all the endophytic fungal species at different levels which has been shown in Table 1.

Table 1: Screening of secondary metabolites of ethanolic extracts of *M. foetida*.

Secondary metabolites	I Season			II Season				
	MFT-C	MFT-F	MFL-Sf	MFT-C	MFT-F	MFT-A	MFL-F	MFL-A
Alkaloids	+	+	+	+	+	+	+	+
Flavonoids	+	+		+	+	+	-	-
Saponins	-	-	+	-	+	-	-	+
Tannins	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Triterpenoids	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	+	-	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+
Carbohydrates	-	-	-	-	-	-	-	-

'+' = Present; '-' = Absent

Where;

MFT- C ➡ *M. foetida* twig *Cladosporium* sp.

MFT - F ➡ *M. foetida* twig *Fusarium* sp.

MFL- Sf ➡ *M. foetida* leaf *Saprophytic fungi*

MFT -A ➡ *M. foetida* twig *Alternaria alternata*.

Analysis of GCMS Studies

M. foetida ethanolic extract of leaf of *Saprophytic fungi* showed the presence of seventy bioactive compounds which have medicinal properties. The identification of compounds are confirmed based on the molecular formula, molecular weight, CAS # and ENTRY # were presented in Figure 1 and Table 2. The chromatogram revealed the presence of 70 compounds in polyherbal preparation. 4H-1,2,4-Triazol-3-amine-4-methyl, Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide; Oxime-, methoxy phenyl; 2-Bromo-4-chloroaniline; Carbamic acid, N-methyl, t-butyl ester; Picolinic acid, 4-chloro-, hydrazide and Carbamimidothioic acid, 1-methylethyl ester were selected for further study.

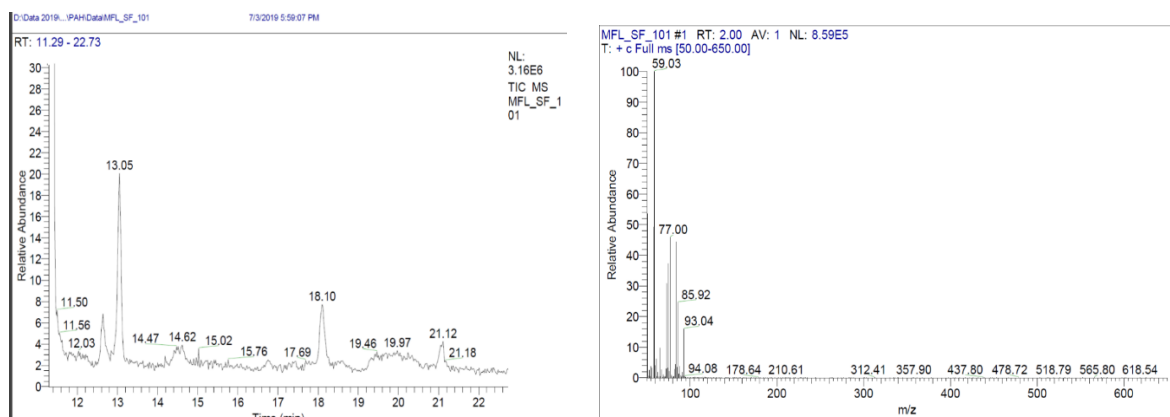


Figure 1: Chromatogram of a polyherbal preparation.

