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In-Vitro and *In-Silico* characterization of *Sophora interrupta* plant extract as an anticancer activity

Pardhasaradhi Mathi[1], Kumar Nikhil[2], Nagavamsikrishna Ambatipudi[3], Partha Roy[2], Venkata Raman Bokka[4] & Mahendran Botlagunta[1]*

[1]Biomedical research Laboratory, Department of Biotechnology, K L University, Green fields, Vaddeswaram, Guntur 522 502, AndhraPradesh, India; [2]Molecular Endocrinology Laboratory, Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee 247 667, Uttarakhand, India; [3]Department of Biochemistry, Acharya Nagarjuna University, Guntur, India; [4]Department of Basic Sciences-Chemistry, Madanapalle Institute of Technology and Science (MITS), Madanapalle 517 325, Chittoor District, Andhra Pradesh, India; Mahendran Botlagunta – Email: bmnchowdary@gmail.com; Phone: +91-7382145827; *Corresponding author

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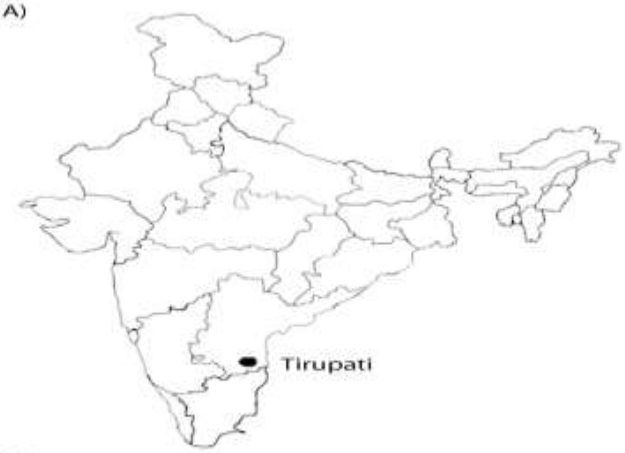
Abstract:
Sophora interrupta belongs to the family of Fabaceae and the species in this genus have a diverse medicinal importance as a folk medicine for preventing many ailments including cancer. In order to evaluate the anticancer activity of *S.interrupta*, we have performed *in vitro* anti-oxidant, anti-inflammatory, anti-proliferative, and cell based anticancer activity in MCF-7 and PC-3 cell lines. Secondary metabolites of *S.interrupta* were used to identify anticancer compounds using Open Eye software. The antioxidant activity of the *S.interrupta* root ethylacetate (SEA) extract at 100 µg/ml is equal to that of ascorbic acid at 50 µg/ml. The anti-inflammatory activity of SEA is half of that of diclofenac at 50 µg/ml. Anticancer activity was detected by measuring the mitochondrial dehydrogenase activity (MTT assay). The half maximal inhibitory concentrations (IC_{50}) for MCF-7 and PC-3 cell lines are 250 and 700 µg/ml respectively. This was supported by the morphological changes such as membrane blebbing, cell detachment and rounded cell morphology when compared to the parental cells. In addition, we observed few green cells (live) over red cells (dead) based on the uptake of acridine orange and ethidium bromide dyes. Kaempferol-3-*O*-b-D-glucopyranoside, a Secondary metabolite of *S.interrupta* form 6 hydrogen bond interactions with Arg 202, Gln 207, Gly 227, Gly 229, Thr 231 and Ala 232 human DEAD box RNA helicase, DDX3 protein and is equivalent to crystal structure of adenosine mono phosphate to DDX3. Overall, it suggests that the SEA extract has anticancer compounds, and it can be used to enhance death receptor mediated cancer cell death.

Keywords: *Sophora interrupta*, Apoptosis, cancer cell lines, DDX3.

Background:
Cancer is the major leading cause of deaths in all over the globe and the etiology of cancer is linked to several lifestyle choices in the modern society. These lifestyle choices include cigarette smoking, excessive alcohol consumption, poor diet, lack of exercise and hormones **[1].** Depending on the lifestyle choices, cancer can originate at any site in the body including breast and prostate. Breast and prostate cancers have shown to be the major leading cause of cancer related deaths in women and men, respectively **[2].** Several methods are being used to treat breast and prostate cancers, such as surgery, chemotherapy, radiation therapy and immunotherapy. Among all, chemotherapy is most commonly used method to treat metastatic cancer using cytotoxic drugs **[3].** However, those agents often have shown to generate side effects by affecting the cell viability of both tumor and normal cells, thereby bring down the quality of human life **[4].** Several anticancer compounds have been derived from natural resources including

