WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 6, 1479-1502. Research Article ISSN 2277– 7105

ANTICANCER EFFECT OF SAPONIN RICH FRACTION FROM *HELIANTHUS ANNUUS*: AN *IN SILICO* AND *IN-VITRO* STUDIES

Desai Tanvi H.*[a] and Joshi Shrikant V.[b]

[a]Shree Dhanvantary Pharmacy College, Kim, Gujarat, India.

[b]Maliba Pharmacy College, Bardoli - Surat, Gujarat.

Article Received on
18 April 2021,

Revised on 10 May 2021,
Accepted on 30 May 2021

DOI: 10.20959/wjpr20216-20588

***Corresponding Author**
Dr. Desai Tanvi H.
Shree Dhanvantary Pharmacy College, Kim, Gujarat, India.

ABSTRACT

Objectives Presented study was performed to explore the anticancer activity of saponin rich fraction of *Helianthus annuus* flower petals using in *silico* studies, MTT assay, shell-less chick embryo culture assay, chromosomal aberration assay and apoptosis assay. **Methods** *In silico* studies of helianthosides (A-C) were performed for the prediction of structure based pharmacological activities using PASS Online and Swiss Target Prediction softwares for the possibilities to be anticancer and apoptogenic along with docking studies using Autodock Vina. MTT assay was executing for antiproliferative action of saponin rich fraction on MCF-7 cells. For the evaluation of antiangiogenic properties, *in vitro* shell-less chick embryo culture assay was studied. chromosomal aberration was studied on normal human blood. Apoptosis assay was performed on MCF-7 cells using cell based ELISHA assay kit. **Result** MTT assay displayed IC50 value in MCF-7 cells at 2µg/mL. Considerable ($p<0.05$) decreases in angiogenic parameters were observed after treatment. Non significant aberrations were noticed following the treatment. Treatment causes activation of caspase 3 and caspase 8 (OD 0.42 and 0.33 respectively at 450 nm) in MCF-7 cells. **Conclusion** Saponin rich fraction of *H. annuus* showed antiproliferative, antiangiogenic and apoptogenic potential in *in vitro* models with non significant chromosomal aberrations in normal cells.

KEYWORDS: *Helianthus annuus*, MTT assay, Antiangiogenic assay, Chromosomal aberration assay, Apoptosis assay.

INTRODUCTION

No solitary has an idea that from when and where plants were instigated to be used to treat disease, but ancient myths seems to hint its establishment from the era of Stone.[1] There is a

proof that different elements of plants were extensively used by the tribal and pastoral communities of the Egyptian, India, Chinese, Greek and Roman civilization for the management of the diseases and for revitalizing body systems.[2] At the present time, rise in population, insufficient provider of drugs, unaffordable price of treatment and drugs resistance in communicable diseases[1]; plants are the nucleus of therapeutic resource not just in developing countries, but also in developed countries where recent medication are mostly used.[3] According to a current estimation of the World Health Organization (WHO), 75-90 % of the global population particularly in rising nations trusted on traditional medication, generally plant drugs for their prime health concern requirements.[4]

Helianthus annuus L. belongs to the Asteraceae (synonym Compositae) family is an ordinary and widespread roadside weed[5] and very flexible to environment, temperature and daylight.[5] *Helianthus annuus* has numerous common names, including Suraj mukhi (Hindi, Gujarati, and Bengali), Suria-mukhi Arkakantha (Sansrit); Surya phul (Mah), and Sunflower (English). It is an annual herb with a vertical, coarse, furry stem, 1- 2 m tall, branched at the top and blossom in delayed summer and autumn.[6] The sunflower is self-sterile, and fertilization is normally effected by insects.[7,8,9]

Helianthus annuus (HA) is believed to have originated from *H. lenticularis*-Douglas (from the Greek Helios, meaning sun, and Anthos, meaning a flower), a feral plant native to Mexico. *Helianthus annuus* is also local to eastern and central North America, and is cultivated globally. It has well recognized in the Argentina, Eastern and Southern Africa, Italy, the Balkans, Uruguay, Caucasus, the USA, Chile, parts of Asia and Australia, Ukraine, France, Canada, and several added nations.[10,11]

The sunflower is the center of therapeutic principles which is useful as food and medicine worldwide. *H. annuus* is cultured chiefly for its seeds, which provide the world's second most essential resource of edible oil. *H. annuus* has a higher nutrients substances like vitamins, portion, dietary fiber, amino acids, olic acid, carbohydrates, peptides, lipids, palmitic acid, quinic acid, other non-protein nitrogen, fatty acids tochophherol, caffeic acid, Stearidonic acid chlorogenic acid, linoleic acid, α-Linoleic acid, carotenoids, total minerals, sulphur, sodium calcium, magnesium phosphorus, and potassium.[12,13]

H. annuus also contains different biologically active non-nutritive chemicals known as phytochemicals that exhibit physiological effects which can be responsible for its curative and defense potential in a broad range of disease conditions. These imperative elements

obtained from *H. annuus* are flavonoids, carbohydrates, saponins, phytosterols, tannins, alkaloids, active proteins, flavanoids and fixed oils.[14] These components may confirm the folkloric medical appliances of the *H. annuus* for the treatment of numeral disease conditions such as cancer in Venezuela, rheumatism, malaria, heart disease, urinary tract infection, spasm of muscles, peptic ulcers, calming in cough, psoriasis, liver disorders, respiratory disorders, and skin lesions.[4,6,10,15,16] The seed oil, herb tincture, and shoot is utilize for antioxidant, diuretic, antigen, antiasthmatic, antimicrobial activities, antihypertensive, aperitif, anti-inflammatory, antihypoglycemic effect, antitumor, cathartic, expectorant, astringent, stimulant, antipyretic, vulnerary purposes, emollient, wound-healing and vermifuge.[17,18]

Sunflower can be consumed wholly by ruminants and no part of sunflower is poisonous. *Helianthus annuus* is well known as an ornamental plant and are very popular in the Chinese market, especially for five-star hotels and famous restaurants.[11,19] After sunflower harvesting, farmers pulverize the leftover sunflower stems and spread them in the farms as an organic fertilizer.

Seeds and petals of *Helianthus annuus* can be used as a snack, spreader on salads, soups and cereals, mixed with vegetables, to garnish cookies and cakes and as a coloring and flavoring agent in different recipes and in various bakery products. The seed oil and petals are used in cooking, candle, lubricants, soaps, paper making, potpourri, dye, as well as biofuel and biodiesel. Sunflower seeds are used medicinally to calm the blood vessels, nerves and muscles.[10] The oil-cake is a precious foodstuff for farm animals and rooster.[8, 9, 20, 21] In Chinese and Iranian ancient medication system herbal teas from sunflower petals were used to lower blood pressure, accelerate childbirth, strengthen the stomach, and heal wounds.[22] The leaves of sunflower have bitter bad taste and used as tonic, emetic, aphrodisiac and emmenagogue. They are also used to lessen inflammation, given in insanity, applied in lumbar pain, used in piles, applied in complaints of the liver, chest, lung and ascites, cure diseases of the kidney and ophthalmia.[23]

Heo et al in 2007 showed that aqueous extract of the Helianthus annuus seed were studied for antiproliferative and apoptogenic activity against SH-SY5Y neuroblastoma cells.[24] Even though no report was found regarding saponin rich fraction of *H. annuus* in anticancer activity.