Table 2: GC-MS Analysis of ethanolic extract from *M. foetida* leaf obtained from *Saprophytic fungi* present

Compound No	LIGANDS	Formula	M.W	CAS#	ENTRY#
1	Tri-ruthenium dodecarbonyl	$C_{12}O_{12}Ru_3$	642	15243-33-1	133
2	Benzonitrile, 4-[(methoxyimino)methyl]-	$C_9H_8N_2O$	160	33499-34-2	119110
3	1,4,7,10,13-Pentaoxa-16-thiacyclooctadecane-3,14,17-trione	$C_{12}H_{18}O_8S$	322	79687-38-0	48066
4	carbon monoxide;2,5-di {tert}-butylcyclopenta-1,3-diene;1,3-di {tert}-butylcyclopentane;molybdenum	$C_{30}H_{42}Mo_2O_4$	662	137680-72-9	24443
5	Pregna-3,5-dien-20-one, 3-hydroxy-, heptafluorobutyrate	$C_{25}H_{29}F_7O_3$	510	18072-21-4	190674
6	Fe(III)Mesoporphyrin 1Xdimethyl ester chloride	$C_{36}H_{40}ClFeN_4O_4$	683	14126-91-1	191276
7	Manganese,tetracarbonyl(ü2-methylmethylenesulfonium)-	$C_6H_5MnO_4S$	228	55268-45-6	27530
8	Oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	151	NA	95096
9	4H-1,2,4-Triazol-3-amine, 4-methyl-	$C_3H_6N_4$	98	16681-76-8	60564
10	trans-(2-Chlorovinyl)methyldiethoxysilane	$C_7H_{15}ClO_2Si$	194	NA	132671
11	Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl]hydrazide	$C_{16}H_{18}N_2O_2$	270	NA	142154
12	9-Acetoxy-1-propyl-3,6-diazahomoadamantane	$C_{14}H_{24}N_2O_2$	252	138223-34-4	34699
13	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	$C_{27}H_{44}O_3$	416	40013-87-4	6295
14	10-Octadecenal	$C_{18}H_{34}O$	266	56554-92-8,	22062

15	2-(Chloromethyl)tetrahydropyran	C ₆ H ₁₁ ClO	134	18420-41-2	46667
16	Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	C ₂₈ H ₄₆ O ₄	446	56599-88-3	66084
17	Acetamide, 2-[[3-cyano-4-(methoxymethyl)-6-methyl-2-pyridinyl]thio]-N,N-di(2-propenyl)-	C ₁₇ H ₂₁ N ₃ O ₂ S	331	NA	1946
18	2-Benzoyl-6-nitrobenzoic acid, methyl ester	C ₁₅ H ₁₁ NO ₅	285	7531-38-6	67580
19	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144	10230-62-3	9854
20	1-Pentene,4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-	C ₂₂ H ₃₀ OSi	338	NA	173826
21	Chloromethyl propanoate	C ₄ H ₇ ClO ₂	122	5402-53-9	22244
22	1-(3-Hydroxy-4-methylphenyl)-1,3,3,6-tetramethylindan-5-ol	C ₂₀ H ₂₄ O ₂	296	107535-11-5	173998
23	Butanal, 2-methyl-	C ₅ H ₁₀ O	86	96-17-3	1892
24	1,3-Dioxolane, 2-(2,4-dimethylphenyl)-2,4,5-trimethyl-, (2S,4S,5S)-	C ₁₄ H ₂₀ O ₂	220	74752-98-0,	147864
25	Diborane(6), æ-mercapto-	B ₂ H ₆ S	60	22548-43-2,	22383
26	N-Thiobutyro-morpholine	C ₈ H ₁₅ NOS	173	5309-97-7	23222
27	1,4-Butanediol, dinitrate	C ₈ H ₈ N ₂ O ₆	180	3457-91-8,	16260
28	Carbamic acid, N-methyl, t-butyl ester	C ₆ H ₁₃ No ₂	131	160066-84-5	22565
29	4-Propylacridine	C ₁₆ H ₁₅ N	221	65753-74-4,	148639
30	Nitric acid, propyl ester	C ₃ H ₇ No ₃	105	627-13-4,	16195
31	2-Bromo-4-chloroaniline	C ₆ H ₅ BrClN	205	873-38-1	149089
32	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	C ₃₉ H ₈₀ O ₂	580	17367-38-3	173819
33	Cyclohexanecarboxylic acid, 2-phenylethyl ester	C ₁₅ H ₂₀ O ₂	232	37139-88-1	65371
34	Chloromethyl 3-chlorononanoate	C ₁₀ H ₁₈ Cl ₂ O ₂	240	80418-72-0,	17655
35	trans-1-Chloropropene	C ₃ H ₅ Cl	76	16136-85-9	2016
36	Propiophenone, 3-phenyl-3-piperidino-	C ₂₀ H ₂₃ NO	293	5350-97-0	46114
37	Cystine	C ₆ H ₁₂ N ₂ O ₄ S ₂	240	56-89-3,	13766
38	3-Dimethylamino-2-(4-chlorophenyl)-thioacrylamide	C ₁₁ H ₁₃ ClN ₂ S	240	NA	148826
39	Carbamimidothioic acid, 1-methylethyl ester	C ₄ H ₁₀ N ₂ S	118	NA	5845
40	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16S,19E)-/Pleiocarpamine	C ₂₀ H ₂₂ N ₂ O ₂	332	6393-66-4	46184
41	Picolinic acid, 4-chloro-, hydrazide	C ₆ H ₆ ClN ₃ O	171	73771-11-6,	75679
42	2-propionylpyridine 4-[4-carbethoxyphenyl]-3-thiosemicarbazone	C ₁₈ H ₂₀ N ₄ O ₂ S	356	70618-79-0,	41459
43	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	C ₁₃ H ₂₂ N ₂ O	222	16454-78-7	148902
44	Arsine	AsH ₃	78	7784-42-	39679

