Tragia plukenetii Radcl.-Sm.: Anticancer, anti-migration potential on A549 cell line and molecular docking analysis on Lung cancer receptors – A systematic study

Manoharan Sowmya Priya, Alagar Yadav Sangilimuthu*, Gnanaselvan Suvathika, Thangavelu Soundara Rajan and Muni Samy Venkataa Suresh
Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore - 641021, Tamil Nadu, INDIA
*smuthu.al@gmail.com

Abstract

Lung cancer is a terrible disease with highest mortality. Although, several modern medications saved many lives but side effects remain dreadful. The natural remedy is needed to overcome the side effects of the lung cancer treatment. Tragia plukenetii belongs to Euphorbeaceae family reported for good ethnopharmacological property which remains unexplored in its anticancer prognosis. This study focusses on cytotoxicity analysis of T. plukenetti extract against A549 cell line and L929 by MTT assay. Scratch assay was done to evaluate their anti-migratory effect against A549 cell line. GC-MS techniques were used to analyse the identification of phytocompounds present in the T. plukenetii leaves and screened their anti-lung cancer mechanism targeting Lung cancer receptors (EGFR, RET, ALK, BRAF, JAK3 and P13k) through molecular docking by SeeSAR 9.2. commercial tool. Best docked ligands were subjected to ADMET analysis and Swiss Target Prediction to check the drug likeliness properties of the ligands.

MTT analysis of T.plukenetii leaves extracts against A549 lung cancer cell line revealed the IC_{50} value as 91.53 ± 0.2 µg and less cytotoxic effect in L929 cell lines. Scratch invasion assay showed 88% inhibition in wound closure at 36 Hrs. T. plukenetii contains fifteen volatile compounds with as Z, Z-3,13-Octadecadien-1-ol (25.71), Oleic acid (16.21) 9-Eicosyne (15.72) and 9,12- Octadecadienoic acid (Z,Z)-(12.62) as major metabolites in the GC-MS analysis. Among best docked ligands, oxacyclododecan-2-one obeyed the major ADMET categories. Top 15 receptor targets predicted from Homo sapiens origin showing T. plukenetii have the anti-cancer potential that may pave the way for the drug discovery.

Keywords: *Tragia plukenetii*, MTT assay, Scratch invasion, GC-MS analysis, Molecular docking, ADMET properties and Swiss target prediction.

Introduction

Cancer is the one of the deadliest groups of disease where cells grow uncontrollably undergoing the metastatic stage. The uncontrolled proliferation occurs when the cell undergoes protein modification in mutation and in over expression. Among the other types of cancer, lung cancer is subsequent in new cases and preeminent in death rate. Lung cancer has gained prominence in twenty-first century from the past 100 years ago[23]. The mortality rate alarms mainly because of continuous usage of tobacco smoking that to be peaked in industrialised countries. 8% of the lung cancer is inherited or genetically pre-dispositional and by the ambient particulate matter $PM_{2.5}$, the causative of air pollution rates up to 41.7% until 2017[28].

Survival rate of lung cancer is estimated up to 10% amongst the diseased in the countries of southern parts of the world. Lung cancer consists of non-small cell lung cancer (80-85%) and small cell lung cancer (10%-15%) followed by the lung carcinoid tumour and other lung cancers[20]. Although, modern medications including chemotherapy, surgery and targeted medicines down regulate the cancer receptors, they become more cytotoxic to the normal cells[2]. Still, the target drug treatments of this particular cancer remain a challenging task to the researchers for development of the drug with lesser side effects. Since ancient era, medicinal plants have served not only as leading drugs but also for production as promising candidates for new drug development[17].

Medicinal plants provide therapeutic phytochemical compounds called as secondary metabolites proven to treat many cancer types. Secondary metabolites promote human health by increasing the immune responses. World Health Organisation states that 80-90 % of world population depends upon traditional medicinal plants as they are safe and ready to use[24]. The recent study done by the Li et al[12] states that medicinal plants belonging to the Euphorbiaceae family have rich source of terpenoids, flavonoids, diterpenoids, acetophenones, phenolic acids, coumarins, steroids and tannins which are more responsible for the anti-tumour, anti-cancer and anti-inflammatory properties. This perennial shrub has the capability to inhibit the proliferation, apoptosis induction and detoxification that serves to treat various tumours including leukaemia and solid tumours[32].

The medicinal plant is *Tragia plukenetii* belonging to the Euphorbiaceae (Castor family) having potential medicinal property. The plant is distributed throughout all over India and found native in all districts of Tamil Nadu[9]. The annual

perennial shrub has also been found in outskirts of Africa and Sri Lanka. The wild shrub is also known as cannabis leaf nettle, climbing/stinging nettle and nose-burn. *Tragia plukenetti* Sm Radcl. has the various synonyms such as *Tragia cannabina* and *Croton hastatus*. This plant has been reported for wide range of medicinal properties such as antinociceptive, antihyperglycemic, curing male impotence, antitumour, antioxidant, skin irritation, anti-diabetic, antiasthmatic, antispasmodic, treatment of polio and gastroentitis, gonorrhoea, tapeworm manifestation and elephantiasis[3].

Structural binding of the targeted protein with the designated ligands through 2D or 3D interaction can be achieved through molecular docking analysis. Molecular docking has been turned to primary analysis to find the covalent bonding (only hydrogen bonding) between the ligand and receptor in *in silico*[22]. A good medication candidate can be ingested at the appropriate time and circulated throughout the body for efficient action and metabolism. Another important factor is toxicity, which often plays a significant role in drug absorption, distribution, metabolism and excretion. Various studies have indicated that drugs failed in clinical trials due to significant side effects and toxicity, which have been proved to be extremely damaging and costly in medication development.

