Alaauldeen S.M.AL-Sallami et al /J. Pharm. Sci. & Res. Vol. 11(4), 2019, 1662-1666

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Cytotoxic Activity of Alkaloids Extracted from *Trigonella foenum graecum* (Fenugreek) against Breast Cancer Cell Line

[1]Alaauldeen S.M.AL-Sallami ; [2]Zuhair Salih Al-Labban; Sanaa mohemmed ali
[1,2] Iraq, [1,2] University of Kufa /[1] Faculty of Science / [2] Faculty of Medicine

Abstract

The present study was investigated to Screening for cytotoxic activity of total alkaloid extracts of *Trigonella foenum graecum* (Fenugreek) against breast cancer cell line Ahmed-Mohammed-Nahi,2003 (AMN-3), AMJ-13 and normal cell line Rat Embryo Fibroblast (REF). The *Trigonella foenum* powders were extracted separately with 80% Ethanol and total alkaloids separated at pH 2 and 10 with HCl and ammonia . Total alkaloids were detected qualitatively by Mayer's, Hager's , and Dragendorff's reagents. The cytotoxic activity was evaluated by crystal violet assay. The results show that The extract of Trigonella foenum graecum had total alkaloid in extract about 1.7% resulting from getting 4.4 gram of dry alkaloid extract from 252 gram of dry powder of *Trigonella foenum – graecum.* The alkaloids reduced the cell viability of all cell lines AMN-3,AMJ-13 and REF, and the inhibitory concentration 50% of cells were 1029 µg/ml, 1069 µg/ml and 1724 µg/ml for AMN-3, AMJ13 and REF respectively. Trigonella foenum alkaloids showed variable cytotoxic activity against cancer and normal cell lines depending on the alkaloid concentrations and cell line types.

Key words: cytotoxic activity , alkaloid , *Trigonella foenum graecum*, breast cancer.

INTRODUCTION

Cancer is a major health problem and is classified as the third leading to death after infectious diseases and cardiovascular [1]. Breast cancer is the second cancer effect on people worldwide and the most common cancer among females [2]. In Iraq, breast cancer accounting for approximately one-third of the registered female cancers, according to the latest Iraqi Cancer Registry [3]. The plant history as a source of anticancer agents is started in the 1950s with the detection and development of the vinca alkaloids [4]. Fenugreek is one of the oldest plant which have been identified as medicinal plant by the researchers around the world. It is therapeutic properties have also which include the treatment of number of diseases such as diabetes,hypercholesterolemia, inflammation, antioxidant, antimicrobial and several kinds of cancers. [5].The Trigonella seeds or Fenugreek are rich source of calcium, iron, b-carotene ,alkaloid, yellow coloring matter, tannic acid, diosgenin,fixed and volatile oils, vitamin A and other vitamins [6] This compound has multiple medicinal properties such as Anticancer, Anti-Inflammatory, Antiseptic, Aphrodisiac, Astringent, Bitter, Demulcent, Emollient, Expectorant, Anthelmintic, Wound healing and Gastro protective[7]. Alkaloids, one of the secondary metabolites which are produced by plants as toxic substances.Out of the 27,000 different alkaloids, more than 17,000 have different pharmacological properties including anticancer activities [8]. Gas chromatography/mass-spectrometry (GC/MS) is a suitable method to investigate complex mixtures of different alkaloid groups (9).

The objective of present study was investigated the complex mixtures of alkaloid extracts of *Trigonella foenum graecum* and its cytotoxic activity against breast cancer cell line AMN-3,AMJ-13 and non-tumorigenic fetal cell line (REF).

MATERIAL AND METHODS

Plants collection: The Trigonella seeds were bought fresh from local market of Kufa, Najaf, Iraq, during May 2016. The plants were classified by specialists in the Botanical. University of kufa .The plant seeds dried under shade for 10 days at room temperature and dried, then the seeds were ground and stored at room temperature until further use.

Total alkaloid extraction: Total alkaloids were extracted according to Harborne [10]. Briefly, 10 g of plant dry powder was extracted with 80% ethanol for 24 h in a continuous extraction by soxhlet apparatus 250 ml volume. The extract was filtered by Whatman No. 1 filter paper and then, the filtrate was concentrated by a rotary evaporator under vacuum at 45°C until the solution reached to 10 ml. The concentrated extract was transferred to a biker and 2 N HCl was added gradually to adjust the pH value up to 2. Then, the pH value of the extract was adjusted to 10 using NH OH, and washed with 10 ml chloroform 3 times. The chloroform portion was dried to obtain the total alkaloid extract. The dried extract was weighed and preserved in a clean container at 4°C for use.