				1	
45	5-(3-Ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl-imidazolidine-2,4-dione	C ₉ H ₁₃ N ₃ O ₄	227	NA	77018
46	Amphibine C	C ₃₆ H ₄₉ N ₅ O ₅	631	38496-01-4	77220
47	Permethyl 4"-O-glucosylisovitexin	C ₃₇ H ₅₀ O ₁₅	734	67549-79-5,	185237
48	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16S,19E)-	C ₂₀ H ₂₂ N ₂ O ₂	322	6393-66-4	46184
49	3(2H)-Benzofuranone, 6-methoxy-2-(phenylmethylene)-	C ₁₆ H ₁₂ O ₃	252	4940-52-7	165699
50	Strychane, 1-acetyl-20-hydroxy-16-methylene-	C ₂₁ H ₂₆ N ₂ O ₂	338	2111-98-0	32366
51	1-Methyl-2-phenylbenzimidazole	C ₁₄ H ₁₂ N ₂	208	2622-63-1,	149119
52	Pentanoic acid, 2-chlorophenyl ester	C ₁₁ H ₁₃ ClO ₂	212	104316-33-8,	2309
53	Butanamide, 2-(1-methyltetrazol-5-ylthio)-3-oxo-N,N-dimethyl-	C ₈ H ₁₃ N ₅ O ₂ S	243	327990-50-1,	34046
54	2-Imidazolidinone	C ₃ H ₆ N ₂ O	86	120-93-4	47439
55	Mucic acid	C ₆ H ₁₀ O ₈	210	526-99-8	39603
56	Acetic acid, 2-(2-furoylhydrazono)-	C ₇ H ₆ N ₂ O ₄	182	139397-87-8	57862
57	l-Alanine, n-propargyloxycarbonyl-, isobutyl ester	C ₁₁ H ₁₇ NO ₄	227	NA	89037
58	Oxalic acid, allyl butyl ester	C ₉ H ₁₄ O ₄	186	NA	1887
59	Propanoic acid, anhydride	C ₆ H ₁₀ O ₃	130	123-62-6	20841
60	Methanesulfonic acid, trifluoro-, [(4-nitrophenyl)sulfonyl]methyl ester	C ₈ H ₆ F ₃ NO ₇ S ₂	349	56177-36-7,	31263
61	Glycine, N-methyl-N-methoxycarbonyl-, nonyl ester	C ₁₄ H ₂₇ NO ₄	273	NA	64487
62	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144	28564-83-2	6348
63	2,6-Piperazinedione, 4-benzoyl-, 2,6-bis(O-benzoyloxime)	C ₂₅ H ₂₀ N ₄ O ₅	456	35975-31-6,	66358
64	s-Triazole, 3,5-dichloro-	C ₂ HCl ₂ N ₃	137	10327-87-4,	99706
65	Pyrazolo[1,5-a]pyridine, 3-methyl-2-phenyl-	C ₁₄ H ₁₂ N ₂	208	17408-32-1,	148907
66	(-)-Myrtenol, tert-butyldimethylsilyl ether	C ₁₆ H ₃₀ OSi	266	NA	39395
67	4-Phthalimidobenzoic acid	C ₁₅ H ₉ NO ₄	267	5383-82-4,	170314
68	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	C ₁₆ H ₃₀ O ₄ Si ₃	370	3618-20-0,	185198
69	Flavone, 3-hydroxy-4',5,7-trimethoxy-	C ₁₈ H ₁₆ O ₆	328	1098-92-6	174293
70	Benzonitrile, 4-[(methoxyimino)methyl]-	C ₉ H ₈ N ₂ O	194	NA	132671

