

Impact of ethanolic extract of *Mikania glomerata* on human breast cancer (MCF 7) cell line

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Abstract

The ethanol extract of *Mikania glomerata* has anti-proliferative effect on the human breast cancer cell lines. The object of the present work is to investigate the anti-cancer effect of *Mikania glomerata* ethanolic extract on breast cancer. Soxhlet fractions using crude ethanolic extract of *Mikania glomerata* was prepared by standard extraction protocols. To check the antiproliferative effect of this extract, the extract chosen was tested for cell viability on the breast cancer cells MCF 7 in different concentrations. Cell viability was evaluated by MTT assay for 24 hour and 48 hours. The LD₅₀ value was calculated and different morphometric assays were performed with the effective dose of the extract. The effect of the extract on the normal cell was evaluated as well. Cell proliferation, cell cycle, Clonogenic survival, Apoptosis and MTT assays were performed. The ethanolic extract showed a dose-dependent and time dependent inhibition on cell proliferation in the breast cancer cell lines. It showed low cytotoxicity in the normal cells and inhibited cellular adhesion and wound healing in treated cancer cells. The present study suggests that the leaf extract from *Mikania glomerata* induces anticancer effect on the breast cancer cells. Further study might help to confirm it as an anti-cancer drug.

Keywords: *Mikania glomerata*, anticancer drugs, breast cancer.

1. Introduction

Herbal medicine is the oldest form of healthcare known to the human race with a history of documentation for almost 4000 years. It has survived for ages and is still a vibrantly alive branch of medicine that is being used actively in many cultures throughout the world today. The World Health Organization estimates that 80% of the world's population, mainly in the developing countries relies on herbs for their primary health care needs. This is principally due to the popular belief that herbal drugs are without any side effects besides being inexpensive and obtainable in the neighborhood.

Cancer is one of the major threats of modern life, which is considered as the second cause of death after myocardial infarction. Millions of people die every year in different types of cancer despite tremendous efforts to find methods of control and cure. In the last century, great advances were made in modern medical science to control disease. But many diseases like cancers are not yet curable fully. To find out new and authentic therapies, scientists are working with traditional or folk medicines in parallel of modern

medicine. *Mikania glomerata* has been used for medicinal purposes for centuries.

The active ingredients of *M. glomerata* have beneficial effects against many diseases, including cancers. There are not so many research works done in this field and very few review articles exist in this area and hence this study.

2. Materials and methods

2.1 Plant Material

Fresh leaves of *Mikania glomerata* was collected through the Central Research Institute for Siddha, Arumbakkam, Chennai. The botanical identity of the species was authenticated at the Botanical Survey of India, Southern Regional Centre, Coimbatore.

2.2 Solvent Extraction

One kilogram of *Mikania glomerata* leaves was collected, shade-dried and coarsely powdered using a pulverizer. This powder was successively extracted with ethanol (80°C) using Soxhlet apparatus. The solvent was removed by vacuum distillation in a rotatory evaporator at 60°C. The extract was filtered through Whatman No. 1 filter paper and concentrated

on a water bath to a syrupy mass. The dried substance was dissolved in ethanol and stored in cold room for future use.

2.3 Cell lines and growth conditions

The cell line used for the proliferation study was MCF7 Human breast cancer cell lines. MCF-7 cells were obtained from the American Type Culture Collection (Rockville, MD) and grown in monolayer in Nunc culture dishes (six wells of 9.6 cm²/plate). Cells were maintained in Dulbecco's minimum essential medium (Gibco) supplemented with 5% PCS (Gibco Bio-Cult, Paisley, Scotland), glutamine (2 mmol/litre), streptomycin (100 ug/ml) and penicillin (100 IU/mL), and phenol red as pH indicator (standard medium). During growth experiments cells were grown in the absence of the pH indicator phenol red (11), and CSFCS (Charcoal stripped foetal calf serum) replaced the normal PCS (experimental medium). The charcoal stripping was performed by shaking 200 mL of FCS with 1.0 g of Norit A charcoal and 0.1 g of dextran (Sigma Chemical Co., St. Louis, MO) at 37°C for 3 h.

The serum was then centrifuged at 9000 x g for 15 min. Growth factors and inhibitors added to the medium were: insulin, EGF, and estradiol-17/3 (obtained from Sigma), interleukin-1 and IGF-1 (Insulin like growth factor 1-gene) and TGF/3 (Transforming Growth Factor-3). The cell layer was briefly rinsed with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contain trypsin inhibitor. 2.0 to 3.0 mL of Trypsin-EDTA solution was added to flask and cells were observed under an inverted microscope until cell layer was dispersed (usually within 5 to 15 minutes). 6.0 to 8.0 mL of complete growth medium was added and cells were aspirated by gently pipetting. Appropriate aliquots of the cell suspension were added to new culture vessels. The cultures were incubated at 37°C.

2.4 Cell proliferation assay

All cells were grown as described earlier. For cell proliferation effects, four different concentrated samples (10, 20, 30, 40 µg/mL) were seeded at 20,000 cells per flask. All cells were grown for 24 hours and cell counts were taken. the cell lines study was used to screen the activity of ethanolic extract of *Mikania glomerata* leaves against for MCF7 Human breast cancer cell line.