plants **[5]** microorganisms and marines **[6].** Plants have a long history of use in the treatment of cancer providing some of the currently used effective anticancer agents such as vinblastine, vincristine, mechlorethamine, prednisone, procarbazine, etoposide, teniposide, paclitaxel, bleomycin, cisplatin and taxanes **[7].** However, cancer cells were found to be resistant to several drugs against chemotherapy **[8].** Therefore, recent focus has been shifted towards the identification of novel natural compounds to suppress the cancer cell growth or to enhance the cancer cell death against chemo-resistance caused by chemotherapy **[9].** In search of novel source of the traditional medicine, we had come across, *Sophora interrupta* as a source of folklore medicinal plant by tribal's in various parts of Andhra Pradesh for the treatment of cancer. *Sophora interrupta* belongs to family Fabaceae, popularly known as Adavi rela or Adavi billa and is very important shrub in the Ayurveda. The generic name *Sophora* is derived from an Arabic word 'Sophera' means a pea-flowered tree **[10].** Species in this genus are spread throughout the tropical and temperate regions of the world. It is especially prevalent in the Eastern Ghats of AndhraPradesh especially Tirumala **[11].** Various parts of *S. interrupta* are traditionally used to treat different diseases such as anti-bacterial, anti-inflammatory, anti-allergic, hepatitis, cardiac arrhythmia and anti-tumor properties of human health as folk medicine **[12-16].** These activities are mainly attributed due to the presence of flavones, flavanones, isoflavanoids etc., in the members of this family **[17].** Hence the present work is undertaken to evaluate the Antioxidant, Anti-inflammatory and Anticancer activity of *S interrupta* using *In-vitro* and *In-silico* experimental models.

A)



B)

C)

Phytochemical constituents	Tests	various solvent extracts					
		PE	CH	EA	BU	ME	AQ
Alkaloids	Mayers test	+ +	+ +	+ +	+ +	+ +	-
Anthraquinones	Borntträger test	-	-	-	-	-	-
Carbohydrates	Molisch's test	-	-	-	-	-	-
Diterpenes	Copper acetate test	+	+ +	+ +	+ +	+ +	+
Flavonoids	Shinoda test	-	-	-	+	-	+
	Alkaline reagent test	-	-	-	-	+ +	-
Glycosides	Modified Borntrager's test	+	+	+	-	+	-
	Keller-Killiani test	+ +	+ +	-	-	-	-
Proteins	Xanthoproteic test	+	+ +	+ +	+	+ +	+
Phenols	Ferric chloride test	+	+ +	+	+	+	+
Saponins	Froth test	-	-	-	-	+	+ +
Sterols	Salkowski test	-	-	-	-	-	-
Tannins	Gelatin test	-	+ +	+ +	-	+ +	+
	KOH test	+ +	+	-	-		

Figure 1: Plant information A) A pictorial representation of *S.interrupta* plant region grown abundantly on tirumala hills; **B)** *S.interrupta* (Fabaceae) plant odd-pinnate, leaflets sub opposite, with strong root in underground in thick hill forest region; **C)** Phytochemical screening of *S. interrupta* root extracts using various solvents. PE = Petroleum ether extract, CH = Chloroform extract, EA = Ethyl acetate extract, BU = n-Butanol extract, ME = Methanol extract, AQ = Aqueous extract. + + = copiously present, + = moderately present, - = absent.

Methodology:

Human breast adenocarcinoma cancer cell line (MCF-7), Prostate adenocarcinoma cell line (PC-3) were obtained from the National Center for Cell Science (NCCS), Pune, India. Cell culture reagents were purchased from GIBCO (Invitrogen USA). Streptomycin, Penicillin, MTT (3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide), DMSO cell culture grade, agarose and analytical grade chemicals are obtained from

HiMedia (Mumbai, India). The woody perennial shrub *S. interrupta* was collected from Tirumala hilltop. Latitude 13.667790 & Longitude 79.345880 readings of the plant collection sites were taken by using a Geographical positioning system (GPS) area of Tirumala Tirupati, Eastern Ghats, AndhraPradesh, India. A Voucher specimen has deposited, at K L University, Guntur, India (Voucher number KLU 1211).

Identification of phytoconstituents present in the S. interrupta

The root part of the plant was thoroughly washed with double distilled water to remove all dust and debris on the surface areas and shade dried properly. Dried plant roots were crushed and milled to powder for extraction of compounds into different solvents with increasing polarity in a ratio 1:3 by the maceration process. Extracts are dried under reduced pressure with rotary vacuum evaporator at 40°C to identify the presence of phytoconstituents present in the extracts. The presence of the phytoconstituents such as Phytosterols, Tannins, Flavonoids, Alkaloids, Saponins, Glycosides and Combined anthraquinones were carried out based on standard protocols **[18-20].**

Evaluation of total antioxidant potential by Phosphomolybdate method

The total antioxidant capacity of the SEA was evaluated by Phosphomolybdate method **[21].** In brief, 3ml of Phosphomolybdate reagent is mixed with a series of 300 µl of SEA of the plant in a test tube. The test tubes were capped with silver foil and incubated in water bath at 95°C for 90 min. Later, the tubes were cooled down to room temperature, and the absorbance measured at 695 nm against the blank. Ascorbic acid was used as a standard for this experiment. The antioxidant activity of SEA was expressed as µg/ml of ascorbic acid equivalents.