ADMET and drug-likeness prediction *in silico* aid in the identification of novel targets and compounds with expected biological activity[4]. This study aims to explore the cytotoxic potential, antimigratory potential of lung cancer cell line A549 in *in vitro* and finding secondary metabolite present in leaves of *T. plukenetii* through GC-MS analysis as key components and against lung cancer specific receptors in *in silico* responsible for the pathway of cell proliferation.

Material and Methods

Plant collection: *T. Plukenetii* was collected in the outer region of Tanjore district, Kangeyampatti (57.9 m of altitude, 10.7759683 of longitude and 78.919249 of latitude) and authenticated in BSI, Coimbatore, Tamil Nadu vide no. BSI/5/23/2013-14/Tech-1768 dated 31st January 2014. Fresh, healthy and insect-egg free leaves of *T. plukenetii* were collected and washed thrice under tap water followed by distilled water to get free from soil and contamination. Thus, cleaned leaves were further made shade-dried for a week. Dried leaves were pulverized in an electric grinder to obtain coarse powder and stored in an air-tight jar.

Extraction: Analytical grade solvent (Carbinol) was procured from Hi Media Laboratories Pvt. Ltd. 50g of plant powder was weighed and made to extract utilizing hot Soxhlet extraction followed according to procedure done by Yadav et al[29]. Methanol extraction was performed as per the previous study showing the presence of better phytoconstituents in methanolic extract. The extract was done by maintaining the temperature under 60ºC in heating mantle continuously for 9 cycles up to 36 hours. The crude extract was made concentrated by distillation method and further stored in the refrigerator at 4ºC.

MTT analysis: To evaluate the cytotoxicity potential of *T. plukenetii*, MTT assay was performed in A549 and L929 cell lines respectively. 10,000 cell per well was seeded in 96 well plates. After 12 hours of incubation, methanolic leaf extract of *T. plukenetii* was added in the range of 25, 50, 75, 100, 125 µg/ml in triplicate to achieve the IC_{50} concentration. 100µl of MTT solution (7mg/ml) was added to form purple formazan crystals followed by 24 hours of drug treatment. This was kept in 5% CO_2 Incubator for 4 hrs at 37ºC. To measure cell viability, 100 µl of DMSO was added and absorbance taken at the wavelength of 570 nm in the ELISA microplate reader (Biotek, USA). Morphological observation was done using inverted microscope (Magnus) for the effect of *T. plukenetii* treatment[16].

Scratch invasion analysis: Cell migration inhibition was demonstrated using the wound healing assay. $3x10^5$ cells were seeded in 6 well plates and left to form monolayer. Cells were treated for fasting with serum free medium for 24 hours. Scratch was made with the help of 1ml tip respectively in all the wells. IC_{50} concentration of *T. plukenetii* methanolic leaf extract was treated in the well in triplicate along with the control. Cells were observed for the migration inhibition in the time interval of 0,16,24 and 36 hours respectively under inverted microscope (Magnus) with 10X magnification[16].

$$\text{Wound Closure \%} = (A_i - A_f) / A_i \times 100\%$$

where A_i is the area of the scratch observed at initial time and A_f is the area of the healed wound at the final time.

Gas chromatography-mass spectrum: Using an Agilent GC-MS (Agilent-GC7890A/MS5975) equipment, the methanolic extract of *T. plukenetii* leaves was examined for bio-active volatile phytochemicals. The phytocompounds were precisely screened using a capillary column DB5MS and a mass analyzer with low energy electron ionisation.

The column was operated using complementary chemical ionisation techniques and had a length of 30 metres, an internal diameter of 0.25 mm and a film thickness of 0.25 microns. It started off at a column temperature of 30 °C and was subsequently heated up to 350 °C at a rate of 10 °C every 4 minutes. Helium was employed as a carrier gas. Exact 1 µl of the sample was injected in a spitless manner.

The ionisation voltage was set at 70 eV. The MS scan's parameters were set at 45-380 (MHz). The entire running time of a sample is roughly 25 minutes. Chemical components were identified using GC-MS. The compound fragmentation patterns of mass spectra were compared to those stored in the spectrometer database using the National Institute of Standards and Technology's (NIST) Mass Spectral Library. The percentage for each component was

calculated using the relative peak area of each component in the chromatogram[11].

Ligand and receptor preparation: The receptors were chosen using the KEGG pathway database (https://www.genome.jp/kegg/ pathway.html) according to the central protein JAK/STAT pathway, P13K/Akt1/mTOR pathway and BRAF/MAPK pathway having the significant role in NSCLC prognosis by cell proliferation. Inhibition of these mutated receptors will shut down the cancer prognosis rapidly. All the receptors were downloaded in the state of 3D format of Protein Data Bank (PDB) files. The EFGR kinase domain (PDB ID: 2GS2), ALK (PDB ID:4TT7) and RET (PDB ID:2IVT) are retrieved[19]. Receptors of BRAFV600E (PDB ID:7P3V) and P13K (PDB ID:1E90) were taken for inactivating the downstream apoptogenic factors initiating cell proliferation[28].

The receptor JAK3 (PDB ID:4H71) was chosen to encounter the JAK3 kinase to inhibit the pathway of JAK/STAT 3 of cell proliferation in lung cancer[13]. Each protein was made dehydrated, pre-processed and external ligand was removed by Biovia Discovery studio (BDS). All the ligands were fetched from the Pubchem data base in 3D SDF format which were selected from the GC-MS analysis.

Molecular docking: Docking was performed to exhibit the molecular interaction between the receptors and ligands with help of commercial software SeeSAR 9.2. The receptors were primarily checked for the binding site - amino acid residues by visualizing the unoccupied pockets. Exact binding site of the amino acid residues was also selected in the protein mode. 3D SDF structure of the ligands was uploaded under the docking mode and allowed to generate poses. Each ligand was pre-setted for 5 poses respectively. Hyde calculation was done to know their estimated binding affinity range and selected for the best protein-ligand complex that can be viewed further for the 2D and 3D interaction in BDS. Docked receptor was saved in .ecf file format. The binding score was calculated using Command Prompt FlexX.4.1[21].