2.4.1 Cells counting using haemocytometer

Assessment of cell viability and distinction of cell types was carried using Neubauer counting chamber. Cover-slip was moistened and affixed to the haemocytometer. Care was taken to ensure that the cover-slip and haemocytometer were clean and grease-free. Alcohol was used for cleaning purpose. A small amount of trypan blue-cell suspension was

transferred to one of the chambers of the haemocytometer by carefully touching the cover slip at its edge with the pipette tip and allowing each chamber to fill by capillary action. Overfilling or under filling of the chamber was avoided.

2.4.2 Determination of the number of cells (total and viable)

The cells were viewed under a microscope at 100x magnification. Focusing was done to see a grid of 9 squares. Then the microscope was focused on one of the 4 outer squares in the grid. Adjustments were made so that the square contained 16 smaller squares. Cell counting was carried in the four 1 mm corner squares. Procedure was repeated based on either concentration or dilution. Trypan Blue is the "vital stain"; excluded from live cells. Live cells appear colourless and bright (refractile) under phase contrast. Dead cells stain blue and are non-refractile. The cells were counted as per the procedure illustrated. 4 corner squares were counted and the average was calculated. Each large square of the haemocytometer, with cover-slip in place, represents a total volume of 0.1 mm³ (1.0 mm X 1.0 mm X 0.1 mm) or 10⁻⁴ cm³. Since 1 cm³ is equivalent to approximately 1 ml, the total number of cells per ml was determined using the following calculations:

$$\% \text{Cell Viability} = [\text{Total Viable cells (Unstained)} / \text{Total cells (Viable + Dead)}] \times 100.$$

$$\text{Viable Cells/ml} = \text{Average viable cell count per square} \times \text{Dilution Factor} \times 10^4$$

$$\text{Average viable cell count per square} = \text{Total number of viable cells in 4 squares} / 4.$$

2.4.3 Assessment of cell density and evaluation of viable cells

The samples were diluted to appropriate concentrations and the absorbance of the sample was measured by means of spectrophotometer at 550 nm. Consistent wavelength must be used while measuring the cell density of a particular cell line. An independent calibration curve was generated for each cell line [1]. After spectrophotometer analysis, actual counting of cells was executed using counting chamber.

2.5 Fluorescence assay for cell cycle distribution

At the end of 24 hours after treatment, the cells were smeared on to glass microscope slides, fixed with methanol and air dried. The cells were then stained with propidium iodide (0.1µg/mL) and scored under fluorescence microscope fitted with PI filter. A total of 100 cells were scored for intensity of DNA staining in the nucleus. Initially the slides were scored for determining the 1x fluorescence intensity using intensity tool from Adobe Photoshop. This was determined by counting the cells and the majority of

cells will fall into this category. This is the G1 cells fluorescence intensity. Based on this intensity other phases of cell cycle were determined

2.6 Clonogenic survival assay

The clonogenic cell survival assay determines the ability of a cell to proliferate indefinitely, thereby retaining its reproductive ability to form a large colony or a clone. This cell is then said to be clonogenic. For cell proliferation effects, cells were seeded at 20,000 cells per flask. Three flasks were used per concentration. All cells were grown for 24 hours. The various treatment conditions on MCF7 breast cancer cell line with different concentration of ethanolic extract of *Mikania glomerata*. At the end of 24 hours' treatment, the cells were removed and plated on to separate flasks. Trypsinize the stock flask of cells containing the cells that have to be tested. Make sure that the cells are in single-cell suspension and obtain an accurate cell count. The cells were counted using a hemocytometer. Using a Pipette, add 20,000 cells (the cell number can vary depending on the cell type) to the 5 mL of medium in each T-25 flask. Shake gently to distribute the cells evenly. The flasks were then incubated for 12-14 days at 37°C. The flasks were then stained with Gentian violet and scored for number of colonies.

Clonogenic assay serves as a useful tool to test whether a given cancer therapy can reduce the clonogenic survival of tumor cells. A colony is defined as a cluster of at least 50 cells which can often only be determined microscopically. Clonogenic assay is the method to determine cell reproductive death after treatment with ionizing radiation, but can also be used to determine the effectiveness of other cytotoxic agents. The following protocol has been modified from a published version [2].

2.7 Apoptosis

Apoptosis induction was determined by measuring Annexin V activity using Annexin V apoptosis kit. The kit was sourced from Promega corporation, Southampton, UK and consisted of Annexin V buffer and lyophilized Annexin V substrate. Cells were seeded at the concentration of 104 cells per well in 96 well microtitre plate and incubated for 48 hours. Aliquot of 100 µL of fresh media containing appropriate concentration of extracts were transferred to the assigned respective wells. Staurosporine 0.1 µg/mL was used as positive control and untreated wells were treated as negative control. Total of six wells were assigned for each treatment. The plate was allowed to equilibrate to room temperature after 24 hours of incubation prior to performing the assay. 100 µL of Annexin V reagent was added to each well and mixed for 60 seconds and incubated for further 1 hour at room temperature. An

aliquot of 100 µL of contents from each well was transferred to white-walled 96 well plate. The light emitted was measured by Packard lumiscount microplate luminometer and measurement was recorded using THERMOmax™ plate reader linked to a computer using SoftMax Pro software (Muñoz-Alonso *et al.*, 2008). The results are depicted in the tabular form.