Cell viability assay

To study the action SEA on cancer cell viability, we seeded MCF-7 and PC-3 cells 5 x 10^3 in 96 well plate and were maintained in Dulbecco's modified eagle's medium (DMEM), F12K medium respectively. Both the cell lines were supplemented with 10% fetal bovine serum (heat inactivated) and 1% antibiotic (100U/ml of penicillin and 100µg/ml streptomycin) gently mixed and placed in a 5% CO_2-humidified incubator at 37°C. The cells were treated with increasing concentrations (1, 10, 50, 100, 250, 300, 500 and 1000 µg/ml) of SEA in DMSO for 24 hrs. Following treatment, 15µl of MTT (5mg/ml) reagent was added to the culture media and further incubated for 4 hrs at 37°C in CO_2 incubator. After an incubation period MTT containing medium was aspirated, 200 µl of DMSO and 25 µl of Sorenson glycine buffer (0.1 M glycine and 0.1 M NaCl, pH 10.5) were added to lyse the cells and solubilize the water insoluble formazan crystals. Absorbance values of the lysates were determined on a Fluostar optima microplate reader (BMG Labtech, Germany) at 570 nm. The percentage inhibition was calculated as: (Mean OD of vehicle treated cells − Mean OD of drug treated cells) / Mean OD of vehicle treated cells x 100. The IC_{50} values were calculated using a graph pad prism, version 5.02 software (Graph Pad Software Inc., CA, USA). Negative controls were maintained with DMSO. In as separate experiment, the effect of *S.interrupta* root extracts on morphological changes such as cell shape, size was captured using a phase contrast microscope (Zeiss, Axiovert 25, Germany).

Acridine orange (AO) /Ethidium bromide (EB) staining

To determine the changes in cell shape and morphology, we stained the live and dead cells using Acridine orange (AO) /Ethidium bromide (EB) dyes. For that, we seeded 0.5 x 10^6 MCF-7 and PC-3 cells were seeded in 6 well plates and cultured as mentioned above. Following 24 hrs of incubation, the media was replaced with fresh media consisting of *S.interrupta* ethyl acetate root extracts and further allowed for incubation of 24 hrs at 37°C in 5% CO_2 incubator. The cells were washed with Phosphate buffer saline (PBS), added 100µl of AO and EB (100µg/ml, Sigma) respectively to each well, and incubated for 15min in CO_2 incubator. Following incubation the medium was aspirated, and washed thrice with PBS. The intensity of fluorescent staining was observed and the images were captured with the help of fluorescent microscope (Zeiss, Axiovert 25, Germany) using appropriate color filters.

Anti-proliferative activity assay

The antiproliferative activity of SEA extract was evaluated using the *Saccharocymes cerevisiae* as a cancer cell mimicking the model according to the previously reported method of **[22].** In brief, *S. cerevisiae* BY4741 strain was obtained from IMTECH, Chandigarh, India. The single healthy colony was inoculated with sterilized yeast peptone and dextrose broth and incubated at 37°C for 24 hrs, referred as seed broth. The seeded broth was diluted with the blank culture media until the absorbance reaches 0.1 at 600nm. To validate the action of SEA, we distributed the overnight yeast culture into three tubes. The first tube kept control without any extract. Whereas second and third tubes were mixed with root extract and standard cisplatin respectively. All tubes were incubated at 37°C for 24 hrs; the anti-proliferative activity of plant extract is calculated by measuring the absorbance at 600 nm using UV-Vis spectrophotometer (DYNAMICA Halo DB20). The culture was spread on plates to count the number of colonies.

Anti-inflammatory assay

The *In-vitro* anti-inflammatory activity of SEA was measured by calculating the Percentage of haemolysis and membrane stabilization of red blood cells (RBC) evaluated by **[23].** In brief, five millilitres of blood sample was obtained from two healthy men (20-30 years old) and added an equal volume of Alsever's solution. The contents were mixed properly and centrifuged at 1500 x g for 10 minutes. Following centrifugation, RBC were washed thrice with isosaline solution for 7 min at 1000 x g and were resuspended in 10% v/v suspension with isosaline. The red blood cells were treated with various concentrations of SEA extract and were incubated at 37°C in continuous shaking water bath for 1 hr. Water containing RBC cells were used as a control. After the incubation period, the cells were centrifuged and the, supernatant was used to measure the absorbance at 560 nm using a UV-Vis spectrophotometer (DYNAMICA Halo DB20). Diclofenac is used as a referral drug. The percentage of haemolysis was calculated using the formula Haemolysis % = (Optical density of Test sample / Optical density of Control) X 100.

Molecular Docking

The three dimensional protein structures along with the crystallized ligand, AMP (Adenosine monophosphate) were obtained from Protein Data Bank (PDB) PDB ID: 2I4I. Hydrogen atoms were added, water molecules were removed from the

cavity to prepare biologically active and stable receptor. The active site within the receptor was identified. Ligands were retrieved from Chemspider and also sketched using tools like Chemdraw. The conformational space of the compounds was employed using an omega (optimized ensemble generation application) program from Open Eye Scientific Software (http://www.eyesopen.com/omega). Ligands were docked onto the receptor and the interactions were checked. The scoring function generates scores depending on which the ligand with the best fit was selected.