ADMET and target prediction: Filtered best docked ligand candidates of *T. plukenetii* leaves were involved for the ADMET analysis and target prediction. ADMET is the abbreviation of absorption, distribution, metabolism, excretion and toxicity. This analysis is very much important to know the druggability of the specific ligands. The free online webserver pkCSM (http://structure.bioc.cam.ac.uk/ pkcsm) is the pharmacokinetic prediction used in the cheminformatics computer programs which save the time and money preventing from the experimental failures in the pre-clinical and clinical trials[25].

Ligand-receptor based target prediction is done to assist finding the efficient drug targets with respective receptors. The *SwissTarget* web tool is used to determine the most likely protein targets of small compounds. In this study, the ADMET responded lead candidate is checked for the receptor target prediction with top 50 hits[6]. This is done for the predicting target proteins which could be able to bind to them with specificity (http://www.swisstargetprediction.ch/)

Results

Soxhlet extraction: Soxhlet extraction has been used widely to obtain heat stable compounds. Thus, crude extract of *T. plukenetii* (TPME) was obtained through extraction with 100% carbinol as solvent which was based on the previous study done by Bonam et al[3]. The extraction yielded up to 8.6 ± 0.2 g from 50g of dry powder. The extract was then analysed for the volatile phytoconstituents by GC-MS analysis.

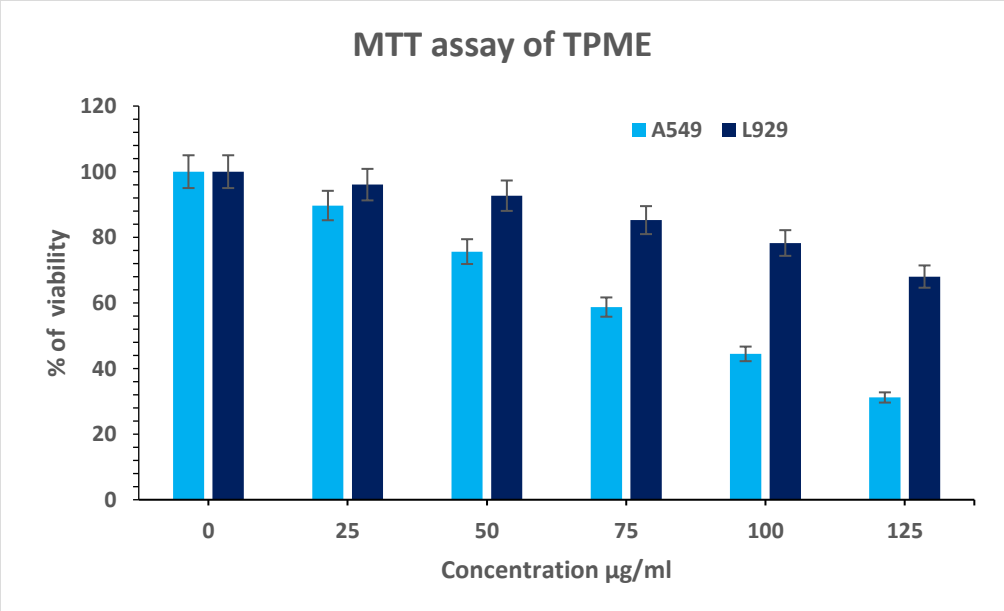

Figure 1: MTT analysis of TPME against A549 and L929 cell line

Anti-cancer property of TPME: Cytotoxic potential of TPME was performed in both A549 (Lung cancer cells) and L929 (Normal cell line). Treatment ranging from 25, 50, 75, 100, 125 µg showed inhibition of cell proliferation in dose-dependent manner under 24 hour incubation. Among the treated range of concentration cytotoxicity of A549 resulted with the IC_{50} concentration as 91.53 ± 0.2 µg and L929 showing lesser cytotoxic activity respectively (Fig. 1).

Cell wall disruptions were observed in the highest concentration 125 µg of TPME treated A549 (Fig. 2). In brief, A549 showed least concentration of IC_{50} contrasting with L929 showing that they have possible elements of anticancer potential of TPME without disturbing the normal cells with less cytotoxic effect.

Anti- migratory potential of TPME: Anti-migratory potential of TPME was analysed using Scratch invasion assay against control and treated A549 cell line. This was treated with IC_{50} Concentration (91 µg) of TPME was observed in timed intervals such as 0,16,24,36 (Fig. 3). The percentage of healed wound reveals the migratory potential of TPME. The *in vitro* scratch assay demonstrated 25%, 37.5%, 62.5% in control and 0%,8.3%,16.6% in treated ones. This demonstrated that the TPME has good antiproliferative property and reduces the migration of A549 cells.