In the present study saponin rich fraction from hydroalcoholic extract of *H.annuus* was separated and used for various *in silico* and *in vitro* anticancer studies. *In silico* structure based pharmacological activity of helianthosides (A-C) was predicted for common actions like stimulation of caspase 3 and 8 by PASS Online software and apoptogenic potential was studied by SwissTargetPrediction Software. Even we docked helianthosides (A-C) with protein structures of caspase 3 and 8 by using Autodock Tool and Autodock Vina. Antiproliferative activity of saponin rich fraction was examined with MTT assay on MCF-7 human breast cancer cells. *In vitro* shell-less chick embryo culture assay was performed on fertile eggs for the study of antiangiogenic properties of saponin rich fraction of *H. annuus* by ImageJ software using different 1µg/ml, 2µg/ml, and 4µg/ml concentrations. Chromosomal aberration assay was carrying out on normal human blood samples for the physical veracity of cells. Apoptosis assay was also studied on MCF-7 cells by using cell based ELISHA assay kit for the activation of caspase 3 and caspase 8.

MATERIALS AND METHODS

Plant extract, cell line, reference standards and solvent

Dried crude hydroalcoholic *Helianthus annuus* flower petals extract was acquired from Shree Hari Life Science Pvt Ltd; Vapi, Gujarat, India. N

Breast cancer MCF-7 cells from human were purchased from NCCS, Pune, Maharashtra, India.

Standard Gallic acid was purchase from Yucca Enterprises, Mumbai, Maharashtra, India.

Cleaved caspase-3 (Asp175) # 9661 and cleaved caspase- 8 (Asp 391) # 9496 were procured from Cell Signaling Technology, USA.

N- Butanol was procured from Loba chemie Pvt Ltd.

Fertile eggs were buying from local egg vendor.

Methods

Isolation of saponin from *Helianthus annuus*

Dried crude hydroalcoholic extract of *Helianthus annuus* (5gm) was added in 50 ml double distilled water. Poured it in separating funnel and added same volume 50 ml of n- butanol and permitted to stand entire night for fractionation. At 45°C n- butanol portion was evaporated for dry saponin rich fraction.[25]

Fingerprinting of *Helianthus annuus*

For Fingerprinting of *H. annuus* CAMAG HPTLC system outfitted with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, restricted by WinCATS- 4 software was utilized. 20 µL of standard gallic acid and crude hydroalcohic extract of *H. annuus* were placed in a bends form on pre-coated silica gel aluminum plate 60F-254 (5×10 cm) of 200 µm thickness with a Camag microlitre syringe by the use of Camag Linomat IV (Switzerland). Gallic acid and crude extracts were dissolved in methanol to get the concentration of 10µg/10µl. 30 min prior Camag twin-trough glass chamber was saturated with Toluene: Ethyl acetate: Formic acid: Methanol (3: 3: 0.8: 0.2 v/v/v/v) mobile phase and plate was then developed in the chamber till 85mm distance. Dried the plate in air to remove mobile phase and scanned it at wavelength of 254nm with a slit dimension of 6×0.40mm and a scanning speed of 10mm/s with a Camag TLC scanner.[26,27]

In silico Pharmacological activity prediction and Molecular docking studies

Pharmacological activity prediction in PASS Online:Structures of helianthosides (A-C) were downloaded obtainable format from websites and changed them in to molecular data file format i.e. mol expansion by the use of software OpenBabel 2.4.1. and mol files were subsequently uploaded in PASS online software for prediction of pharmacological actions. Results were scrutinized for Pa (probability to be active) and Pi (probability to be inactive) values of Caspase 3 and Caspase 8 stimulations.[28, 29, 30]

For Molecular docking with AutoDock Vina. Molfiles of helianthosides (A-C) were renewed to protein data bank (.pdb format) with OpenBabel 2.4.1. Protein structures of Caspase 3 (PDB ID - 3DEI) and Caspase 8 (PDB ID – 3KJQ) were getting in. pdb format from Research Collaboratory for Structural Bioinformatics (RCSB) website and processed through AutoDock Tools 4.2.6 to get. pdbqt file format. On Caspase 3 and Caspase 8 ligand binding place were recognized by MetaPocket 2.0 and grid box parameters were determined. Obtained values were used for docking of corresponding proteins with helianthosides in Autodock Vina.[31,32]

MTT assay

The MTT[3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] antiproliferative assay was carry out to study cell viability and cell toxicity of saponin rich fraction of *H. annuus* by using different (0.001µg/ml, 0.01µg/ml, 0.1µg/ml, 1µg/ml and 10µg/ml) concentrations on MCF-7 cells of breast cancer. 10mg saponin rich fraction was added in DMSO of 10 ml for the dissolution of test compound and successively diluted with growth

media to get the different concentrations. DMSO was used < 0.1% concentration for all samples. MCF-7 cells maintained. in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) and incubated at 37°C of 5 % CO2 were placed in 96 well plates and treatment was given to cell with different concentrations of saponin rich fraction and incubated at 37°C, 5% CO2 till 96 hours. MTT dye was then added in wells and again incubated for four hours. Dark purple formazan product were formed by cells were dissolved with DMSO and measured spectrophotometrically at wavelength 550 nm. IC50 value was calculated by plotting the graph of Percentage inhibitions against concentrations.[33]

Shell less chick embryo culture assay

Fertilized eggs were incubated for 72 hrs and permitted them to cool for 25 min. After that eggs were wiping up with alcohol (70% v/v) to reduced contamination on shell surface. Albumin of the unfertilized egg was placed in sterile Petridish and act as a cushion for cultures. Subsequently incubated eggs were break from the above by the use of spatula and eggs contained were quietly released in the Petridish on the cushion. Effectively placed embryos were then separated in to control and different concentrations of (1µg/ml, 2µg/ml, and 4µg/ml) of saponin rich fraction of *H. annuus* treated groups. Saponin rich fraction was added in dose of 10 µl in the area where blood vessels developed. Then all Petridish were closed by lid and again incubated at 37.5°C and 80% humidity. Later than 3hrs and 6 hrs photographs of embryo were captured and processed with ImageJ 1.50 b angiogenesis analyzer to study the differentiations in number of branches, nodes, junctions, extremities and total branches length.[34]

Chromosomal aberration (CA) assay

2.5 ml fresh blood samples were collected from non-smoking, healthy and randomly selected female individuals of the age between 20-25 years for chromosomal aberration study. For study one vial was used as control and other three were used as different concentrations (1µg/ml, 2µg/ml and 4µg/ml) of saponin rich fraction of *H. annuus* treated vials in a dose of 50 µl. In each vials 5ml PB-MAX TM karyotyping media, 50µl Heparin, 0.6 ml peripheral blood were added and incubated for 72 hrs. After 24 hrs of incubation saponin rich fraction of *H. annuus* of different concentrations was added in three vials. At the end of 69th hr or starting of 70th hr 100µl of colchicine was added in all vials. And after completion of 72 hr, the vials were centrifuged for 8 min at 1200 rpm, discarded the supernatant and 5ml of 0.56%

KCl was added. These mixtures were incubated for 25 min at 37°C which allows the cells to swell. The tubes were centrifuged again at 1200 rpm for 8 min. The supernatant was removed, cells were fixed with 6 ml carnoy's fixative (methanol: glacial acetic acid 75:25v/v) and incubated for 1 hr in refrigerator. The samples were centrifuged again for 8 min at 1200 rpm, Supernatant was removed and using 3ml fixative, the washing step was continued till white pellet obtained. The slides were prepared by dropping 4-5 drops of cell suspensions from a suitable height on clean pre-chilled slides. The slides were coded and used for scoring after staining with 2% giemsa stain for 7-8 min and rinsing in distilled water. 100 metaphases were scored in each group using compound light microscope at magnification of 100x for CAs.[35]

Apoptosis assay

Apoptosis assay was performed on human MCF-7 breast cancer cells using cell based ELISA assay kit for the assessment of cleaved caspase-3 and caspase- 8. MCF-7 cells were positioned in 96 well plates and after incubated overnight cells were treated with saponin rich fraction of *H. annuus* at a dose concentration of 10µg/ml. Cells were incubated again for 6 hours at 37°C and 5% CO_2. Latterly growth medium was discarded from the wells and cells were cleansed with PBS. Washed cells were filling with 0.5% triton X for 2 minutes and fixed in methanol for 10 minutes. The cells were again washed three times with PBS and treatment of cleaved caspase-3 (Asp175) # 9661 and cleaved caspase- 8 (Asp 391) # 9496 was given for couple of hours. Further cells were rinse with PBS and treated with corresponding HRP conjugated secondary antibodies for 30 minutes. After 30 minute wells were cleaned thrice and detect with TMB substrate and ODs were measured at 450 nm by using Fluostar (BMG Germany).[36]

Statistical analysis

The data were articulated as mean ± S.E.M. (standard error of the mean) investigational groups. Statistical evaluation amongst treated and control groups were carrying out by one-analysis of variance (ANOVA) followed by turkey's post hoc test. Minimum considerable level was recognized at $p<0.05$ (95% confidence interval).