Result:

Phytochemical analysis

Phytochemical analysis showed the presence of phytochemical excipients in different solvent extractions. Several secondary metabolites were identified and their presence / absence of the phytochemical excipients in different solvent extracts were scored as **+ + / + / -** based on color change/precipitation. Petroleum ether and Ethyl acetate extracts confirm the presence of Alkaloids, diterpenes, glycosides, proteins, phenols, and in undetermined state or absence of rest all phytoconstituents. Chloroform extract confirms the presence of alkaloids, diterpenes, glycosides, proteins, phenols, tannins and rest all in undetermined state or absence. The n-Butanol extract gave positive results for alkaloids, diterpenes, flavonoids, proteins, phenols and rest all constituents are absent. The ethylacetate extract showed the presence of all phytoconstituents and aqueous extract confirmed the presence of diterpenes, flavonoids, proteins, phenols, saponins, tannins and rest all absent. In all extracts Anthraquinones and sterols are completely absent or may be in undetectable range **(Figure 1).**

Antioxidant, Anti-inflammatory and Anti-proliferative activity of SEA extract

The total antioxidant capacity of the SEA was calculated based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH, which was measured spectrophotometrically at 695 nm. Our results showed that the antioxidant activity of SEA increases in a dose dependent manner at a concentration of 100 µg/ml to 1mg /ml. The antioxidant activity of SEA at 100 µg/ml is similar to ascorbic acid at 50 µg/ml concentration. **(Figure 2A & 2B).** To study the anti-inflammatory activity, we have evaluated haemolysis and membrane stabilization of human red blood cells (RBC). For that blood cells were treated with varying concentrations (100, 250, 500, 700 and 1000 µg/ml) of plant extract using Diclofenac is used as a positive control. Our results suggest that the percentage (%) of haemolysis is decreased in a dose dependent manner throughout the concentration range of 50-1000 µg/ml. It is evident from the increasing membrane stabilization of red blood cells. Diclofenac (50-1000 µg/ml) was used as a reference drug which also demonstrates concentration dependent inhibition of haemolysis. It suggests that the % of haemolysis/membrane stabilization of RBC in the presence of the SEA extract at 1000 µg/ml is similar to that of Diclofenac concentration of 50 µg/ml **(Figure 2C & 2D)**. Next, we studied the anti-proliferative activity of SEA extract using actively proliferating yeast (BY4741 strain) as a model system for studying the anti-proliferative activities in eukaryotes **[24].** Our results showed that, the cells exposed to concentrations of SEA at 250 µg/ml showed growth arrest during a 24 hrs period, with respect to control cultures **(Figure 2E).** However, the concentrations above 250 µg/ml concentration had little or no effect on proliferation of cells, even at 48 hrs (data not shown). Same cultures were spread on Yeast peptone dextrose (YPD) agar plates and further incubated for 24 hrs to measure the number of colonies **(Figure 2F).** As shown in **Figure 2F**, the number of colonies is greatly reduced as compared to control cultures and is equal to the levels of anticancer drug, cisplatin. It suggests that the SEA extract contains anti-oxidant, anti-inflammatory and anti-proliferative activity and it might be attributed due to presence of medicinal compounds.

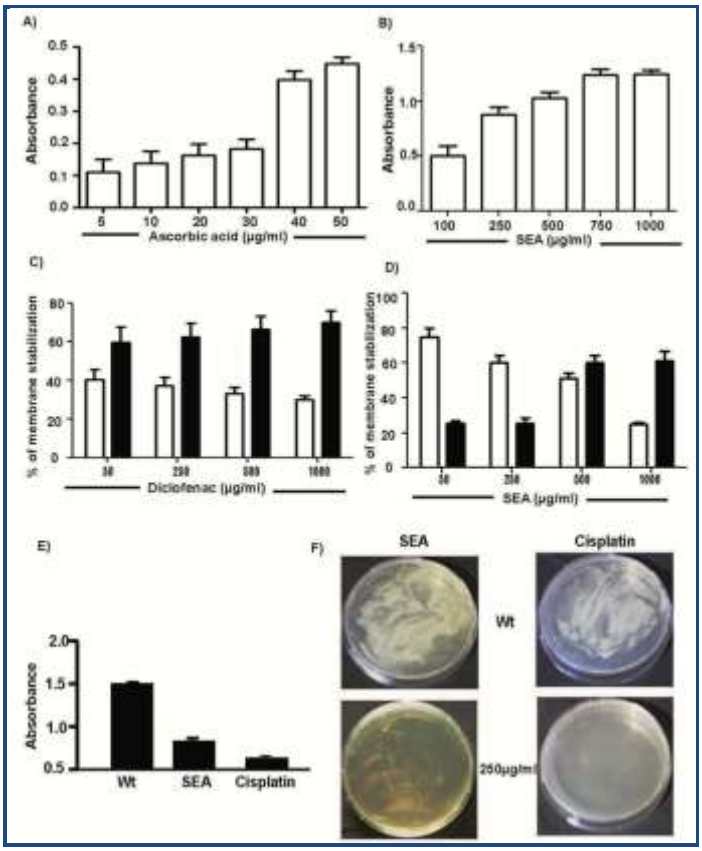


Figure 2: Antioxidant, Anti-inflammatory and Antiproliferative assay. The total antioxidant activity of **A)** Ascorbic acid; **B)** SEA extract. The membrane stabilization of HRBC; **C)** standard Diclofenac; **D)** SEA extract**.** The antiproliferative activity of SEA extract using yeast cells as eukaryotic models; **E)** and their growth on YPD plates; **F)**. Results are presented as mean ± SEM of three independent experiments. % = Percentage, SEA = *Sophora interrupta* ethylacetate extract.