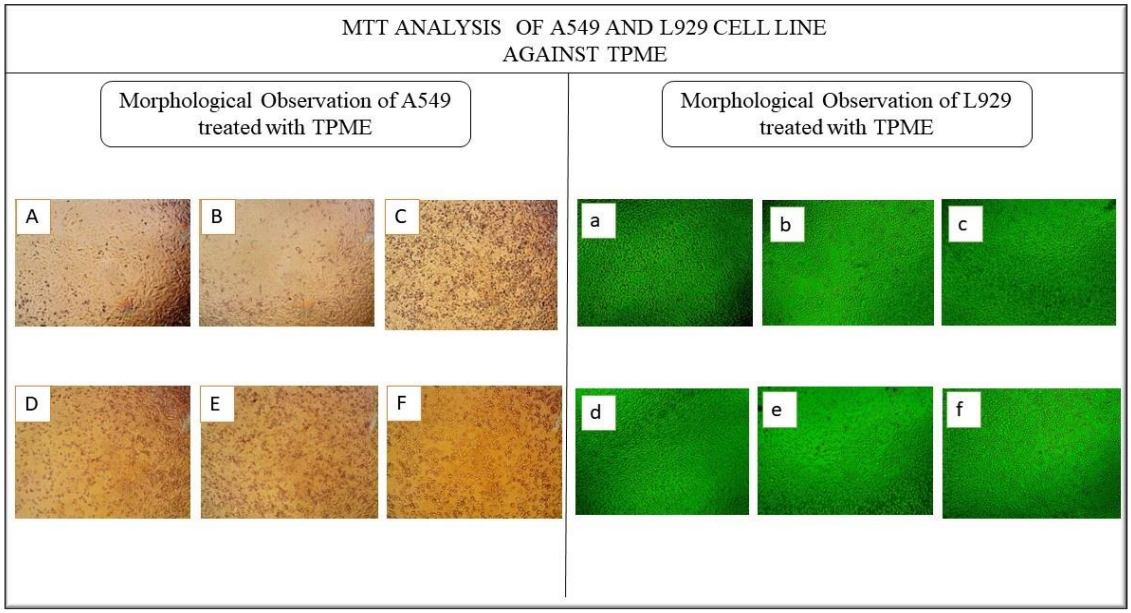

Figure 2: Morphological observation of A549 and L929 cell line treated with TPME (A,a, B,b, C,c, D,d and E,e : control, 25 µg, 50µg, 75µg, 100 µg and 125µg in A549 and L929 cell lines respectively)

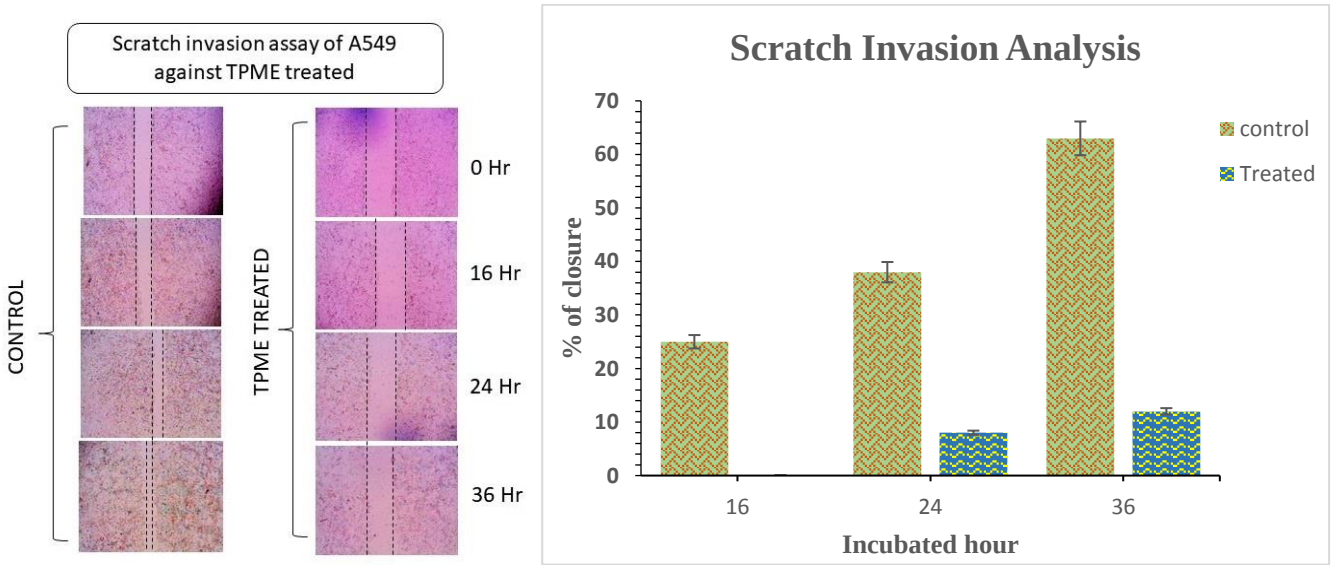

Figure 3: Scratch invasion analysis of A549 cell line treated with TPME and the graph denotes the percentage of wound closure

Identification of volatile metabolites: Fifteen volatile compounds were fetched from TPME using the GC-MS (Agilent) analysis. The compounds were compared with the retention indices and mass spectra with similarity search from the NIST library (https://webbook.nist.gov/chemisty/name-ser/) where already compound indices are available. The tentative structure of the identified compound with IUPAC name, retention time, peak percentage area, molecular formula and molecular weight are shown in the table 1. The secondary phytocompounds 9,12-Octadecadienoic acid (Z,Z)-, Z,Z-3,13-Octadecadien-1-ol, 9-Eicosyne and oleic acid were found abundant in the TPME. Compounds such as phytol, palmitic acid, dichloro acetic acid and gamma tocopherol have also been found in the GC-MS study done by kalaivanan et al[10] in the ethanolic extract.

These compounds are reported to possess cytotoxic, anti-oxidant and antitumour properties respectively[1]. Total chromatogram and fragments of abundant peaks have been shown in the figure 4. The tentative structure of the compounds was made docked with pathway related receptors of molecular interaction.