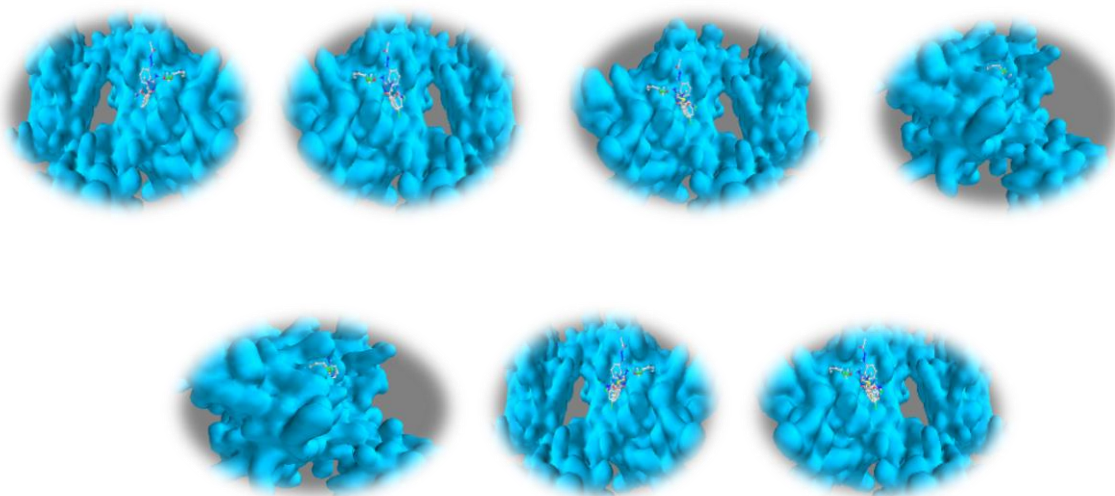
Molecular Docking:

Table 3: Results of docking performed with a software iGEMDOCK and Autodock Vina between the drug targets with ligands and Arachidonate 5-lipoxygenase (PDB ID: 1G83)