Anticancer activity of SEA extract on MCF-7 and PC-3 cells

To evaluate the anticancer activity of *S. interrupta*, we treated the breast (MCF-7) and prostate cancer (PC-3) cell lines with increasing concentrations of SEA extract (1, 50, 100, 250, 500 and 1000 µg/ml) for 24 hr. The cell viability was determined using a standard MTT assay and the results were depicted in **(Figure 3).** As shown in **Figure 3** top panel, petroleum ether (PE) and n-butanol (BU) root extracts showed higher percentage of cell viability as compared to ethyl acetate (EA) extract. Whereas, ethyl acetate (EA) extract inhibited 75% of the cell viability in both the cell lines, MCF-7 and PC-3 at variable concentrations. It is evident from the calculated dose of 50% inhibition of cell

viability (IC_{50}). The ethyl acetate extract showed lower IC_{50} value for both MCF-7 (250µg/ml) **(Figure 3A)** and PC-3 (700µg/ml) **(Figure 3B)** cell lines as compared to other solvent extracts. It suggests that growth inhibitory principles are present in all organic fractions and we rank them as EA>BU>PE. In a separate experiment we have captured the phase contrast photomicrographs for both MCF-7 and PC-3 cells. The cell morphology studies showed a smooth, flattened morphology with normal nuclei in normal culture conditions. Whereas, in treated cells showed a typical morphological changes with membrane blebbing and detached from the surface were noticed in both MCF-7 and PC-3 cell lines **(Figure 3C).**

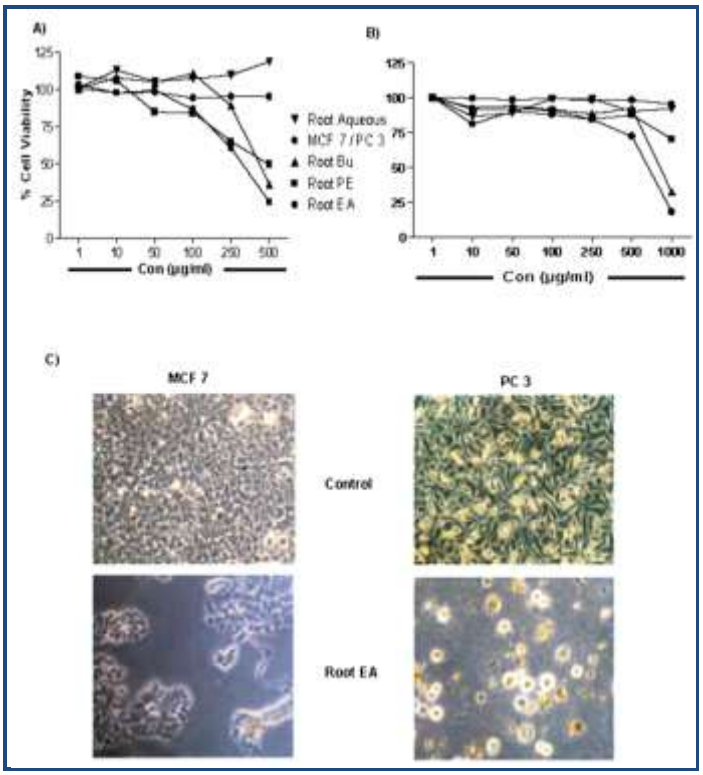


Figure 3: Cell viability with MTT assay. A) Cytotoxicity curves *S.interrupta* root subsequent extracts on MCF-7 cell line; **B)** Cytotoxicity curves *S.interrupta* root subsequent extracts on PC-3 cell line. Data are expressed as means ± SDs; **C)** Cell morphological study of *S.interrupta* root EA extract induced membrane blebbing and detachment were observed in both MCF-7 and PC-3 cells. The cells were incubated for 24 hrs with IC_{50} value.

Live/Dead Cell Viability Assay

Based on the IC_{50} values the cell lines were treated with 250 µg/ml and 700 µg/ml of root ethyl acetate extract and the percentage of live/dead cells were counted based color change using acridine orange (AO) and Ethidium bromide (EtBr) staining. As shown in **Figure 4**, bright red fluorescent cells were observed in MCF-7 cell line as compared to vehicle treated cells. On the other hand, orange fluorescent cells were observed in PC 3 cells, it indicates that those cells are at the early stage of apoptosis. Moreover, the number of PC 3 cells in the field of view is lesser than vehicle treated cells. It is suggested that, the large number of cells completely ablated from the cell attachment, a few cells are adherent and they also about to die, which is evident from condensed chromatin granules, the change of cell shape **(Figure 4A).** This was further confirmed by DNA fragmentation assay. As results shown in **Figure 3B**, ethyl acetate extracts caused an intensified fragmentation of DNA in both MCF-7 and PC-3 cancer cell lines at higher concentration. This further supports our hypothesis regarding the toxic effects of the SEA extract on cancer cell growth.