Molecular interaction: The profound phyto molecules present in the methanolic extract of *T. plukeneti* were subjected to the molecular interaction studies along with the standard drug of lung cancer named as Pemetrexed[27]. The docking was done on the basis of receptor pathway interaction involved in the prognosis of lung cancer. The best docked ligands with each receptor were selected on the basis of doc score as mentioned in the table 2. The receptor EFGR has the stronger interaction with hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with 4 hydrogen bonds in the binding residues of ASN A:732, ALA A: 726 and THR A:761 at the µM binding affinity 2.90, 2.94, 2.76 and 2.91. The receptor ALK bonded with ligand oxacyclododecan-2-one having a hydrogen bond at the position ASP A: 1023 in the range of µM with the binding affinity of 2.80. The same ligand bonded with the RET receptor with lesser than mM affinity with 2 hydrogen bonds TYR A: 928 at the affinity range of 2.48 and 2.72.

Table 1
Molecular interaction of volatile metabolites identified from *T. plukenetii* by GC-MS analysis against lung cancer receptors

S. N.	RT	Name of the Compound	Molecular Weight (g/mol)	Molecular formula	Peak Area %	Name of the receptors and binding energy values Kcal/M					
						EGFR (2GS2)	ALK (4TT7)	RET (2IVT)	BRAF (7P3V)	JAK3 (4H71)	P13K (1E90)
1.	8.653	3- Dimethylsilyloxy-6-ethyloctane	215.43	$C_{12}H_{27}Osi$	3.02	-0.620	4.220	1.340	-3.970	-0.590	-2.960
2.	12.819	Methoxyacetic acid ,2 ethylhexyl ester	202.29	$C_{11}H_{22}O_3$	1.37	-1.760	0.110	-8.500	-7.530	-4.210	-7.530
3.	14.752	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	13.25	$C_{10}H_{18}$	1.31	-1.040	-	-0.090	-6.030	-3.770	-6.370
4.	15.808	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	4.53	-1.470	1.110	-9.320	-4.270	-7.630	-9.850
5.	16.985	Phytol	296.5	$C_{20}H_{40}O$	3.18	-1.800	3.200	-6110	-3.050	-	-4.270
6.	17.163	9,12- Octadecadienoic acid (Z,Z)-	352.6	$C_{21}H_4O_2si$	12.62	0.050	-	3.220	-1.870	4.530	-0.780
7.	17.207	Z,Z-3,13-Octadecadien-1-ol	266.5	$C_{18}H_{34}O$	25.71	-6.320	2.050	-9.510	0.310	2.740	-5.500
8.	17.385	9-Eicosyne	278.5	$C_{20}H_{38}$	15.72	2.890	10.00	5.840	4.580	5.490	2.450
9.	19.563	Methyl 10-trans. 12-cis-octadecadienoate	294.5	$C_{19}H_{32}O_2$	1.84	2.260	5.600	-4.980	-0.190	-4.310	-6.130
10.	19.840	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	330.5	$C_{19}H_{38}O_4$	2.65	**-6.490 #**	-0.860	-11.440	-6.600	-7.500	**-14.430 #**
11.	20.996	Oleic acid	282.5	$C_{18}H_{34}O_2$	16.21	-2.720	0.810	-7.590	-3.490	-7.360	-13.210
12.	21.151	Oxacyclododecan-2-one	184.27	$C_{11}H_{20}O_2$	3.13	-6.980	**-5.450 #**	**-11.450 #**	-7.490	-5.490	-10.820
13.	21.696	Squalene	410.7	$C_{30}H_{50}$	2.78	3.130	10.650	-	-3.190	2.160	-1.600
14.	23.273	Gamma.-Tocophero	416.7	$C_{28}H_{48}O_2$	2.12	-1.430	-3.510	-9.710	**-10.830 #**	-7.330	-8.290
15.	23.640	Dichloroacetic, heptadecyl ester	367.4	$C_{19}H_{36}Cl_2O_2$	3.79	1.710	4.380	-2.640	-1.440	0.830	-2.820

RT is the retention time obtained from the GC-MS chromatogram. # - Denotes the best docked Receptor against liga