RESULTS

Extraction yield for isolation of Saponins

Isolation of saponin rich fraction was performed by using water and n-butanol separation procedure. We obtained the yield of 3.2% of saponins from crude hydroalcoholic extract of *H. annuus* flower petals.

Fingerprinting of *Helianthus annuus*

Chromatograms of standard gallic acid and crude hydroalcoholic extract of *H. annuus* were obtained by using solvent system of Toluene: Ethyl acetate: Formic acid: Methanol (3: 3: 0.8: 0.2 v/v/v/v). Rf values were same for the compound of 0.84 at 254 nm. (Fig. 1and 2A, 2B)

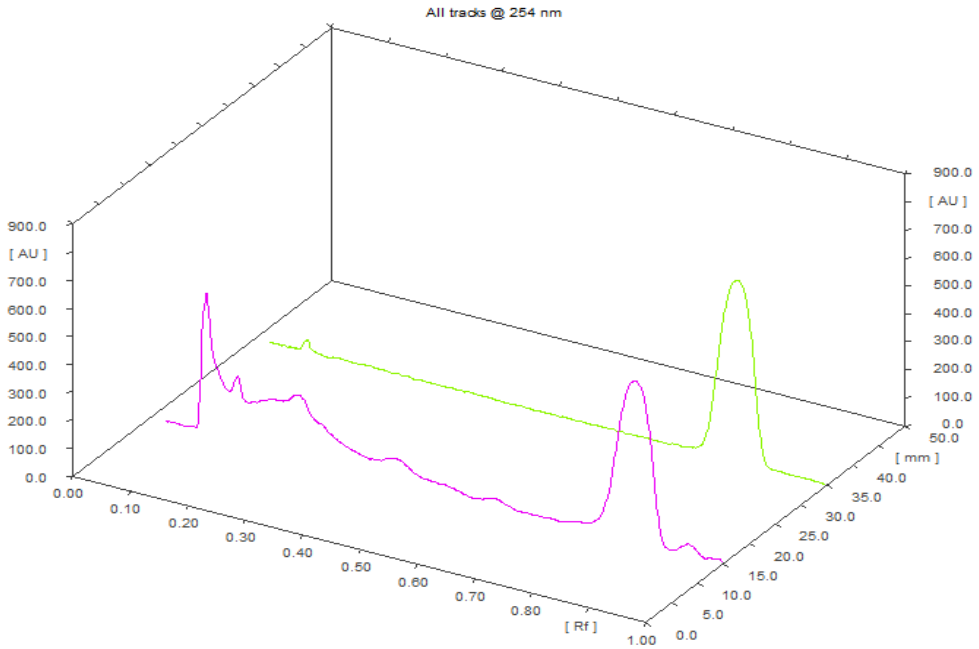
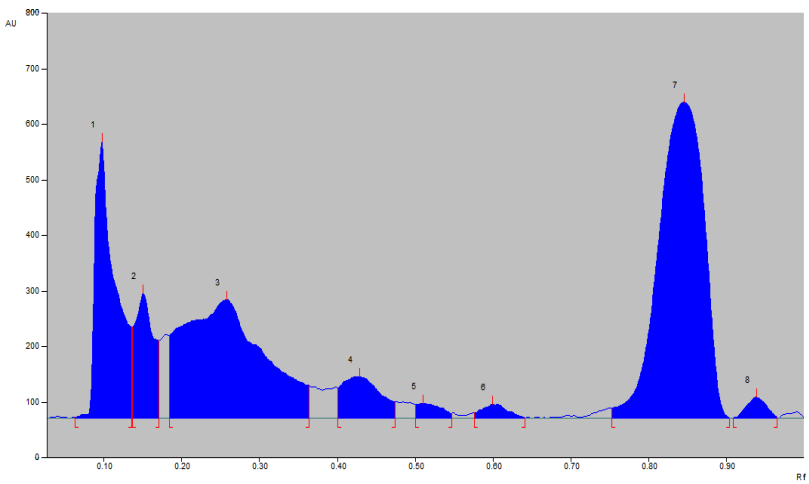


Fig. 1: 3D HPTLC Cromatogram for *H.annuus* extract and standard Gallic acid.

A



B

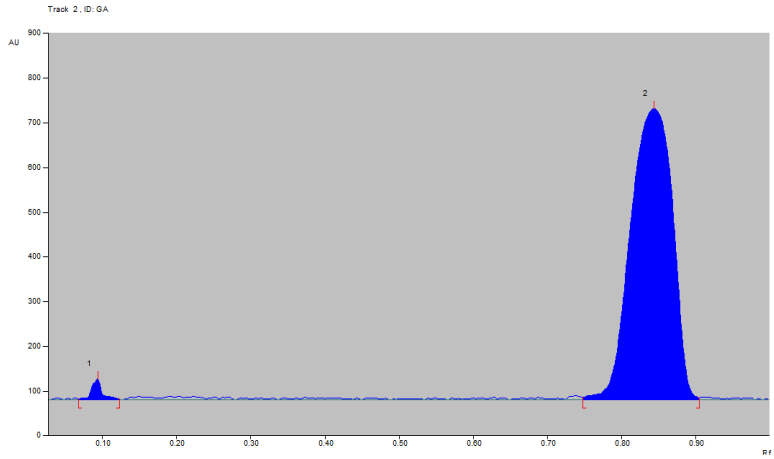
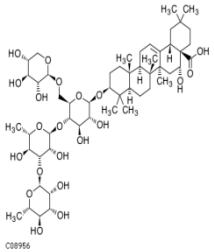
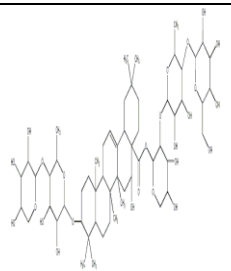
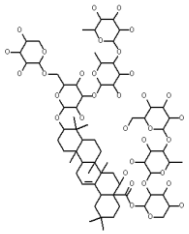


Fig 2. (A) HPTLC chromatogram for *H.annuus* extract; (B) HPTLC chromatogram of standard Gallic acid.