Qualitative detection of alkaloids: To detect the presence of alkaloids in plant extracts some qualitative tests were performed using Mayer's, Hager's and Dragendorff 's reagents. Mayer's reagent used to screen all types of alkaloids, that prepared by Harborne [11]. Hager's test, appeared a yellow color precipitate that indicate to the presence of alkaloids [12].Furthermore, Dragendorff 's reagent was used to investigate alkaloids in plant extract. The formation of an orange color indicated the test was positive [13].

Gas Chromatography–Mass Spectrometry (GC/MS) Analysis: The GC/MS were recorded on a Hewlett Packard 5890/MSD 5972A instrument operating in EI mode at 70 eV. A HP5 MS column (30 m × 0.25 mm × 0.25 µm) was used. The temperature program was 80 to 250°C at 10°C min-1 and held for 10 min at 250°C. The injector

temperature was 250°C. The flow rate of carrier gas was 0.8 ml min1. The identification of the alkaloids was confirmed by comparing the mass spectral data with those of authentic compounds and with data obtained from the literature.

Preparation of cell line : The cell lines used in this study were supplied by tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) maintained in RPMI- 1640. Ahmed-Mohammed-Nahi-2003 (AMN-3 cell line) The cell line was supplied by tissue culture unit / ICCMGR, Baghdad, Iraq (passage number 222). The origin and description of this cell line was first mentioned by [14]. The specimen was taken from murine mammary adenocarcinoma, and then transplanted in immune suppressed rat by cortisone. It was kept at -169˚C (in liquid nitrogen). In preparation to any in vitro assay, the frozen cell line was withdrawn and maintained in RPMI-1640 containing 10% bovine calf serum, when the in vitro cells culture forms a monolayer. These cells were treated with trypsin/ versine mixture in order to pursue subculture process. The AMJ13 cell line was established from the primary tumor of a 70-year-old Iraqi woman with a histological diagnosis of infiltrating ductal carcinoma. The cells were morphologically characterized by elongated multipolar epithelial-like cells [15] (passage number 25). Rat Embryo Fibroblast (REF) The normal culture of the rat embryo is the most important source for the undifferentiated fibroblastic culture. This cell line was supplied by tissue culture unit / ICCMGR, Baghdad, Iraq (passage number 320). The specimen was taken from rat embryo then killed and analyzed by using Trypsin, then maintained in RPMI-1640 medium with 20% bovine calf serum, when it becomes confluent monolayer, the cells treated with Trypsin-Versine mixture in order to pursue subculture process .

The cell lines were maintained in RPMI-1640 (Sigma-Aldrich, Taufkirchen, Germany) supplemented with 5% calf bovine serum,100 units/mL penicillin, and 100 _g/mL streptomycin. Cells were passaged using Trypsin-EDTA (USbiological, Salem, MA, USA), reseeded at 50% confluence twice a week, and incubated at 37 _C.

Cytotoxicity Assay: Cell cultures in microtitration plate (96wells) were seeded with (200 µl/well) Vero cell suspension (3 - 5 × 10^4 cells/ml). The plates were incubated at 37°C in humidified 5% CO2 incubator till a confluent monolayer was determined by inverted microscope (DM IL LED Leica, Germany). The liquid medium above the monolayer was aspirated and the cells were washed once and incubated with RPMI-1640, containing 5% FBS (200 µl/well) for further 72h. When the cells of micro- titer plates are in log phase, the medium was removed, and the cells were exposed to a range of seed extracts concentrations. Three replicates were used for each concentration of the alkaloid extract that used for treatment of tissue cultures were; (100000, 50000, 25000, 12500, 6250, 3125,1562, 781.3,390.6, 195.3 and 97.7) µg/ ml, At the end of recovery time, 150µl of crystal violate were add to each cultured well, the plates incubated in an incubator at 37^{o}C for 25 minutes. then, the plates were washed with water and left to dry. The optical density of each well was read by using a micro – ELISA reader at a transmitting wavelength of 492 nm [16]. The proliferation rate was measured as the formula according to [17] as follows:

PR% =B/A x 100

PR=proliferation rate, A= the optical density of control, B= the optical density of test

Statistical analysis

Data were expressed as means ± S.E. Statistical analyses were performed using graghpad prism program followed by LSD test. The $p \leq 0.05$ were considered a significant for all data of the results.