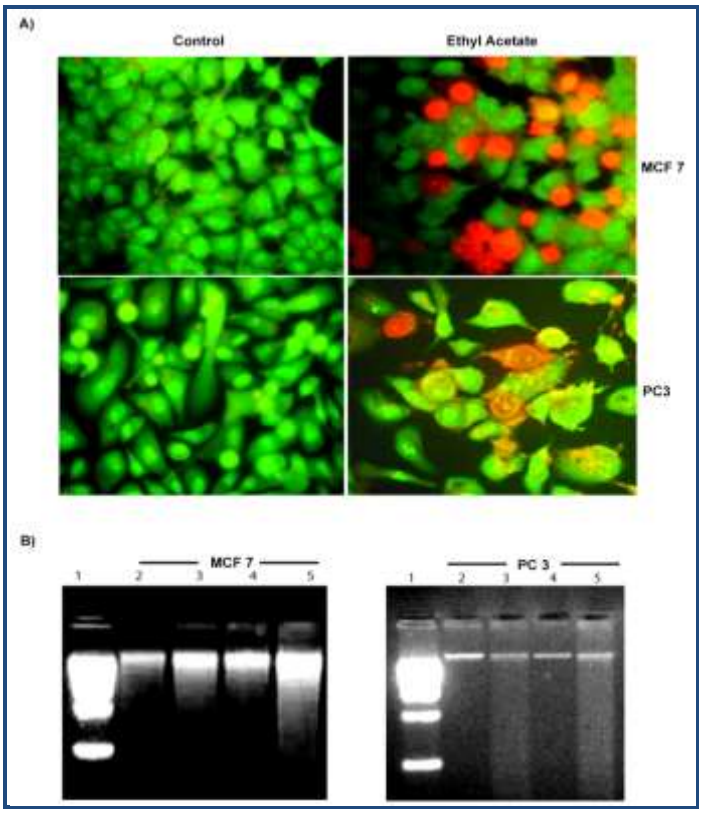


Figure 4: Apoptotic study with Acridine orange/Ethidium bromide and DNA fragmentation assay. A) Induction of apoptotic effect upon incubation of cells with *S.interrupta* root EA extract on both MCF-7 and PC-3 cells stating the early and late apoptosis depicted by orange and red colour respectively which was visualized by fluorescence microscope; **B)** Induction of DNA fragmentation by *S.interrupta* root extracts in MCF-7 and PC-3 cell lines. Lane1.1kb DNA Marker, Lane2. Control cells, Lane3. Root petroleum ether, Lane4. Root n-Butanol and Lane 5. Root ethyl acetate.

Molecular docking against TRAIL induced anti-apoptotic complex gene, DDX3

In search of anticancer compounds present in the *S. interrupta* we have identified four isolated compounds from the recently published paper **[25].** They are 1) O-Prenylated flavonol, 2) 2'-hydroxy-3, 4-dimethoxychalcone 3) biochanin A and 4) kaempferol-3-*O*-b-D-glucopyranoside (kaempferol). Among all kaempferol and biochanin A has shown to sensitize or enhance tumor necrosis factor-related *apoptosis-inducing* ligand (*TRAIL)* mediated cancer cell death **[26].** Biochanin A is belonging to a member of the class of 7-hydroxyisoflavones and is isolated from red clover *Triflolium pretense* **[27].** The molecular weight of this compound is 284.2 Da. Whereas, Kaempferol-3-O-β-D-glucopyranoside is a molecular weight of 448.37 Da and is isolated from ''*Phytolacca americana*'' (the American pokeweed). **[28].** TRAIL is a type II membrane protein and has shown to

enhance apoptosis by multi-component regulation via DEAD box RNA helicase, DDX3 **[29].** Therefore, we evaluated the interaction of *Sophora interrupta* flavones against cancer resistance marker, DDX3 by rational molecular docking approach. Five flexible amino-acid constraints (Tyr 200, Gln 207, Gly 229, Gly 227, and Thr 231) were detected with Crystallized ligand. The pyrimidine portion of adenine and sugar portion of nucleoside interacted with arginine 202, Glutamine 207 and with the side chain of Tyr 200 respectively. The phosphate group interacted with Thr 231, Gly 229 and Gly 227. On the other hand, *S.interrupta* flavones bound deep within a narrow pocket formed by the inner lobe cleft as similar to AMP **(Figure 5A)**. Among all four compounds tested kaempferol formed six (6) hydrogen bond interactions. Some important interactions between kaempferol and DDX3 include H-bonding between (1) Gly 227-NH---OH at 4th position of kaempferol (2) Gly 229 NH-OH at 4th position of kaempferol (3) Thr 231- NH-OH, CH-OH at 4th position of kaempferol (4) Gln 207-NH=OC at 8th position of kaempferol (5) Arg 202-C=O-HO at 9th position of kaempferol and (6) Ala 232-NH-OH at 5th position of kaempferol **(Figure 5B).**