In silico Pharmacological activity prediction and Molecular docking studies

For the prediction of structure based pharmacological actions of helianthosides (A-C) PASS Online and SwissTargetPrediction Softwares were used. Results obtained in PASS Online software with Pa (probability to be active) and Pi (probability to be inactive) values for Caspase 3 and Caspase 8 stimulation were reported. SwissTargetPrediction Software expects apoptogenic action for helianthosides. (Table 1)

Table 4: PASS Online prediction and Molecular Docking studies of triterpenoids from *H. annuus.*

Sr. No.	Name of Triterpenoids	Structure	Caspases 3 stimulation					Caspases 8 stimulation				
			Passonline Prediction Data		Binding energy (Kcal/mol)	Amino acid residue	Hydrogen Bond	Passonline Prediction Data		Binding energy (Kcal/mol)	Amino acid residue	Hydrogen Bond
			Pa	Pi				Pa	Pi			
1	Helianthoside A		0.997	0.000	-9.1	Glu123 Gly122 Rxb300 Gly165 His121 Tyr204	1	0.994	0.000	-8.0	Thr149 Pro415 Tyr412 Ser316 His317	11
2	Helianthoside B		0.998	0.000	-9.4	Glu123 Gly122 Rxb300 Gly165 His121 Tyr204	1	0.996	0.000	-8.7	Thr149 Pro415 Tyr412 Ser316 His317	11
3	Helianthoside C		0.997	0.000	-10.8	Glu123 Gly122 Rxb300 Gly165 His121 Tyr204	1	0.994	0.000	-7.4	Thr149 Pro415 Tyr412 Ser316 His317	11

MTT assay

Cell viability and cell toxicity assay of saponin rich fraction of *H. annuus* was performed on human MCF-7 breast cancer cell line by the use of MTT antiproliferative assay method. Cells were placed in 96 well plates and treated them with different (0.1µg/ml, 0.01µg/ml, 0.001µg/ml, 1µg/ml and 10µg/ml) concentrations of saponin rich fraction. IC50 value of 1 µg/ ml was observed by spectrophotometrically at 550nm. (Table 2 and Fig. 3).

Table 2: MTT assay of saponin rich fraction of *H. annuus*.

Concentrations	*H. annuus*
10	65.32
1	44.25
0.1	35.68
0.01	22.47
0.001	2.39
IC50 value µg/ml	2

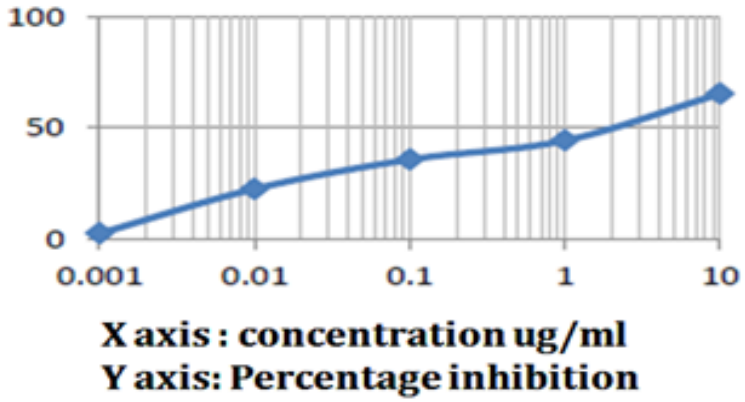


Fig 3. MTT assay of saponin rich fraction of *H. annuus*.

Shell less chick embryo culture assay

Shell less chick embryo culture assay showed significant ($p<0.05$) decrease in no. of Extremities, no. of nodes, no. of junctions, no. of branches and Total branches length after the treatment of saponin rich fraction among 0-3 hour and 0-6 hour. (Table 3 and Fig. 4).

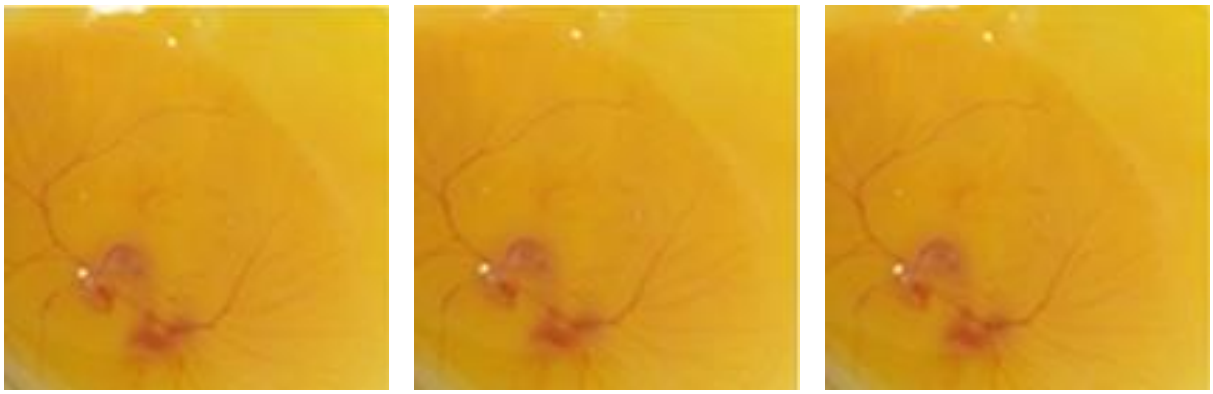
Table 3: Effect of saponin rich fraction of *Helianthus annuus* on Angiogenic parameters in Shell-Less Chick Embryo Fibroblast Culture System.

Concentrations	% Decrease in no. of Extremities		% Decrease in no. of Nodes		% Decrease in no. of Junction		% Decrease in no. of Branches		% Decrease in Total branch length	
	0-3 hrs	0-6 hrs	0-3 hrs	0-6 hrs	0-3 hrs	0-6 hrs	0-3 hrs	0-6 hrs	0-3 hrs	0-6 hrs
Control	0	0	0	0	0	0	0	0	0	0
1 µg/ml	17.00 ± 1.26*	35.76 ± 1.93*	26.16 ± 1.89*	43.47 ± 1.17*	24.78 ± 1.41*	43.55 ± 2.35*	23.18 ± 1.24*	38.04 ± 2.03*	29.74 ± 0.97*	44.66 ± 2.93*
2 µg/ml	29.67 ± 0.67*	48.41 ± 1.54*	37.43 ± 1.83*	52.26 ± 2.38*	33.67 ± 2.73*	35.52 ± 1.55*	34.24 ± 2.80*	48.05 ± 3.82*	39.61 ± 1.23*	61.47 ± 2.83*
4 µg/ml	38.54 ±1.63*	58.99 ± 3.18*	45.78 ± 1.86*	59.57 ± 1.83*	46.79 ± 2.23*	56.59 ± 2.81*	47.21 ±3.00*	57.78 ± 1.42*	49.34 ± 4.54*	63.47 ± 1.73*

Each values and bar expressed as mean ± SEM (n=6) in each group. Statistical analysis: One-way ANOVA followed by Turkey's post hoc test.