RESULTS

The soxhlet extraction procedure using the soxhlet showed that the total alkaloid getting 4.4 gram of dry alkaloid extract from 252 gram of DW of plant. Table 1 showed the qualitative detection of alkaloids present in the plant extracts using different reagent. The qualitative analysis of extracts appears the presence of alkaloids by changing color in reagents.

Present study investigated the alkaloid composition of seeds by GC/MS showed the presence of biologically active alkaloids to confirm the traditional use of the plant in medicine treatment . Ten compounds were identified and to the best of our knowledge they are reported for Table 2:

Table 3 showed the effects of the total alkaloid extract treatment against AMN-3,AMJ13 and REF. The concentration of the alkaloid extract that used in the study was chosen depending on the result IC50 which revealed the most cytotoxic effective concentration ranged between 195.3-100000 µg/ml**.** Results shows a high significant ($P < 0.05$) effect of these concentrations in inhibiting the growth of AMN3 tumor cell lines as compared to the cell lines of AMj-13 and REF , while moderate significant occurred only in ANJ-13 cell lines compared with REF cell lines .

The results showed no significant ($P > 0.05$) toxic effect in the three cancer cells lines and the percentage of cytotoxicity effect of cell proliferations especially in high concentration used in present study (50000 –100000 µg/ml)

Table 1: Qualitative detection of alkaloids in plant extract using different reagents

Reagent	Result	Resulted color
Mayer's reagent	+	Creamy precipitate
Hager's reagent	+	Yellow color
Dragendorff's reagent	+	Orange color
+: Indicate the positive results		

Table 2: The compounds that isolation from the alkaloid by GC/MS

PEAK#	RT	Area%	Compound name
2	4.167	0.57	3,7-Dimethyl-3-octyl methylphosphonofluoridate
3	4.232	0.54	2,3,6-Trimethylhept-6-en-1-ol
4	4.292	1.96	4-methyl-1-piperazinyl(decahydro-1-napthyl)methanone
5	6.974	0.51	p-Phenylenediamine, N,N,N'-trimeth y1-N'-[2-(N-methylanilino)ethyl]-
6	7.063	0.32	15-Trimethylsilyloxydehydroabietic acid, trimethylsilyl ester
7	9.010	0.51	Theobromine, tert-butyldimethylsilyl deriv.
8	9.064	0.17	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5,5-tris(trimethylsiloxy)tetrasiloxane
9	9.354	0.23	1H-Phosphole, 2,5-dihydro-1,3-dimethyl-
10	9.426	0.06	1H-Phosphole, 2,5-dihydro-1,3-dimethy-
11	10.452	2.28	Propanoic acid, pentamethyldisilanyl ester
12	10.690	0.28	Silane, trimethyl(2-pentenyloxy)-,(Z)-
13	10.761	0.32	Silandrone
14	10.862	1.15	Indolo[2,3-b]quinoxaline, 1-fluoro
15	12.263	1.04	.alpha.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-
16	12.405	0.38	Pentadecanoic acid
17	12.874	0.38	3-Amino-2-phenazinol ditms
18	13.610	7.02	Hexadecanoic acid, methyl ester
19	14.845	6.40	Hexadecanoic acid, ethyl ester
20	15.937	0.37	5-Iodocytosine
21	17.201	6.47	9,12-Octadecadienoic acid (z,z)-,methyl ester
22	17.326	9.55	9-Octadecenoic acid ,methyl ester,(E)-
23	17.907	1.58	Methyl stearate
24	18.305	0.60	Palmidrol
25	18.708	6.92	n-propyl 9,12-octadecadienoate
26	18.827	10.02	Ethyl Oleate
27	19.397	1.52	Octadecanoic acid, ethyl ester
28	19.581	0.45	Pyrazolo[1,5-a]pyrimidine, 2,5,7-trimethyl-3-phenyl-
29	22.003	2.15	6-[3-Piperidino-1-hydroxypropyl]-2-phenylbenzothiazole
30	22.139	0.98	Nonadecanoic acid, 18-oxo-, methyl ester
31	22.721	0.43	3,3-Diethylheptadecane
32	23.504	5.78	Hexanedioic acid, mono(2-ethylhexyl)ester
33	23.860	0.38	9-Octadecenamide, (Z)-
34	23.949	0.14	8-Methyl-6-nonenamide
35	24.543	1.01	Fumaric acid, 2-dimethylaminoethyl undecyl ester
36	26.151	1.28	Bis(2-ethylhexyl) phthalate
37	27.243	0.36	1-Monolinoleoylglycerol trimethylsilyl ether
38	27.849	0.26	1-Monolinoleoylglycerol trimethylsilyl ether
39	29.083	0.08	Fumaric acid, 2-butyl dodecyl este
40	29.867	0.33	4a,5,6,7,8,8a,10,10a-Octahydro-2H-1-oxa-9a-azaanthracen-9-one
41	30.864	0.58	Silane, diethylhexadecyloxy(2-phenylethoxy)-
42	31.173	1.28	Silane, diethylhexadecyloxy(2-phenylethoxy)-
43	33.737	1.01	Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl-
44	34.134	1.23	1H-Indole, 2-methyl-3-phenyl-
45	37.256	1.66	Pyrido[2,3-d]pyrimidine, 4-phenyl-
46	37.874	4.78	Imidazo[1,2-b]1,2,4-triazine, 6-(4-methoxyphenyl)-7-methyl-2-phenyl-
47	38.497	0.11	2-Bromo-4-fluorobenzyl 2,3,4,5,6-pentafluorobenzoate
48	39.375	7.68	.beta.-Sitosterol
49	39.826	0.11	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene