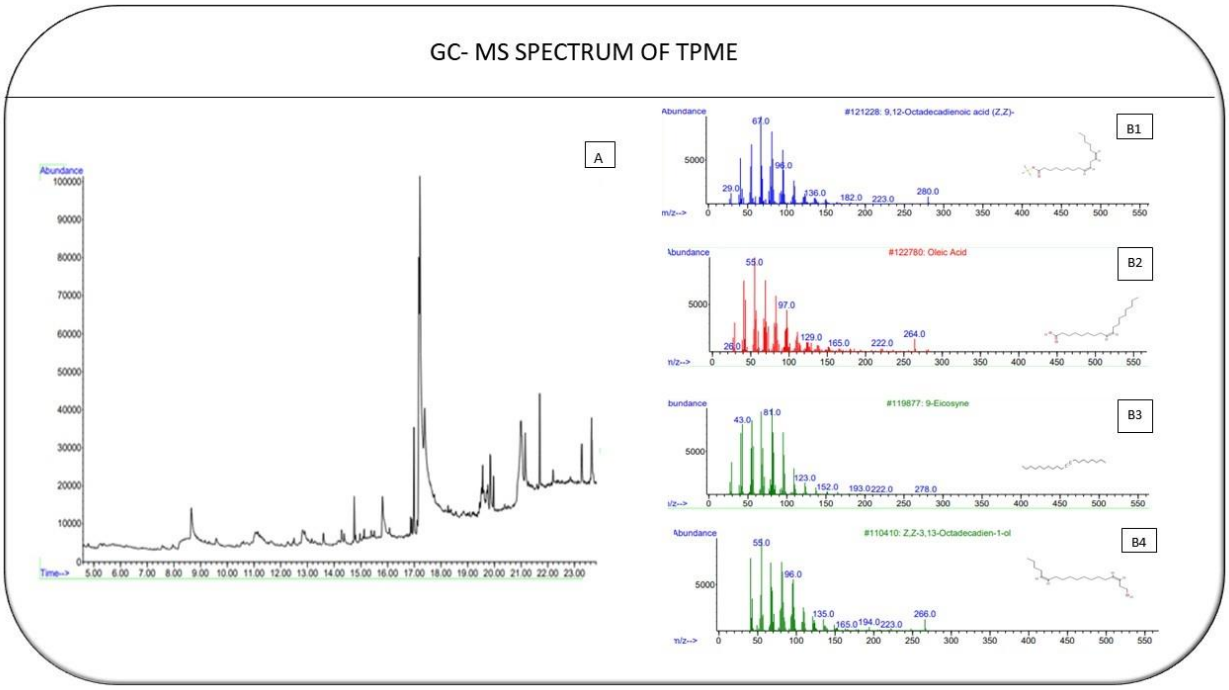

Figure 4: Total GC-MS chromatogram with abundance peak of the *T. plukenetii*-methanolic extract. A represents total chromatogram. The highest similarity fragments shown as B1, B2, B3 and B4 representing 9,12-Octadecenoic acid (Z,Z), Oleic acid, 9-Eicosyne and Z,Z-3,12-Octadecadien-1-ol respectively

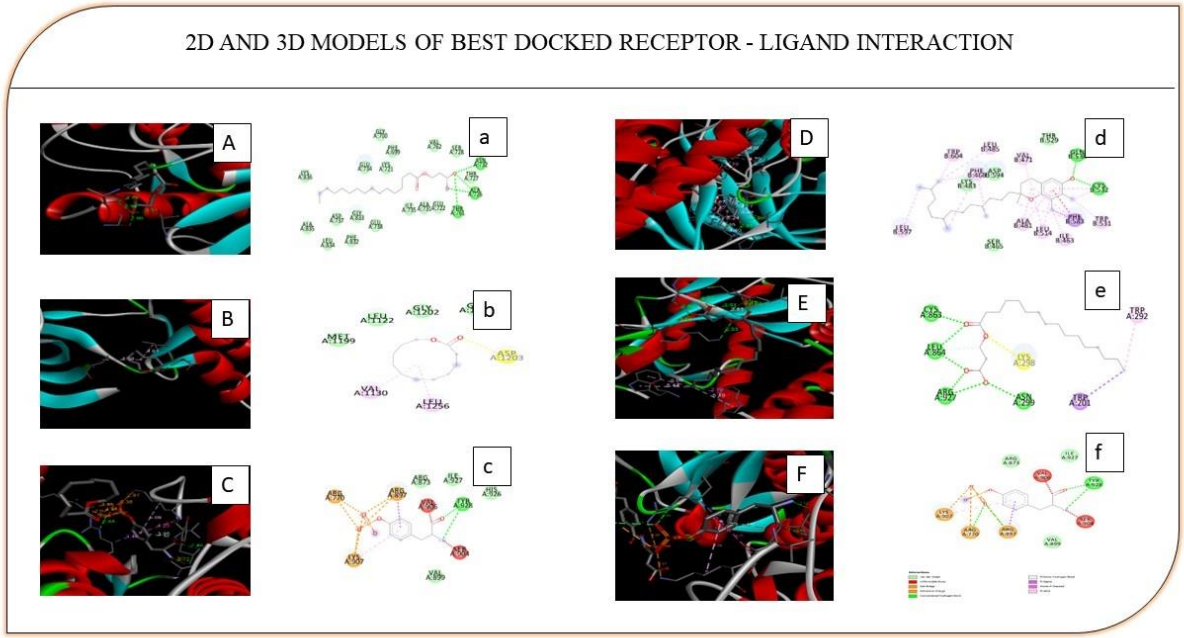

Figure 5: Molecular interaction of Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with EGFR (A and a), Oxacyclododecan-2-one with ALK (B and b), Oxacyclododecan-2-one with Ret (C and c), Gamma-Tocopherol with BRAF (D and d), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with P13k (E and e) and Standard Pemetrexed complexed with RET (F and f). Where A1-F1 denotes the 3Dstructure and A2-F2 denotes the 2D interactions with respected receptors

Gamma-tocopherol exhibited interaction with BRAF receptor with the affinity range of greater than Pico molar having 2 hydrogen bonds of 2.89, 2.85 at the residue of GLN B:530 and CYS B:532 in which the receptor JAK3 does not show the interaction with selected candidate bicyclo[3.1.1]heptane, 2,6,6-trimethyl- which had the binding affinity in the range of µM. The last receptor P13K showed the binding interaction with hexadecanoic acid, 2-

hydroxy-1-(hydroxymethyl) ethyl ester with major 7 hydrogen bonds at the residue destination of the of CYS A: 8.63,LEU A: 864, ARG A: 927, ASN A: 299 and LYS A: 298 in the estimated affinity range of µM which have shown the highest efficacy among all other compounds (Table 2).

The standard pemetrexed showed the maximum binding affinity with 6 H-bond interaction with the receptor ALK as shown in figure 5. Those bonded amino acid residues are THRA:766, ASPA:831, GLU A:738, LYS A:721, THR A: 830, GLN A: 767. The compounds hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Oxacyclododecan-2-one and Gamma.-Tocopherol exhibited the good binding interaction with the selected receptors of lung cancer (Figure 5).

ADME/TOX and swiss target analyisis Among the three ADMET screened ligands, oxacyclododecan-2-one satisfied the highest 21 categories as shown in highlights from the table 3. The physicochemical parameters of oxacyclododecan-2-one suit all the parameters including LogP and zero violation of Lipinski rule. In absorption category, $CaCO_2$ permeability, Human Intestinal absorption (HIA), Skin permeability, P-glycoprotein I and II inhibitors showed positive results. The HIA of all screened compounds suggested that they could be absorbed in the intestine. The BBB and CNS permeability of oxacyclododecan-2-one shows the important category in distribution parameters possessing 0.337 logBB and -3.036 logPS respectively. There is no metabolism parameter violation except CYPD26 and CYP3A4 substrate.