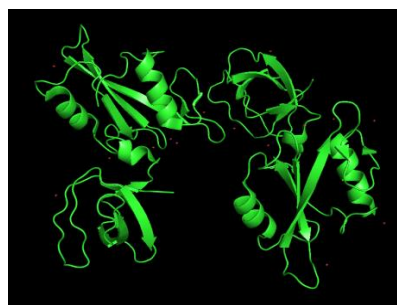
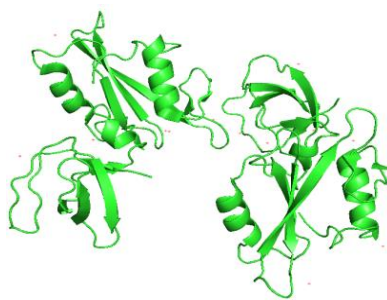
Sl.No	Ligand	Binding Affinity	rmsd/ub	rmsd/lb
1	1g83_606396_uff_E=341.05	-6.9	0	0
2	1g83_72711_uff_E=75.66	-5.6	0	0
3	1g83_119838_uff_E=113.32	-5.5	0	0
4	1g83_70110_uff_E=56.18	-4	0	0
5	1g83_290276_uff_E=262.21	-3.9	0	0
6	1g83_527635_uff_E=62.11	-3.9	0	0
7	1g83_445319_uff_E=44.26	-3.3	0	0

The binding affinity values obtained by Autodock Vina oxime-, methoxy phenyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide; Carbamic acid, N-methyl, t-butyl ester; 2-Bromo-4-chloroaniline; Carbamimidothioic acid,1-methylethyl ester; Picolinic acid, 4-chloro-, hydrazide and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- respectively.

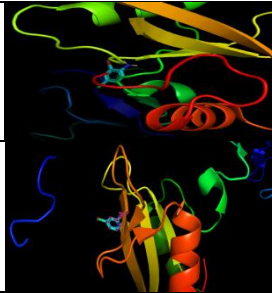
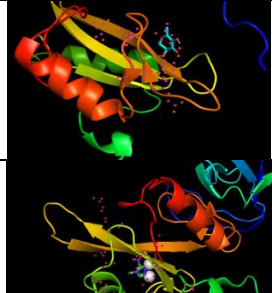
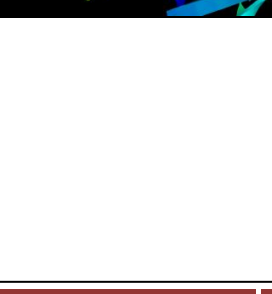
The target proteins, namely ALOX5 (Arachidonate 5-Lipoxygenase) is a Protein Coding gene were docked with oxime-, methoxy phenyl; 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide; Carbamic acid, N-methyl, t-butyl ester; 2-Bromo-4-chloroaniline; Carbamimidothioic acid,1-methylethyl ester and Picolinic acid, 4-chloro-, hydrazide by iGEMDOCK and Autodock Vina. The energy values and the binding affinities are presented in Table 3. The energy values obtained by iGEMDOCK of the drug targets of oxime-, methoxy phenyl; 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide; Carbamic acid, N-methyl, t-butyl ester; 2-Bromo-4-chloroaniline; Picolinic acid, 4-chloro-, hydrazide and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- were -6.9, -5.6, -5.5, -4, -3.9, -3.9, -3.3 Kcal/mol, respectively.

**Figure 2:** Docking of Arachidonate 5-lipoxygenase with 7 different compounds

The above figures display the docking stages of various compounds with their protein drug targets. The docking stages were analysed, and the amino acid residues involved in the various interactions were evaluated.



Protein and ligand docking Visualization by Pymol

SL.NO	LIGANDS	Protein-1G83
1	Oxime-, methoxy-phenyl- _	
2	4H-1,2,4-Triazol-3-amine, 4-methyl-	
3	Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl]hydrazide	
4	Carbamic acid, N-methyl, t-butyl ester	
5	2-Bromo-4-chloroaniline	
6	Picolinic acid, 4-chloro-, hydrazide	
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	

Drug likeness and other properties**Table 4:** General properties of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide

Molecules	Name of the Ligand/compound	Chemical formula	SMILES	IUPAC Name
1	4H-1,2,4-Triazol-3-amine-4-methyl	C ₃ H ₆ N ₄	CN1C=NN=C1N	4-methyl-1,2,4-triazol-3-amine
2	Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl]hydrazide	C ₁₆ H ₁₈ N ₂ O ₂	CC(=O)NNC1=CC=CC(=C1)C(C)(C2=CC=CC=C2)O	N'-[3-(1-hydroxy-1-phenylethyl)phenyl]acetohydrazide
3	Picolinic acid, 4-chloro-, hydrazide	C ₆ H ₄ ClN ₃ O ₂	C1=CN=C(C=C1Cl)C(=O)NN	4-chloropyridine-2-carbohydrazide

SMILES: Simplified Molecular Input Line Entry Specification,

IUPAC: International Union of Pure and Applied Chemistry.