*P<0.05 as compared to control

For control

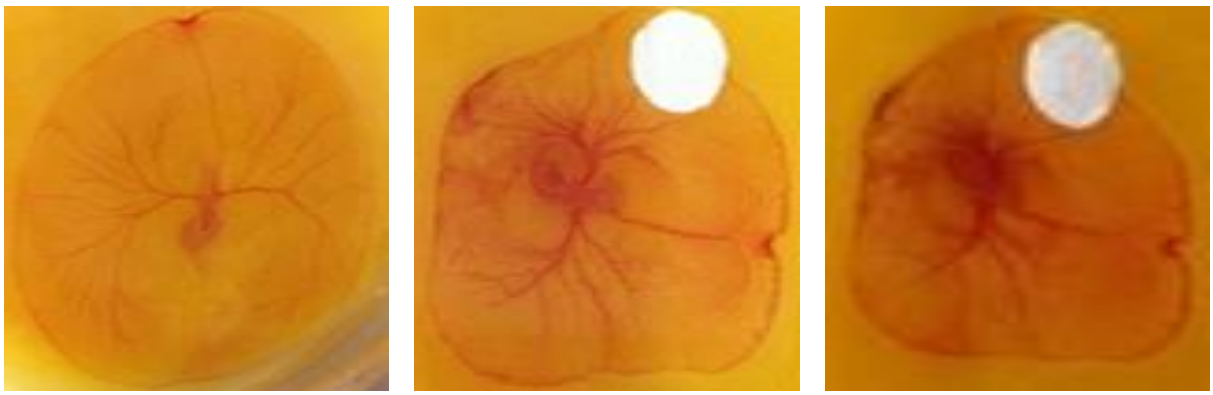


0 hrs **3 hrs** **6 hrs**

For 1 µg/ml saponin rich fraction of *H. annuus*

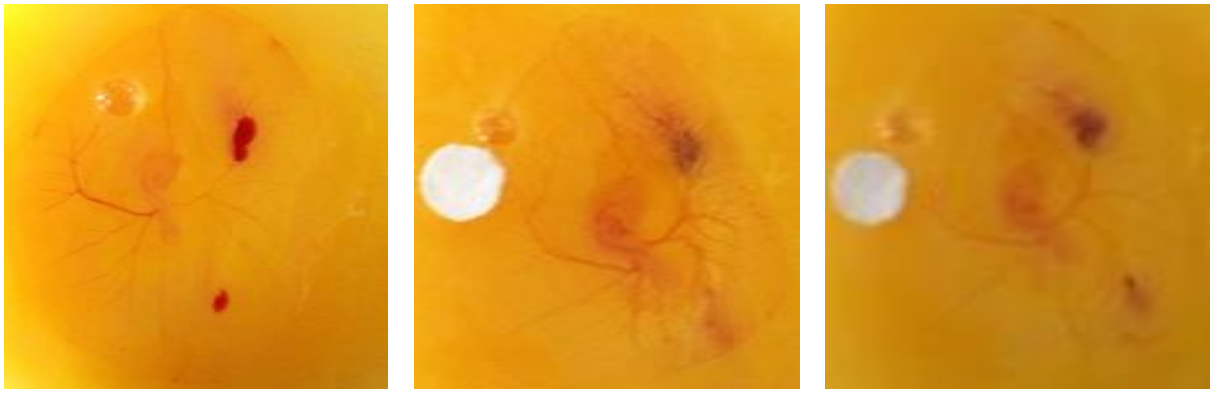
0 hrs **3 hrs** **6 hrs**

For 2 µg/ml saponin rich fraction of *H. annuus*



0 hrs **3 hrs** **6 hrs**

For 4 µg/ml saponin rich fraction of *H. annuus*



0 hrs **3 hrs** **6 hrs**

Fig. 4: Photographic images of Shell–less Chick Embryo Fibroblast culture treated with different concentrations of saponin rich fraction of *H. annuus* at different exposure time and different concentrations.

Chromosomal aberration (CA) assay

Saponin rich fraction of *H. annuus* was added in dose of 50 µl of dissimilar concentration (1 µg/ml, 2 µg/ml, 4 µg/ml) in cultured vials showed no significant change in number of total chromosomal aberrations as compared to control group (Table 4 and Fig 5).

Table 4: Chromosomal aberration observed in *in vitro* cultured human blood after addition of saponin rich fraction of *H. annuus.* (Figures indicated total aberration per 100 cells).

Aberration	Normal (Control)	saponin rich fraction of *H. annuus* (1 µg/ml)	saponin rich fraction of *H. annuus* (2µg/ml)	saponin rich fraction of *H. annuus* (4 µg/ml)
Chromatid Break	8.66 ± 0.88	6.33 ± 1.20	7.00 ± 1.15	6.00 ± 1.52
Chromosomal Gap	7.66 ± 0.33	6.33 ± 0.88	6.66 ± 1.45	7.00 ± 1.52
Chromosomal Break	8.66 ± 088	7.33 ± 1.20	7.33 ± 0.66	8.00 ± 1.15
Dicentric Fragments	4.00 ± 0.57	4.66 ± 0.66	4.00 ± 0.57	4.33 ± 1.20
Acentric Fragment	8.33 ± 0.88	7.33 ± 0.88	8.66 ± 0.33	6.66 ± 1.20
Premature Separation	4.00 ± 1.15	2.66 ± 0.66	2.66 ± 0.66	4.33 ± 0.33
Hypodiploid	10.33 ± 0.66	9.33 ± 1.45	9.66 ± 0.88	9.66 ± 0.88
Hyperdiploid	1.33 ± 0.33	3.33 ± 0.33	3.00 ± 5.77	4.33 ± 2.02

Each values and bar expressed as mean ± SEM (n=3) in each group. Statistical analysis: One-way ANOVA followed by Turkey's post hoc test. *P<0.05 as compared to control.

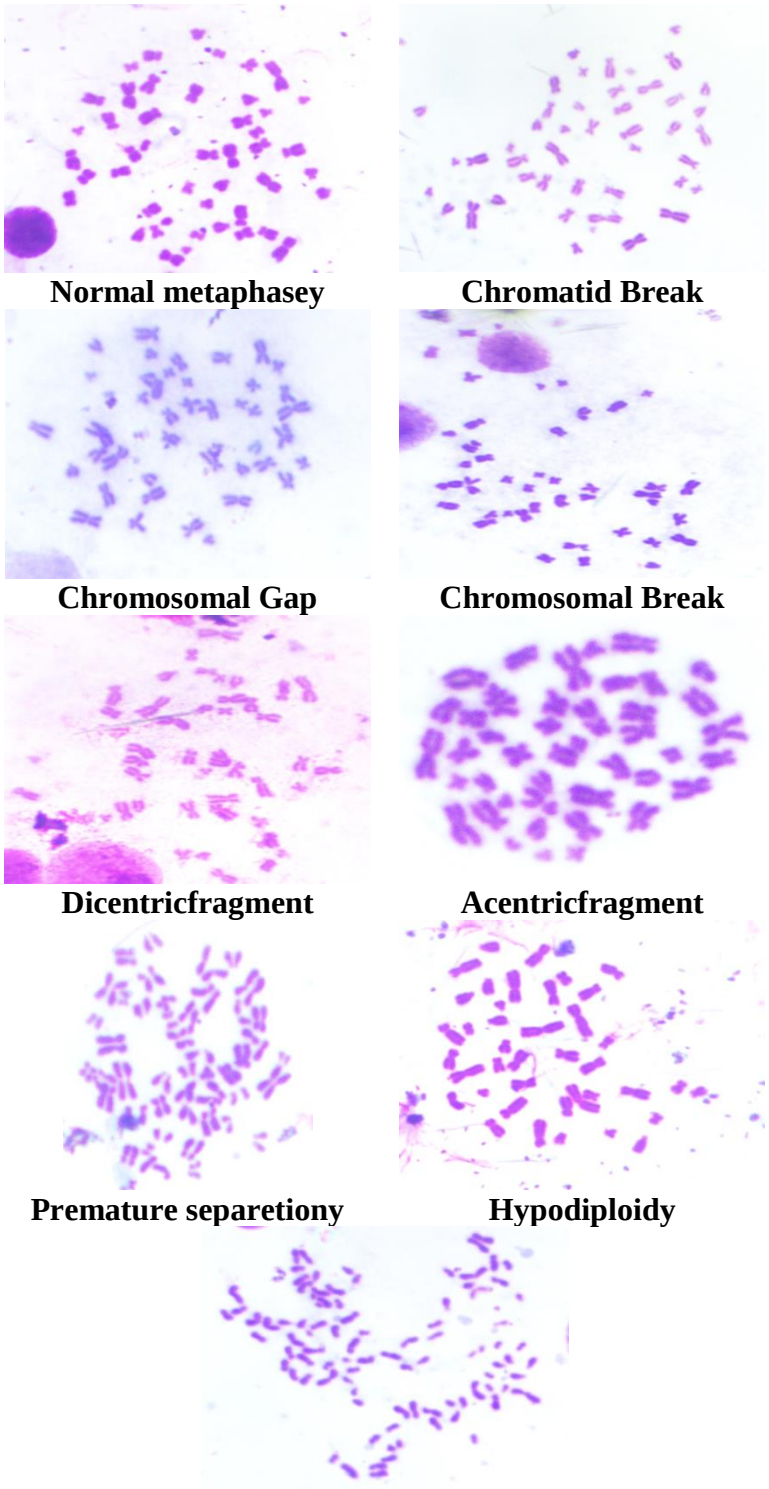


Fig. 5: Representative pictures of Chromosomal Aberration.