In toxicity analysis, Gamma-tocopherol persisted the druggability with four criteria's such as AMES toxicity, hERG II Inhibitor, Hepatotoxicity, Skin sensitization. With the results derived from the ADMET analysis, oxacyclododecan-2-one is further checked for the suitable targets using Swiss Target Prediction.

Table 2
List of best docked ligands on receptors with interactive residue, Distance of H_2 bonds.

S.N.	Name of the Ligand	Receptor	Estimated Binding affinity range (pM-mM)	Distance in Å	No. of H Bonds	Binding Residues
1.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	EGFR	µM	2.90 2.94, 2.76 2.91	4	ASN A:732 ALA A: 726 THR A:761
		P13K	µM	2.75 2.88, 3.01 2.92, 2.89 3.06 2.93	7	CYS A: 8.63 LEU A: 864 ARG A: 927 ASN A: 299 LYS A: 298
2.	Oxacyclododecan-2-one	ALK	µM	2.80	1	ASP A: 1023
		RET	>mM	2.48, 2.72	2	TYR A: 928
3.	Gamma.-Tocopherol	BRAF	<pM	2.89, 2.85	2	GLN B:530 CYS B:532

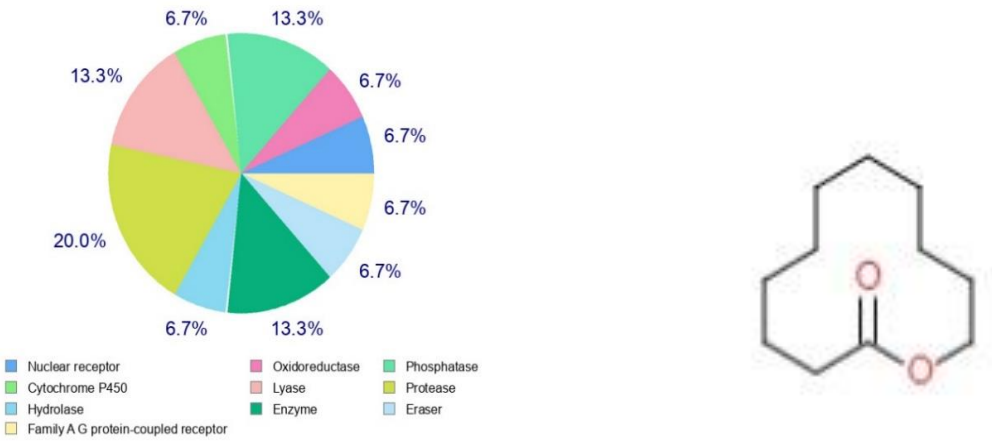

Figure 6: Top 15 target receptor (*Homo sapiens*) hits of Oxacyclododecan-2-one using Swiss target Prediction with 2D picture.

Table 3
Pharmacokinetic (ADMET) analysis of best docked ligands from *T.plukenetii* leaves

ADMET Properties	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Oxacyclododecan-2-one	Gamma.-Tocopherol
Physico-chemical Parameters			
LogP	4.7117	3.0541	11.547
MW	330.509	184.279	598.953
HBD	2	0	0
HBA	4	2	4
Rotatable Bonds	17	0	12
Surface Area	142.172	80.658	265.023
Absorption			
Caco2 permeability (log Papp in 10^{-6} cm/s)	0.428	1.618	1.358
Water Solubility	-5.463	-2.385	-7.122
Human Intestinal absorption (% absorbed)	90.441	95.692	91.395
Skin permeability (log Kp)	-2.822	-2.596	-2.749
P-glycoprotein substrate	No	No	No
P-glycoprotein I inhibitor	Yes	No	Yes
P-glycoprotein II inhibitor	No	No	Yes
Distribution			
Human VDss (log L/kg)	-0.321	0.113	0.074
BBB permeability (log BB)	-0.857	0.337	-0.008
CNS permeability (log PS)	-3.256	-3.036	-2.361
Metabolism			
CYP2D6 substrate	No	No	No
CYP3A4 substrate	Yes	No	Yes
CYP1A2 inhibitor	Yes	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Excretion			
Total Clearance (log ml/min/kg)	1.936	1.328	-0.126
Renal OCT2 substrate	No	No	No
Toxicity			
Ames Toxicity	No	No	No
Human Maximum tolerated dose (log mg/kg/day)	0.372	0.748	0.182
hERG I inhibitor	No	No	No
hERG II inhibitor	No	No	Yes
Oral Rat Acute Toxicity – LD50 (mol/kg)	1.659	2.059	2.008
Oral Rat Chronic Toxicity – LOAEL (mg/kg_bw/day)	2.881	1.941	2.221
Hepatotoxicity	No	No	No
Skin Sensitisation	Yes	Yes	No
Minnow toxicity	-0.622	1.638	0.364

Top 50 targets of *Homo sapiens* origin receptors were retrieved and given as supplementary material. Figure 6 resembles the pie chart of top 15 receptor target hits from the origin. 20% of the predicted targets comprise the class of enzymes in which the target P13 Kinase p110-delta/p-85 alpha (Uniprot ID: O00329/P27986) remains the main protein responsible for the cancer prognosis in a wide range. Family A – G protein coupled receptors (GPCR) comprise

14% of the target site protein by oxacyclododecan-2-one where the modern medicine always targets to modify the structural changes when bind to alpha, beta and gamma subunits to send or receive cellular messages.