Table 5: Physicochemical properties of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide.

Molecules	Molecular Weight (g/mol)	No. heavy atoms	No. arom. heavy atoms	Fracti on CSP3	No. rotatable bonds	No. H-bond acceptors	No. H-bond donors	Molar refractivity	TPSA (°A ²)
1	98.11	7	5	0.33	0	2	1	25.69	56.73
2	270.33	20	12	0.19	5	2	3	78.86	61.36
3	171.58	11	6	0	2	3	2	40.14	68.01

TPSA: Topological Polar Surface Area

Table 6: Lipophilicity and hydrophilicity of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide

Lipophilicity		Hydrophilicity											
Molecules	Consensus Log P	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class	Ali Log S	Ali Solubility (mg/ml)	Ali Solubility (mol/l)	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility (mg/ml)	Silicos-IT Solubility (mol/l)	Silicos-IT class
1	-0.57	-0.4	3.93E+01	4.01E-01	Very soluble	0.21	1.60E+02	1.63E+00	Highly soluble	0.02	1.02E+02	1.04E+00	Soluble
2	2.3	-3.15	1.92E-01	7.11E-04	soluble	-3.34	1.23E-01	4.56E-04	Soluble	-5.21	1.65E-03	6.10E-06	Moderately soluble
3	0.51	-1.39	7.00E+00	4.08E-02	Very soluble	-1.33	7.98E+00	4.65E-02	Very soluble	-2.28	9.07E-01	5.28E-03	Soluble

o/w: octanol/water

Table 7: Pharmacokinetics properties of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide.

Molec	GI	BBB	Pgp	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3	Log Kp
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ules	absorption	permeant	substrate	inhibitor	inhibitor	inhibitor	inhibitor	A4 inhibi tor	(cm/s)
1	High	No	No	No	No	No	No	No	-7.55
2	High	Yes	No	No	No	No	Yes	No	-6.24
3	High	No	No	No	No	No	No	No	-7.11

GI absorption: Gastrointestinal absorption, BBB: Blood Brain Barrier, CYP: cytochrome P

Table 8: Druglikeness and leadlikeness of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide

Molecules	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score	PAINS #alerts	Brenk #alerts	Leadlikeness #violations	Synthetic Accessibility
1	0	4	0	0	2	0.55	0	0	1	1.54
2	0	0	0	0	0	0.55	0	0	0	2.9
3	0	1	0	0	1	0.55	0	2	1	1.56

Table 9: Tabulates the toxicity profile of the compounds of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide, which were non-toxic in hERG, AMES toxicity, acute oral toxicity, and Human oral bioavailability.

Name of ligand	<i>hERG</i> inhi bition	AMES toxicity	Carcinogenicity(C lass III)	Acute oral toxicity (kg/mol)	Human-oral bio availability
4H-1,2,4-Triazol-3-amine-4-methyl	0.7348	0.6000	0.6995	1.04	0.9286
Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl] hydrazide	0.6215	0.5600	0.4687	2.734	0.6286
Picolinic acid, 4-chloro-, hydrazide	0.7422	0.7500	0.5285	1.703	0.6571

hERG: human Ether-a-go-go related gene.

From the table 5 physicochemical properties shows the number of atoms, molecular weight, fraction CSP3, topological polar surface area and number of rotatable bonds, molar refractivity. 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide; Picolinic acid, 4-chloro-, hydrazide representing their importance is good oral bioavailability properties.