Normal metaphase: Number of chromosomes is from 44 to 48, **Chromatid break:** Break in one sister-chromatid at any one locus, **Chromosomal break:** Breaks in both sister-chromatids at any one locus, **Chromosomal gap:** Gap between the chromosomes from centromere**, Dicentric fragment:** Abnormal chromosome with two centromeres. It is formed

through the fusion of two chromosome segments, **Acentric fragment:** A segment of a chromosome that lacks a centromere, **Premature separation:** Premature loss of cohesion between centromeres results in their independent segregation at meiosis I, **Hypodiploidy:** Number of chromosomes are >48, **Hyperdiploidy:** Number of chromosomes are <44.

Apoptosis assay

Stimulation of caspase 3 and 8 was observed in human MCF-7 breast cancer cells after the treatment of 10µg/ml saponin rich fraction of *H. annuus* (Table 5 and Fig 6).

Table 5: Apoptosis assay of saponin rich fraction of *H. annuus* for Caspases 3 and 8.

Sample	Concentration (µg/ml)	OD at 450 nm	
		Caspase 3	Caspase 8
Saponin rich fraction of *H. annuus*	10	0.42	0.33
Staurosprin	1	2.47	2.65

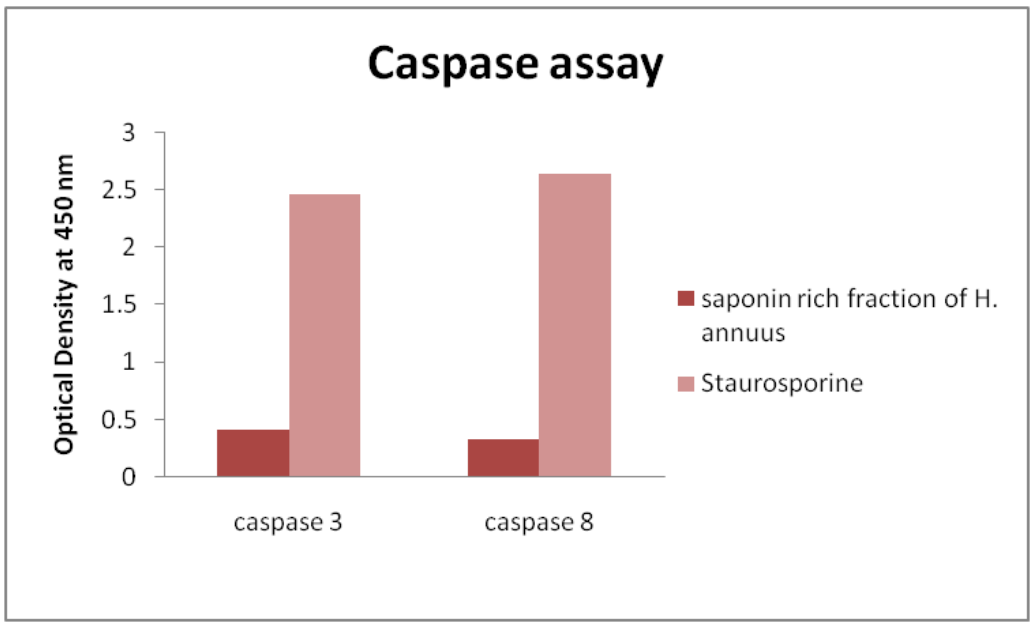


Fig. 6: The OD 450nm corresponding to cleaved caspase-3 (Asp 175) and caspases-8 (Asp 391) with saponin rich fraction of *H. annuus* at 10 µg/ml and reference standard staurosporine 1 µg/ml.

DISCUSSIONS

Nature has extensive, an imperative resource of medical agents[37] and medicinal plant have been acknowledged universally for curing of ailment in conventional mode form numerous years. The relations among traditional medication and advance biotechnological tools are to be found towards development of newer treatment.[38] Recently different Phytochemical offered defense mechanisms against variety of chronic ailments including cardiovascular

diseases, obesity, diabetes, and cancer.[39,40] Saponins are common in a variety of higher plants[41] and secondary metabolites of a diverse group of compounds with high chemoprevention potential. It is also reported that saponins having broad spectrum of anticancer activity, antiproliferative activity and stimulate the apoptosis in cancer cells.[42] Therefore in presented study isolated saponin rich fraction from *H. annuus* was studied for its cell viability and cytotoxicity, anti angiogenesis effect, aberrations in chromosomes of normal cells and responsibility in stimulation of caspases in apoptosis assay.

For *in silico* studies from the available sources we find out helianthosides (A-C) structures, for prediction of pharmacological activities uploaded in PASS Online software. Predicted results were filtered for stimulation of caspase 3 and caspase 8 and examine for further processing. For docking studies binding pocket on caspase 3 and caspase 8 were identified using Metapocket 2 and docking of these three compounds were done aiming these pockets through AutoDock Vina and AutoDock Tool. The least binding free energy of -9.1, -9.4 and -10.8, was shown by helianthoside A, helianthoside B, helianthoside C respectively for caspases-3. And -8.0, 8.7 and -7.4 by by helianthoside A, helianthoside B, helianthoside C respectively for caspases-8 (Table 1).

Cell-based assay was used to display the potency of saponin rich fractions by incorporating cell culture under restricted conditions with uncomplicated procedures. *In vitro* cytotoxicity test was use to evaluated drug effectiveness based on mitoc1hondrial enzyme activity and MTT assay was the method for the prediction of drug response On MCF-7 cells.[43] MTT antiproliferative assay is a colorimetric assay (measures colour changes)[44] to determine amount of viable cells in the 96 wall plate.[45] In these assay capacity of mitochondrial succinate dehydrogenase enzymes of viable cells to transform the MTT (3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide) into its blue formazan products was measured.[46, 47] Change in quantity of formazan product formed via cells, showing the cytotoxicity and IC50 value of drug. The lesser the IC50 value, the more cytotoxicity of the substance. In this study MCF-7 breast cancer cells were treated with different concentrations (0.1µg/ml, 0.01µg/ml, 0.001µg/ml, 1µg/ml and 10µg/ml) of the saponin rich fraction of *H.annuus* IC50 value was calculated by using percentage inhibitions. IC50 of saponin rich fraction of *H.annuus* was found to be 2 µg/ml.

An angiogenesis is the event of the development of new blood vessels from existing ones. It is an important and normal procedure concerned in several physiological actions, such as,

wound healing, embryonic development, organogenesis and inflammation.[48] Sometimes human being losses command among angiogenesis stimulators and inhibitors, which directed the unwarranted angiogenesis.[49] Cancer development and metastasis are identified to dependable on the angiogenesis and therefore, targeting angiogenesis might become an important strategy to hold tumor development and attack on tissues.[50] For these here we studied anti-angiogenic parameters for saponin rich fraction of *H. annuus* in shell–less Chick Embryo Fibroblast Cultures. In shell-less culture method, chick embryo is taken out from an egg shell and cultivated in an artificial atmosphere. This method is easy to access, examine and treat.[51] Here by treating the shell–less Chick Embryo with various concentrations of saponin rich fractions we observed considerable ($p<0.05$) decreases in number of Extremities, nodes, junctions, branches and total branches length within 0-3hours and 0-6 hours.