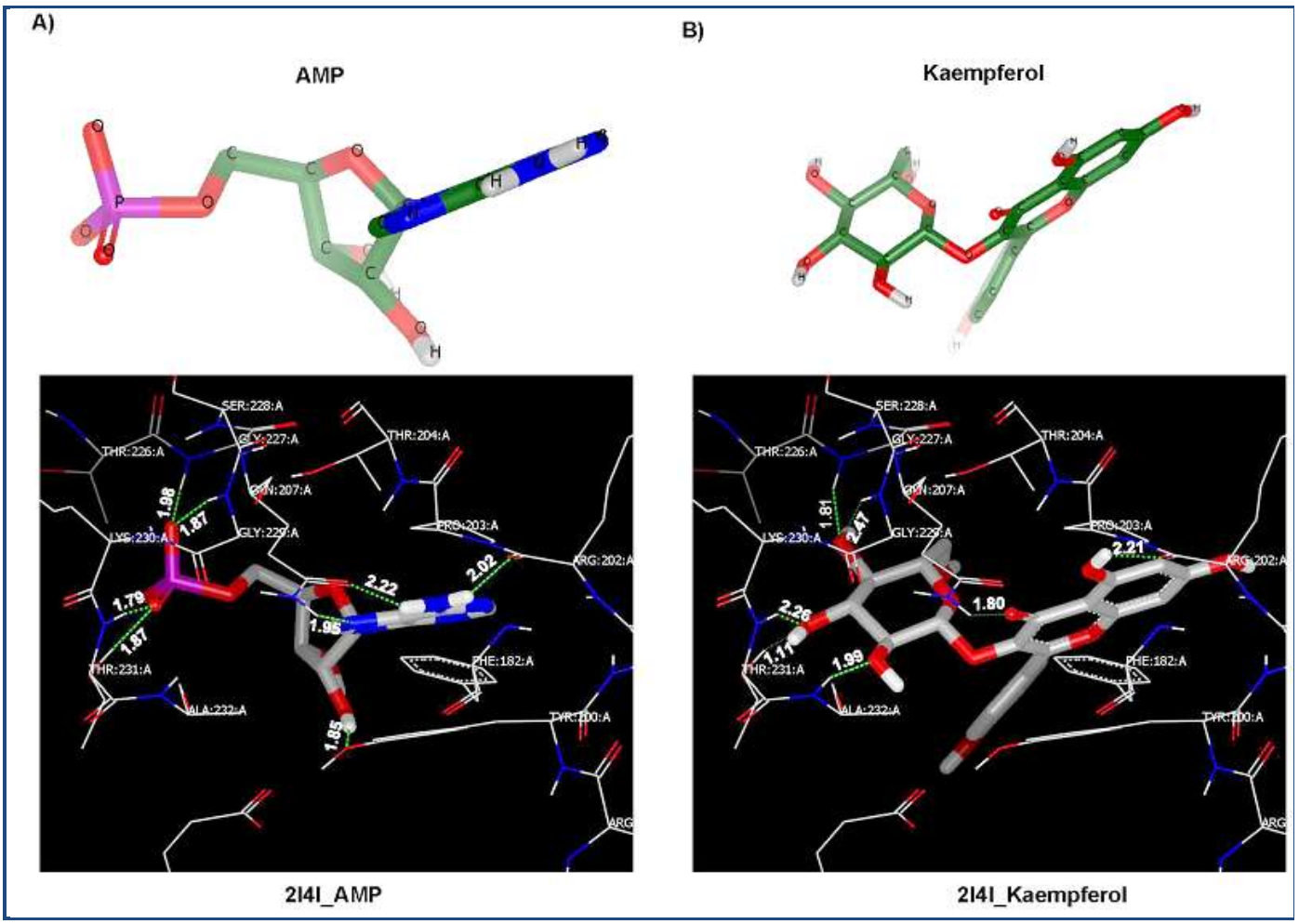


Figure 5: ***Insilico*** **molecular docking. A)** Protein-drug interaction against DDX3 (PDB ID: 2I4I) with AMP; **B)** Protein-plant compound interaction against TRAIL induced anti-apoptotic complex gene, DDX3 using molecular docking analysis. Kaempferol with 2I4I. Green dotted lines indicate direct hydrogen bond interaction with flexible aminoacid residues. Numbers indicates the hydrogen bond distance in Angstroms with their respective aminoacid constrains.

Discussion:

Herbal medicine is one of the oldest forms of health care known to mankind. In Ayurveda it is also known as phytomedicine, which uses various phytoprinciples such as alkaloids, steroids, tannins and flavonoids from medicinal plants to treat various human ailments. Flavonoids, alkaloids and glycosides has shown to be responsible for cure diabetes, obesity, inflammation associated diseases, cardiovascular diseases, cancer **[30].** These phytoprinciples differ from plant to plant due to vast biodiversity. An enormous number of plants, even though identified, their medicinal values are still with local people and tribal populations. Our interaction with local tribal people at forest area, we could come across one of the medicinal plant *Sophora interrupta* as an anticancer plant. *Sophora interrupta* commonly called as Adavibilla belonging to Fabaceae family and is distributed in temperate regions. This plant is abundant of alkaloids, tannins, flavonoids and phenol derivatives **[31].** In search of anti-oxidant potential of *S. interrupta* we have extracted the total phytochemical constituents from the root using ethyl acetate as an organic solvent. This extract is nominiclated as *S. interrupta* root ethyl acetate (SEA) extract. The anti-oxidative potential of SEA is comparable to that of Ascorbic Acid, a well-known anti-oxidant molecule. Oxidative cellular damage have shown to be primary cause of cancer **[32]**

and anti-oxidative mechanisms have shown to reverse the oxidative damage by regulating tumor supressor genes and their products **[33].** Cancer is not a single disease, it is a multifactorial disease, it can be also caused by inflammation. Inflammation has shown to diseemeniate the cancer cells from the site of origin, it is called as a metastasis. In order to understand the anti-inflammatory potential of SEA extract we have used red blood cells as an experimental model to determine the % of haemolysis and membrane stabilization. Because inflammation has shown to destabilize the membrane of red blood cells, thereby release inflamatory cytokines such as CXCR4 and others in the process of cancer dessimination **[34].**