From the table 6 Lipophilicity and hydrophilicity demonstrates the octanol-water partition coefficient values of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide. As indicated in this table, these values were within the permissible range of -0.4 to +5.6, implying a good lipophilic compound. 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide compounds were mostly soluble in aqueous medium as the log S was less than -4.0.

From the table 7 the pharmacokinetic properties of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide. According to the results, the oral bioavailability was high for 4H-1,2,4-Triazol-3-amine-4-methyl, Picolinic acid, 4-chloro-, hydrazide and low for; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide.

From the table 8, Druglikeness and leadlikeness shows that the 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide followed the Lipinski's rule of 5 and other filters, like Veber^[32] and Egan^[33], with four violations for Ghose filter^[34] of 4H-1,2,4-Triazol-3-amine-4-methyl, one violations for Picolinic acid, 4-chloro-, hydrazide and two violations of Muegge filter of 4H-1,2,4-Triazol-3-amine-4-methyl^[35]. For a new drug molecule, the bioavailability score^[36] is to expect the probability of a new different drug that have at least 10% oral bioavailability in rodents. The filters for lead likeliness, like pains filter^[37] and brek filter^[38], were obeyed for 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide. A new drug compound with a molecular weight of 250-350, XLog P less than 3.5, rotatable bonds of 7, and synthetic accessibility 2.5 can be an Investigational New Drug (IND).

From the table 9, The toxicity profile of the compounds of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide and Picolinic acid, 4-chloro-, hydrazide, which were non-toxic in hERG, AMES

toxicity, acute oral toxicity. Regarding the carcinogenicity, 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide was found to be non-carcinogenic, and Picolinic acid, 4-chloro-, hydrazide was revealed to be an alarming sign as the median toxic dose was above 10 mg per kg body weight per day.

DISCUSSION:

In developing countries Cancer has become very dangerous disease. Some of the drugs are used to cure cancer. Discovery of anticancer agents are helpful for curing cancer. Several medicines are used for the treatment of cancer. The improvement of many natural compounds through biological activity is required for curing many infections. *In-silico* analysis used for the improvement of new drugs to cure many infections. Endophytes play a major role since endophytes are resistance against insects, animals, pests and pathogens. *In-silico* analysis used for the improvement of new drugs to cure many infections. In this study *M. foetida*- endophytic fungal leaf extract were identified majority of bioactive compounds from GCMS analysis. This plant has anticancer properties which has made it an endangered species. Docking analysis were done using autodock vina and pymol. 7 bioactive compounds are selected which undergo anticancer property. These compounds were docked against 1G83 protein (ALOX5- Arachidonate 5-Lipoxygenase). All the seven compounds had a good inhibitory potential. Among them Oxime-, methoxy-phenyl- showed good binding affinity. "Wet lab" experiments are required to confirm. Hence these compounds can be analysed by further through *in vitro* studies and can be a lead in the designing of potential drug in the treatment of cancer disease. This study encourages the utility of the compound for further drug discovery through advanced techniques.

Autodock vina is the calibrated well accepted open source software tool for molecular docking analysis of molecular interaction between ligand and protein for specificity and druggability. The prediction on ligand target is based on the orientation, receptor, electrostatic interactions and Van der Waals interactions.

Drug likeness in the acceptable criteria of accepting active small molecules as oral active compound. Irrespective of the source from either natural or synthesis the process of drug discovery need a high throughput screening to consider active compound as a potential lead molecule. The process of drug development involves evaluation of ADME-absorption, distribution, metabolism and excretion. The limitations can be minimized

by simple and accurate online tool which predict the pharmacokinetic behaviour of the drug in the more similar pattern of the experimental analysis.

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CONFLICTS OF INTEREST

The authors of the current study declare not any conflicts of interest.

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