Genomic unsteadiness and Chromosomal aberrations (CAs) are one of the imperative biological damage revealed that populace with major number of CAs in their peripheral blood lymphocytes have a considerably higher possibility of growing tumor.[52] For chromosomal aberrations assay Human peripheral blood lymphocytes offer an excellent investigation method. This method is easily cultured, rise speedily to offer more numbers of mitotic cells, and preserve a diploid karyotype in short-term culture.[53] CAs comprises chromosomal exchanges and breaks visually within arrested metaphases and are generally separated into chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs), which are morphologically different with each other. CSAs engage the similar position on both sister chromatids on one or numerous chromosomes, while CTAs involve one sister chromatids of a chromosome or more chromosomes.[54] This study was carry out in vitro Cultured Human Lymphocytes with and without saponin rich fraction of *H. annuus* for chromosomal aberrations. Results of this assay illustrated non-significant relationship between control and treated cultures for chromosomal aberration like chromatid break, chromosome breaks, chromosomal gaps, acentric fragments, dicentric fragments, premature separation, hypodiploidy and hyperdiploidy.

Cell fatality can be a reason of necrosis and apoptosis.[55] Apoptosis is the mechanism of programmed cell death that eliminates unnecessary cells in biological system.[56] Contraction of cells, morphological alteration, growth of apoptotic bodies, decreases of cell organelles and reduction of chromatin are connected with apoptosis.[57] Apoptosis needed the activation of cysteine aspartyl proteases (caspases) family[58] and initiation of apoptosis through

intrinsic and extrinsic pathways is one of the best strategies for cancer treatment.[59] There are mainly two caspases, caspase 8 and caspase 9 which catalyze the stimulation of caspase 3.[60] For an extrinsic pathway caspase 8 is considering being the initiator while caspase 9 is an inventor for the intrinsic pathway.[61] Therefore, searching of natural composite through the capability to bring apoptosis in cancer cells has achieved more concentration.[62] Here we studied caspase activation by natural compound saponin rich fraction of *H. annuus* by *in vitro* ELISHA cell based assay on MCF-7 cells. Results of apoptosis assay showed that elevate in caspases-3and caspase-8 levels in MCF-7 cells (optical density of 0.42 and 0.33 respectively at 450 nm) after the treatment with saponin rich fraction *of H. annuus* (10µg/ml).

CONCLUSION

Results of different in vitro studies clear the antiproliferative, anti-angiogenic and apoptotic action of the saponin rich fraction of *H. annuus* flower petals. Chromosomal aberrations in normal human blood lymphocyte was non-significant suggested that saponin rich fraction of *H. annuus* did not causes any DNA damage or toxicity in normal cells. Hence, current studies scientifically confirm the predictable consumption and eventual utilization of *Helianthus annuus* in management of cancer.

Conflict of interest

The authors hereby declare that there is no conflict of interest.

REFERENCES

1. Singh A, Sharma M. Medicinal Herbs of Punjab (India). Biol Forum, 2018; 10(2): 10-27.
2. Al-Jumaily RMKh, Al-Shamma NMJ, Al-Halbosiy MMF, Al-Shamma LMJ. Anticancer Activity of Sunflower *(Helianthus annuns* L.*)* Seeds oil against cell lines. Iraqi J Sci., 2013; 54(4): 1003-1009.
3. Dwivedi A, Sharma GN. A Review on Heliotropism Plant: *Helianthus annuus* L. J Phytopharmacol, 2014; 3(2): 149-155.
4. BashirT, Mashwani ZUR, Zahara K, Haider S, Tabassum S, Mudrikah. Chemistry, Pharmacology and Ethnomedicinal Uses of *Helianthus annuus* (Sunflower): A Review. Pure Appl Biol., 2015; 4(2): 226-235.
5. Guo S, Ge Y, Jom KN. A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus* L.). Chem Cent J., 2017; 11: 95-104.

6. Liang Q, Cui J, Li H,Liu J, Zhao G. Florets of Sunflower (*Helianthus annuus* L.): Potential New Sources of Dietary Fiber and Phenolic Acids. J Agric Food Chem., 2013; 61: 3435−3442.
7. Kirtikar KR, Basu BD. Indian medicinal plant. Vol. 2. New Delhi, India: Sri Satguru Publications, 2006; 1370-1371.
8. Chopra RN, Chopra IC, Varma BS. Glossary of Indian medicinal plants. New Delhi, India: Publicationand Information Directorate, CSIR, 2006; 131.
9. Anon. The Wealth of India: A dictionary in Indian raw material and industrial products. In B. N. Sastri (Ed.). New Delhi, India: Publication and Information Directorate, CSIR, 2001; 17-26.
10. Pal D. Sunflower (*Helianthus annuus* L.) Seeds in Health and Nutrition. Nuts and Seeds in Health and Disease Prevention, 2011; 1097–1105.
11. Gonzalez-Perez S, Vereijken JM. Sunflower proteins: overview of their physicochemical, structural and functional properties. J Sci Food Agric, 2007; 87: 2173–2191.
12. Holliday R, Phillips K. Health benefits of the sunflower kernel. Cereal Foods World, 2001; 46: 205-208.
13. Boriollo MF Souza LS, Resende MR, Silva TA, Oliveira Nde M, Resck MC, Dias CT, Fiorini JE. Nongenotoxic effects and a reduction of the DXR-induced genotoxic effects of *Helianthus annuus* Linné (sunflower) seeds revealed by micronucleus assays in mouse bone marrow. BMC Complement Altern Med., 2014; 14(1): 121.
14. Ibrahim TA, Ajongbolo KF, Aladekoyi G. Phytochemical screening and antimicrobial activity of crude extracts of *Basella alba* and *Helianthus annuus* on selected food pathogens. J Microbiol Biotechnol, 2014; 3(2): 27-31.
15. Al-Shamma LMJ, Bu-risha R, Al-Shamma NMJ, Batol K Effect some of sunflower genotypes oil on some pathogenic bacteria. Iraqi J Sci., 2010; 51(4): 565–570.
16. Saini S. Sharma S. 2011. *Helianthus annuus* (Asteracea) Review. Inter J Phar Profess Res., 2011; 2(4): 465–470.
17. Owler MW. Plants, medicines and man. J Sci Food Agric, 2006; 86(12): 1797–1804.
18. Ukiya M, Akihisa T, Yasukawa K, Koike K, Takahashi A, Suzuki T, Kimura Y. Triterpene Glycosides from the Flower Petals of Sunflower (*Helianthus annuus*) and Their Anti-inflammatory Activity. J Nat Prod, 2007; 70: 813-816.
19. Sun Z, Chen J, Ma J, Jiang Y, Wang M, Ren G, Chen F Cynarin-Rich Sunflower (Helianthus annuus) sprouts possess both Antiglycative and Antioxidant activities. J Agric Food Chem., 2012; 60: 3260−3265.

20. Bruneton, J. Pharmacognosy, phytochemistry of medicinal plants, 2nd edn. Andover, UK: Intercept Ltd, 1999; 151-152.
21. LaGow, B. PDR for herbal medicines. Montvale, NJ: Thomson Healthcare PDR, 2004: 803-804.
22. Mirzabe AH, Cheginib RG, Khazaei J. Sunflower petals: Some physical properties and modeling distribution of their number, dimensions, and mass. Inf Process Agric, 2018; 5: 185–198.
23. Khan NI, Shinge JS, Naikwade NS Antilithiatic effect of *Helianthus annuus* linn. leaf extract in ethylene glycol and ammonium chloride induced Nephrolithiasis. Int J Pharm Pharm Sci., 2010; 2(4): 180-184.
24. Heo JC, Woo SU, Kweon MA, Park JY, Lee HK, Son M, Rho JR, Lee SH. Aqueous extract of the *Helianthus annuus* seed alleviates asthmatic symptoms in vivo. Int J Mol Med., 2008; 21(1): 57–61.
25. Pal BC, Achari B, Yoshikawa K, Arihara S. Saponins from *Albizia lebbeck*. Phytochemistry, 1995; 38(5): 1287–1291.
26. Venkatesh P, Mukherjee PK, Kumar NS, Bandyopadhyay A, Fukui H, Mizuguchi H, Islam N. Anti-allergic activity of standardized extract of *Albizia lebbeck* with reference to catechin as a phytomarker. Immunopharmacol Immunotoxicol, 2010; 32(2): 272–276.
27. Bobby MDN, Wesely EG, Johnson M. High performance thin layer chromatography profile studies on the alkaloids of *Albizia lebbeck*. Asian Pac. J. Trop. Biomed, 2012; 2(1): S1–S6.
28. Lagunin, A, Filimonov D, Poroikov V. Multi-targeted natural products evaluation based on biological activity prediction with PASS. Curr Pharm Des., 2010; 16: 1703-1717.
29. Poroikov V, Filimonov DA, Borodina YV, Lagunin AA, Kos A. Robustness of biological activity spectra predicting by computer program PASS for noncongeneric sets of chemical compounds. J Chem Inf Comput Sci., 2000; 40: 1349–1355.
30. Poroikov VV, Filimonov DA, Ihlenfeldt WD, Gloriozova TA, Lagunin AA, Borodina YV, Stepanchikova AV, Nicklaus MC. PASS biological activity spectrum predictions in the enhanced open NCI database browser. J Chem Inf Comput Sci., 2003; 43: 228-236.
31. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem., 2010; 31: 455–461.
32. Laskowski RA, Swindells MB. LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. J Chem Inf Model, 2011; 51: 2778-2786.