Our results have shown that SEA can inhibit the percentage of haemolysis by preserving the stability of RBC membrane. Similar observations also found using a well known anti-inflammatory compound Diclofenac. Moreover, our results have shown that SEA can effectively inhibit the active proliferation of yeast cells, as a model system for studying the anti-proliferative activities in eukaryotes. Uncontrolled cell growth (Proliferation) is one of the major hallmark of cancer **[35].** Since SEA extract effectively inhibited the growth of proliferating cells, we evaluated its potential on two cancer cell lines of breast (MCF-7) and prostate (PC-3) origin. The cell viability assay detects the reduction of MTT by mitochondrial dehydrogenase to a blue formazan product, which infers the normal function of mitochondria and cell viability **[36].** SEA extract showed more suppression of MCF-7 than PC-3 growth as compared to other n-butanol and petroleum ether (PE) extracts, and is dose dependent **(Figure 3A & 2B).** However, the concentration below 100 µg/ml does not show any cytotoxicity against either cell line. To visualize the cancer cell viability under experimental conditions, we used in-vitro live/dead assay using Acridine orange (AO) and Ethidium Bromide (EB) dye method. Acridine orange is a cationic dye that enters only live cells and stains DNA green while Ethidium bromide stains dead cell's DNA orange and is excluded by live cells **[37].** Adopting this method, we found that, MCF-7 displayed more number of red fluorescent cells and PC-3 displayed few adherent cells. It suggests that the SEA extract has potent anticancer compounds, thereby regulating a primary homeostatic mechanism of cell division and cell death **[38].** This process is called as apoptosis and it can be determined by DNA fragmentation assay. The DNA fragmentation in presence of SEA extract is marked by the formation of tailing (200–300 kbp) on electrophoretogram resulting from DNA breaks. Overall, It suggests that, phytoconstituents of SEA extract possesses the anti-oxidant, anti-inflammatory and anti-proliferative activity, which is also evident from formation of apoptotic bodies and membrane distortions in MCF-7 and PC-3 cell lines. Similar observations were observed in phytoconstituents of *Pterocarpus marsupium* **[39].** Apoptosis is a cell death mechanism, by which cell number and cell cycle events are coordinated in many physiological settings under normal conditions. However, cancer cells are exceptional to normal cell cycle events, and they can easily escape from the cytolytic pathway, proliferate, metastasize and can bring down the quality of human life. Several cytotoxic drugs have shown to induce apoptosis in tumor cells.

However, those agents often have shown to generate side effects by affecting the viability of both tumor and normal cells. In search of compounds to trigger apoptosis in tumor cells without much affecting the normal cell functions. Towards that goal, researchers have identified member of the Tumor Necrosis Factor (TNF) family of "death receptors" and its ligand TRAIL (Tumor necrosis factor-Related Apoptosis-Inducing Ligand). TRAIL is a type II membrane protein and has shown to induce apoptosis by multi-component regulation via death receptor 4 (DR4, TRAIL-R1) and 5 (DR5, TRAIL-R2). The compounds present in the *Sophora interrupta* has shown to sensitize or enhance TRAIL mediated apoptosis in cancer cells, and it may dissolve the cancer resistance caused against TRAIL therapy **[40].** One of the markers associated in TRAIL induced cancer death is a DEAD box RNA helicase, DDX3 **[41].** DDX3 is a DEAD box RNA helicase family member and has shown to play a role in RNA metabolism and protein translation. To understand the nature of *S. interrupta* compounds towards TRAIL induced cancer cell death, we have performed molecular docking analysis. Among all the selected compounds, kaempferol form four hydrogen bond interactions with Gly 227, Gly 229, Thr 231 and Ala 232 as similar to the AMP catalytic binding site. Moreover, non-bonding interactions around the cavity are similar to the experimental data obtained from crystal structure as reported in earlier studies **[42].** The docking scores against selected natural compounds showed higher binding affinity towards DDX3, which is evident from the hybrid chemgauss4 score for Kaempferol (-8.786 K.cal/mol), and Biochanin A (-5.906 K.cal/mol) are significantly equal to the crystallized ligand, AMP (-6.233 K.cal/mol). Collectively, our results suggest that selected natural compounds from the *S. interrupta* may inhibit the function of DDX3 and kaempferol-3-O-b-D-glucopyranoside can be considered as potential bioactive molecules to treat various DDX3 associated diseases.

Conclusion:

Studies revealed that *S.interrupta* has significant antineoplastic effects on MCF-7 and PC-3 cell lines. The compounds present in *S.interrupta* root extract may enhance TRAIL mediated cancer cell death via interacting with anti-apoptotic protein, DDX3. More extensive studies need to be done with the active compounds to elucidate the mechanism of action of the compounds against cancer cells and apoptosis.

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