Table (3): Comparison among the alkaloid extracts cytotoxicity on cell lines (cell proliferation as the % of control) after 72hr.

Cytotoxicity of extract (cell proliferation as the % of control)				LSD value
Concentration (µg/ml)	**AMN3 Mean ± SE**	**AMJ-13 Mean ± SE**	**REF Mean ± SE**	
195.3	ABCDEFG 117.1 ± 2.6	ABCDEFGHI 121.7 ± 2	ABCDEFGH 112.7 ± 6.7	15
390.6	ABCDEFG 116.3 ± 2 ab	A BCDEFG 81 ± 2.3	ABCDEF 74 ± 2	7.3
781.2	ABCDEFG 110.6 ± 5.7 ab	A BCDEFG 77.6 ± 1.3	ABCDEF 73.8 ± 1.6	12.4
1562	ABCDEF 65.2 ± 3.2	A BCDEF 56.5 ± 2	ABCDE 56 ± 3	9.6
3125	ABCD 18.8 ± 3.9 a b	A BCDE 45.4 ± 1.7	ABCD 38.7 ± 2	9.5
6250	ABCD 14.9 ± 6.3 a	ABCD 25.1 ± 1	ABC 28.3 ± 1.1	13.1
12500	1.2 ± 0.5 ab	A 7.4 ± 0.7 c	A B 22.9 ± 1.7	4
25000	1.2 ± 0.8 ab	A 7.3 ± 0.6 c	A 15.1 ± 1.1	3.4
50000	1.1 ± 0.8 ab	5.4 ± 0.6 c	8.15 ± 0.7	2.7
100000	0.9 ± 0.2 a	1.9 ± 0.1 c	4.2 ± 0.5	1.3
LSD value	9.8	4.3	8	

Small letters in the same row are meaning significant with next cell lines
Capital letters in the same column are meaning significant with next concentration

DISCUSSION

Medicinal plants contain some organic compounds which provide definite physiological action on the human body, and these bioactive substances include alkaloids [18]. Total alkaloids were detected by changing the color of specialized reagents .The method described in this study can be used for the determination of a Cytotoxicity of total alkaloid against cell lines performed using a crystal violet assay, this methods describes a quick and reliable screening method that is suitable for the examination of the impact of chemotherapeutics or other compounds on cell survival and growth inhibition [19]. The results of this study indicate that the alkaloid extract of the Trigonella plant has cytotoxity on cancer cell lines, this alkaloids may be similar to the alkaloids used in the manufacture of anticancer drugs ,these alkaloid classified by [20] according to its effects to the microtubule on two main groups: Microtubule – destabilizing agents that inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass and Microtubule –stabilizing agents which promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass [21] Another mechanism of Alkaloids are capable of modulating key signaling pathways involved in proliferation, cell cycle, and metastasis making them the chief components of several clinical anticancer agents [22]. PI3k/Akt signal transduction cascade is one of the several cellular proliferative pathways which promote a normal cell cycle progression by modulating cyclins and pro-apoptotic proteins. Many cytotoxic agents target DNA and the Akt pathway to block cell proliferation and induce apoptosis [23].Nuclear Factor-Kappa B (NF-kB) pathway is an inducible nuclear transcription factor that activates genes involved in cell survival and proliferation [24] . Alkaloids have been found to suppress tumorigenesis by targeting the NF-kB pathway such as Taxol that targgeting NF-kB pathway [25]. Some alkaloids, derived from plants, activate caspases , are important agents in the programmed cell death pathways. Such as scutebarbatine A , a major alkaloid in *Scutellaria barbata*., was found to exhibit its anti-proliferative activity against human lung carcinoma cells through the cleavage of caspases-3 and -9 [26] .