33. Plumbs JA. Cell sensitivity assay: The MTT assay. Methods in molecular medicine, cytotoxic drug resistance mechanisms, Humana Press, New Jersey, 2004. Vol 28
34. Brahma J, Dhumal K. Evaluation of Anti-angiogenic properties of *Tridax procumbens* leaves extract using shell less chick embryo culture. Int J Bioassays, 2014; 3(02): 1765-1767.
35. Alsatari ES, Azab M, Khabour OF, Alzoubi KH, Sadiq MF. Assessment of DNA damage using chromosomal aberrations assay in lymphocytes of waterpipe smokers. Int J Occup Med Environ Health, 2012; 25(3): 218-224.
36. Xu JX, Song H, Bu Q, Feng D, Xu X, Sun Q, Li X. Isoflavone Attenuates the Caspase-1 and Caspase-3 Level in Cell Model of Parkinsonism. Behav Neurol, 2015; 2015: 725897.
37. Itharat A, Ooraikul B. Research on Thai medicinal plants for cancer treatment. Adv Med Plant Res., 2007: 287-317.
38. Dixit S, Ali H. Anticancer activity of Medicinal plant extract-A review. J Chem & Cheml Sci., 2010; 1(1): 79-85.
39. Dhanamani M, Lakshmi Devi S, Kannan S. Ethno medicinal Plants for Cancer therapy – A review. J D Med, 2011; 3(1): 1-10.
40. Kaur R, Kapoor K, Kaur H. Plants as a source of anticancer agents. J Nat Prod Plant Resour, 2011; 1(1): 119-124.
41. Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia, 2010; 81: 703–714.
42. Koczurkiewicz P, Czyż J, Podolak I, Wójcik K, Galanty A, Janeczko Z, Michalik M. Multidirectional effects of triterpene saponins on cancer cells —mini-review of in vitro studies. Acta Biochimica Polonica, 2015; 62(3): 383-393.
43. Hayon T, Dvilansky A, Shpilberg O, Nathan I. Appraisal of the MTT based Assay as a Useful Tool for Predicting Drug Chemosensitivity in Leukemia. Leuk Lymphoma, 2003; 44(11): 1957-1962.
44. Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. J Immunol Methods, 1986; 94: 57-63.
45. Alam F, Najum Us Saqib Q, Waheed A. Cytotoxic activity of extracts and crude saponins from Zanthoxylum armatum DC. against human breast (MCF-7, MDA-MB-468) and colorectal (Caco-2) cancer cell lines. BMC Complement Altern Med, 2017; 17: 368.
46. Ferrari M, Fornasiero MC, Isetta AM. MTT colorimetric assay for testing macrophage cytotoxic activity in vitro. J Immunol Methods, 1990; 131: 165-172.

47. Watanabe W, Konno K, Ijichi K, Inoue H, Yokota T, Shigeta S. MTT calorimetric assay system for the screening of antiorthomyxo- and anti-paramyxoviral agents. J Virol Methods, 1994; 48: 257-265.
48. Auerbach R, Lewis R, Shinners B, Kubai L, Akhtar N. Angiogenesis assays: A critical overview. Clin Chem., 2003; 49: 32–40.
49. Mistry J, Boghani N, Mistry D, Pithawala M, Chakraborty S. Monitoring angiogenic property of aqueous extract from *Blumea balsamifera* leaves using *in vitro* shell-less chick embryo culture system. Int J Pharm Biol Sci., 2015; 5(4): 150-154.
50. Naik M, Brahma P, Dixit M. A Cost-Effective and Efficient Chick Ex-Ovo CAM Assay Protocol to Assess Angiogenesis. Methods and Protoc, 2018; 1: 19.
51. Nguyen QX, Dang LT. Experimental culturing of chick embryo in shell-less culture system – the first research in Vietnam. Biomed Res Ther., 2017: 4(S).
52. Obe G, Pfeiffer P, Savage JR, Johannes C, Goedecke W, Jeppesen P, Natarajan AT, Martínez-López W, Folle GA, Drets ME. Chromosomal aberrations: formation, identification and distribution. Mutat Res., 2002; 504: 17–36.
53. O'Hare S, & Atterwill CK. In Vitro Toxicity Testing Protocols. Humana Press Inc, Totowa, New Jersey, 1995; 43.
54. Hagmar L, Strömberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C, Norppa H. Impact of Types of Lymphocyte Chromosomal Aberrations on Human Cancer Risk: Results from Nordic and Italian Cohorts. Cancer Res., 2004; 64: 2258 2263.
55. Chien SY, Wu YC, Chung JG, Yang JS, Lu HF, Tsou MF, Wood WG, Kuo SJ, Chen DR. Quercetin-induced apoptosis acts through mitochondrial- and caspase-3 dependent pathways in human-dependent pathways in human breast cancer MDA-MB 231 cells. Hum Exp Toxicol, 2009; 28(8): 493-503.
56. Jiang X, Jiang H, Shen Z, Wang X. Activation of mitochondrial protease OMA1 by Bax and Bak promotes cytochrome c release during apoptosis. Proc Natl Acad Sci U S A, 2014; 111(41): 14782–14787.
57. Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene, 2008; 27(48): 6194–6206.
58. Strasser A, O'Connor L, Dixit VM. Apoptosis signaling. Annu Rev Biochem, 2000; 69: 217–245.
59. Rana C, Piplani H, Vaish V, Nehru B, Sanyal SN. Downregulation of PI3-K/Akt/PTEN pathway and activation of mitochondrial intrinsic apoptosis by Diclofenac and Curcumin in colon cancer. Mol Cell Biochem, 2015; 402(1-2): 225–241.

60. Wetzel B, Green DR. Caspases induce cytochrome c release from mitochondria by activating cytosolic factors. J Biol Chem., 1999; 274: 17484-7490.
61. Awad AB, Roy R, Fink CS. ß-sitosterol, a plant sterol, induces apoptosis and activates key caspases in MDA-MB-231 human breast cancer cells. Oncol Rep., 2003; 10: 497-500.
62. Samarakoon SR, Ediriweera MK, Nwokwu CDU, Bandara CJ, Tennekoon KH, Piyathilaka P, et.al. A Study on Cytotoxic and Apoptotic Potential of a Triterpenoid Saponin (3-O-*α*-L-Arabinosyl Oleanolic Acid) Isolated from *Schumacheria castaneifolia* Vahl in Human Non-Small-Cell Lung Cancer (NCI-H292) Cells. Biomed Res Int, 2017; 2017.