Discussion

Lung cancer prevalence remains increased and the etiology made complex so far. Smoking addiction, dietary malnutrition, air pollution, genetic factors decreased immune function[5]. Mortality is further projected to be 22% higher both in men and women as of the year 2025. This can be changed by the introduction of the alternative plant based phyto-medicine medicine rather than the chemotherapy existence as it tends to more side-effects[30,31]. Ethanolic extract of *T. plukenetii* arial part has the antitumour activity in Ehrlich Ascites carcinoma bearing mice according the study demonstrated by Muthuraman et al[15]. Chloroform extract of this plant possesses antinociceptive and analgesic activity comparable to aspirin (77%) revealing that it may have the morphino-mimetic property by entering on to the peripheral receptors. Methanolic extract shows promising antioxidant activity in DPPH, carotein complex and ion chelation assays.

The study proved that *T. plukenetii* has no cytotoxicity in normal mice at the dosage of 5000mg/kg[26]. T. *plukenetii* has significance as it has lesser cytotoxicity against normal cells. TPME has the potential anticancer activity in 91 µg concentration showing that it is potential anticancer agent when compared to the L929 cell line. *In vitro* scratch assay proved that TPME has good antimigratory potential revealing that it is abundant in antiproliferative property which paves important role in drug likeliness. In addition, the molecular docking mechanism of the phytocompounds of TPME has been explored by analysing the GC-MS analysis. Fifteen volatile compounds were identified in this study in which the long chain fatty alcohol Z,Z-3,13-Octadecadien-1-ol was found with the highest peak percentage followed by the oleic acid, palmitic acid (n-Hexadecanoic acid), oxacyclododecan-2-one and Gamma.-Tocopherol as identified in the *Ficus sycomorus* fruit and leaf extract proving that they are potent antioxidant and anticancer metabolites[7].

Thus, volatile phytoconstituents were made to screen *in silico* molecular interaction with the cancer receptors such as Epithelial Growth Factor Receptor (EGFR), Anaphylatic Lymphoma Kinase (ALK), Tyrosine Kinase Target (RET), B-type Raf kinase (BRAF), Janus Kinasae 3(JAK3) and Phosphoinositide 3-kinases (P13k) involved in the cell proliferation which turns in cancer. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester also known as 2-palmitoylglycerol had 2 hydroxyl atoms and oxygen atom bonded to the EGFR and P13k receptors in the binding score range of -6.490 and -14.430 respectively. This shows the *T. plukenetii* has the stronger efficacy to inhibit the EGFR protein that activates the P13k which is the central protein of the overexpression of lung cancer cells by the action of the Akt pathway. Knocking down of these proteins at the initial stage may reduce the cancer cell proliferation[13].

Oxacyclododecan-2-one also called as undecalactone, a type of fatty ester intercalates with both receptor of RET and ALK proving that they can shut down the pathway of signal transduction of cells which undergo blocking of energy access by binding to the abnormal pocket of the ALK protein. Gamma tocopherol with -10.830 binding affinity energy interacted with the BRAF receptor downregulating the anti-apoptotic proteins such as MCl-1 that may initiate the apoptosis. Inhibition of the BRAF mutation receptors is one of the important hooks for stacking the cell metastasis. As bicyclo[3.1.1]heptane, 2,6,6-trimethyl- did not show the binding site of the JAK receptor, Gamma- tocopherol has been found as the next compound targeting the antiapoptotic proteins such as BCl2, Bax and Bad proteins which are responsible for the cell proliferation[28]. Inhibition of these proteins may result in the controlling of over expression of the cancer cells. This works states that methanolic extract of *T. plukenetti* has the lead role in the inhibition of the lung cancer prognosis.

The ultimate aim of each and every drug is to obey the ADMET, Lipinski rule and Veber's rule to check druggable nature and to avoid the clinical failure. Lipinski's rule of five states that excellent absorption or penetration is more likely when the molecular weight (MW) is 500 Da and the number of hydrogen bond donors (HBDs) is 5. LogP<5 and the number of hydrogen bond acceptors (HBAs) should be lesser than 10 as demonstrated in molecular docking experiments with two more significant descriptors: the number of rotatable bonds (NBR) 10 and the polar surface area (PSA) 140< $Å^2$ [14]. Among the diagnosed 3 best docked ligands, oxacyclododecan-2-one obeyed the majority of ADMET properties. Oxacyclododecan-2-one is also known as undecalactone and undecanolide is commonly present in cassia species especially in fruits of *Cassia fistula* that are most responsible for antitumour, antidiabetic, anti-inflammatory and antiplatelates[8]. This also can be synthesized synthetically by five step process of lactonization from the methyl pentanoate in acidic medium[18].

As mentioned earlier, synthetic medicine causes severe damage and side effects, the chemically synthesized oxacyclododecan-2-one causes eye irritation, respiratory irritation and skin irritation if took directly (Pubchem database). To the surprise, methanolic extract of *T. plukenetii* comprises of oxacyclododecan-2-one and may act as a synthetic and natural anticancer compound proving from the *in silico* data. The concretive additional analysis of Swiss target prediction shows that they are potent active binding to the proteins *in silico* of human beings. This current study paves the way to explore the plant *T. plukenetii* having the potency of anticancer property against lung cancer and may be responsible for the drug likeliness property.

Conclusion

Methanolic extract of *T. plukenetii* has a potential anticancer activity revealing the IC_{50} concentration at 91.53 ± 0.2 in A549 cells with antimigratory inhibition of 88% upto 36 hrs. Among fifteen identified volatile compounds from GC-MS, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, oxacyclododecan-2-one and gamma.-tocopherol showed elevated interaction against lung cancer receptors by inhibiting the major central proteins through *in silico* molecular docking. ADMET analysis and target prediction revealed that oxacyclododecan-2-one from *T. plukenetii's* methanolic extract contains good drug likeliness property.

Anticancer targeting efficacy of *T. plukenetii* may be due to the presence of active phytocompounds in the extract which might be used as the future drug invention in continuation of the *in vitro* and pathway related protein approaches. This is the lead to develop novel natural medicine with less side effects from *T.plukenetii*.

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