The alkaloid toxicity of cancer cells different from AMN3 cell line to AMJ-13 due to the different cellular cell lines in their receptors. It is possible to find more than one compound in the extracts that has the inhibitory properties against the cancer cells by affecting certain receptors on the surfaces of these cells and through these receptors the cells are responsive to do many stimulants for apoptosis [27].

This result indicated to the less cytotoxicity of extract on normal cell line REF comparing with its cytotoxicity on cancer cell line that may be related to the selective cytotoxicity of extract towards cancer cells [28]. Furthermore, another study revealed that alkaloid have ability inhibits breast stem cell renewal without cause toxicity to differentiated cells [29],also interact with DNA or RNA to form an alkaloid-DNA or a alkaloid-RNA to prevent damage such as berberine make as anticancer by formation complex with DNA and RNA to prevent damage [30].

GC-MASS analysis also showed two anticancer compound in crude alkaloid such as Quinoline that plays an important role in anticancer drug development as their derivatives have shown excellent results through different mechanism of action such as growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell

migration, and modulation of nuclear receptor responsiveness[31]

Also compound is Indole are natural products which are well known for its anti-cancer activity due to its ability to induce cell death for many cancer cell lines[30] Indoles function as efficient group of numerous biochemical molecules and compounds, such as alkaloids. Indols have been verified to inhibit proliferation, expansion of new blood vessels (angiogenesis) and invasion of human cancer cells by many mechanisms of apoptosis stimulation to stimulating of caspase-9 and caspase-3 [32] .

CONCLUSION

Plant alkaloids appeared variable cytotoxic activity against cancer and normal cell lines depending on the alkaloid contents, concentrations, purity, and type of cell lines.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2):74-108.
2. Parkin DM, Fernández LM. Use of statistics to assess the global burden of breast cancer. Breast J 2006;12 Suppl 1:S70-80.
3. Iraqi Cancer Board. Results of the Iraqi Cancer Registry 2004. Baghdad: Iraqi Cancer Registry Center, Ministry of Health; 2007.
4. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol 2005;100(1-2):72-9.
5. Goyal Sh, Gupta N, and Chatterjee S. Investigating Therapeutic Potential of Trigonella foenum-graecum L. as Our Defense Mechanism against Several Human Diseases. Journal of Toxicology 2016; 10 pages.
6. Moniruzzaman, Shahinuzzaman , Haque A, KhatunR and Yaakob Z. Gas chromatography mass spectrometry analysis and in vitro antibacterial activity of essential oil from Trigonella foenum-graecum. Asian Pac J Trop Biomed 2015; 5(12): 1033–1036
7. Toppo F.A., Akhand R., Pathak A.K. Pharmacological action and potential uses of Trigonella foenum-graecum : A Review. Asian Journal of Pharmaceutical and Clinical Research.2009;2(4):29-38.
8. Habli Z, , Toumieh G, Fatfat M, Rahal O and Muhtasib H. Emerging Cytotoxic Alkaloids in the Battle against Cancer: Overview of Molecular Mechanisms. Molecules 2017; 22, 250.
9. Kreh M, Matusch R, Witte L (1995). Capillary gas chromatographymass spectrometry of Amaryllidaceae alkaloids. Phytochemistry 38(3):773-776.
10. Harbone JB. Phytochemical methods. A Guide to Modern Techniques of Plant Analysis. 2nd ed. London. Chapman and Hall; 1984. p. 307.
11. Harborne JB. Phytochemical Methods. 2nd ed. New York: Chapman and Hall; 1984. p. 288.
12. Neelima N, Gajanan N, Sudhakar M, Kiran V. A preliminary phytochemical investigation on the leaves of Solanum xanthocarpum. Int J Res Ayurveda Pharm 2011;2(3):845-50.
13. Antherden LM. Textbook of Pharmaceutical Chemistry. 8th ed. London:Oxford University Press; 1969. p. 813-4.
14. Al-Shamery A M H (2003). The study of Newcastle disease virus effect in the treatment of transplanted tumor in mice. MSc Thesis College of veterinary medicine University of Baghdad Iraq.
15. Al-Shammari A, Alshami M, Umran M, Almukhtar A, Yaseen N, Raad K and Hussien A. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer (Dove Med Press). 2015; 7: 223–230.
16. Freshney, R. I.(1994):Culture of Animal Cells.(3rd. Ed.).Wiley-Liss,U.s.A.,pp:267-308.
17. Chumchalova, J. And Smarda, J. (2003): Human Tumor Cells are Selectively Inhibited by Colicins. Folia Microbiol., 48: 111-5.
18. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents some Nigerian medicinal plants. Afr J Biotechnol 2005;4(7):685-8.
19. Feoktistova M., Geserick P., Leverkus M. (2016). Crystal Violet Assay for Determining Viability of Cultured Cells. Cold Spring Harb Protoc . pdb.prot087379. doi: 10.1101/pdb.prot087379.
20. Mukherjee, A., Sourar, B., Nabainta, S., and Anil, C. (2001). Advance in canser therapy with plant based natural product.curent medicinal chem.1467-1486.
21. Checchi, P. M.; Nettles, J. H.; Zhou, J.; Snyder, J. P.; Joshi, H. C. (2003). Trends Pharmacol Sci, 24: 361-365.
22. Millimouno, F.M.; Dong, J.; Yang, L.; Li, J.; Li, X. Targeting apoptosis pathways in cancer and perspectiveswith natural compounds from mother nature. Cancer Prev. Res. **2014**, 7, 1081–1107.
23. Fruman, D.A.; Rommel, C. PI3K and Cancer: Lessons, Challenges and Opportunities. Nat. Rev. Drug Discov. **2014**, 13, 140–156.
24. Ma, B.; Hottiger,M.O. Crosstalk betweenWnt/_-Catenin and NF-_B Signaling Pathway during Inflammation. Front. Immunol. **2016**, 7, 378.
25. Kampan, N.C.; Madondo, M.T.; McNally, O.M.; Quinn, M.; Plebanski, M. Paclitaxel and its evolving role in the management of ovarian cancer. Biomed. Res. Int. **2015**, 2015, 413076.
26. Yang, X.K.; Xu, M.Y.; Xu, G.S.; Zhang, Y.L.; Xu, Z.X. In Vitro and in Vivo Antitumor Activity of Scutebarbatine A on Human Lung Carcinoma A549 Cell Lines. Molecules **2014**, 19, 8740–8751.
27. Moteki, H. Hibasmai, H. Yamada, K.H. Imai, K. and Komija, T.(2002).Specific of apoptosis 1,8-cineole in two human leukemia cell line but not in human stomach cancer cell lines. Oncology. Reports., 9:757-760.
28. Cho RJ, Campbell MJ, Winzeler EA, Steinmetz L, Conway A, Wodicka L, Wolfsberg TG, Gabrielian AE, Landsman D, Lockhart DJ, Davis RW.. (1998) Parallel analysis of genetic selections using whole genome oligonucleotide arrays. Proc Natl Acad Sci U S A 95(7):3752-7.
29. .Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier et al. Targeting breast stem cells with the cancer preventive compound curcumin and piperine. J Breast Cancer Res Treat 2010;122(3):777-83 .0.
30. Li XL, Hu YJ, Wang J. Molecular spectroscopy evidence for berberin binding to DNA: Comparative binding and thermodynamic profile intercalation. J Biomacromolecules 2012;13(3):873-80.
31. Afzal, S. Kumar, M.R. Haider, M.R. Ali, R. Kumar, M. Jaggi, S. Ba wa Eur. J. Med. Chem., 97 (2015), p. 871
32. *Mardia El-sayed, Nehal A Hamdy, Dalia A Osman, Khadiga M Ahmed* ; 2015 :1(1)