2.8 MTT assay

MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole), is sliced by mitochondrial dehydrogenase of viable cells, resulting in a purple formazan product that could be measured spectrophotometrically. The formazan product formed is in proportion to the number of viable cells and inversely proportional to the amount of cytotoxicity. The absorbance of formazan product can be measured at a wavelength between 510 and 600 nm using a spectrophotometer [3][4]. The positive control treatment consisted of 1 % phenol. Positive control reagents are chemicals known to bring a standard positive response in a test system. The positive control chemical is used to show that the test system is responding satisfactorily and that the test is valid. The negative control used consisted of the sterile distilled water. Qualitative and quantitative evaluations of viability of cells were determined using MTT assay. Cells were seeded at the density of approximately 1×10^5 cells per well in 96 well plates in 100 µL medium. After an incubation of 48 hours' culture medium was removed and replaced with aliquots of 100 µL of medium containing appropriate concentration of extracts particles. 1 mg/mL of phenol was treated as positive control and untreated cells served as negative control. Total of six wells were assigned for each treatment. The cells were treated with the test material and positive control for 24 hours. Following the incubation period the cells were examined microscopically to assess the morphological alterations indicative of qualitative evaluation of toxicity. A 100 µL of MTT reagent was added to each well and mixed at least for 60 seconds and the plates were incubated for approximately 4 hours. 100 µL of Dimethyl Sulfoxide (DMSO) was transferred to each well to lyse the cells. Absorbance of the lysate was measured at 560 nm to determine the quantitative evaluation of cytotoxicity using the THERMOmax™ plate reader linked to a computer using SoftMax Pro software.

The culture medium was removed from the plate and the wells were washed with phosphate buffered saline. Aliquots (100 µL) of the culture medium containing appropriate concentrations of the test article, positive controls, and negative control were applied to the plate wells. Plate was incubated at

approximately 37°C in a humidified atmosphere of 5% (v/v) CO₂ in air for approximately 24 hours. Following the incubation period, the cells on the plate was examined microscopically to assess any morphological alterations indicative of toxicity. All measurements were recorded using the THERMOmax™ plate reader linked to a personal computer using SoftMax Pro software. Programming of the plate reader included setting up a template to which blanks, controls, empty and test article wells were assigned. The raw data were exported and analysis performed using Microsoft Excel. Statistical analysis of Mean and standard deviations (SD) were calculated and viability of test item treated cultures was compared to the negative control cultures. The assay was normally considered acceptable for the evaluation of the test results valid if the following criteria were met: No significant cytotoxicity was observed in the negative controls. The positive control compound induces a statistically significant cytotoxicity compared to the negative controls. The test extract is declared as a cytotoxic substance if, there was a noticeable increase (>50%) in cytotoxicity even after normalization to the negative control.

3. Results and Discussion

Different classes of compounds were previously isolated from various *Mikania* parts, which can be associated to this plant's pharmacological activities. The main groups are: coumarins and derivatives, sesquiterpenes, sesquiterpenes lactones, diterpenes, phytosterols/terpenoids and flavonoids. Caffeoylquinic acid derivatives beyond other chemical compounds are found in smaller amount. Diterpenes such as kaurenoic acid and benzoylgrandifloric acid (class of kauranes), have also attracted interest for their pharmacological action. Moreover, detailed screenings revealed the presence of other substances in species of *Mikania* as alcohols, acids, esters, aldehydes and organic esters [5][6]. These ingredients and their derivatives are found to have analgesic activity [7][8], antibacterial [9][10] and anticancer properties [11]. One of the main species of genus *Mikania* is *M. glomerata* popularly known as 'guaco' with a long standing record application and has a reasonable reputation to have many therapeutic properties was taken for screening of any anti-cancer activities. Hence, the effects of alcoholic of *M. glomerata* were investigated on cell proliferation, cell cycle analysis, clonogenic survival assay, apoptosis and cytotoxicity using MCF7 (human breast cancer cell lines) cell lines.

3.1 Cell proliferation assay

In the cell proliferation assay on MCF7 cells using ethanolic extract of *Mikania glomerata*, initial

cell counts were done in the triplicate culture vessels and 24hrs counts were made to ensure cell proliferation in the taken test cell line. The initial count had a mean value of 20,777 cells and the 24hr count had a mean value of 50,914 cells. Control cell lines with a mean value of 50,444 cells were set up without the test material to assess the inhibitory effect. The results indicated that the ethanolic extract of *Mikania glomerata* on the MCF7 human breast cancer cell line did not produce cell growth inhibitory effect in the cell proliferation assay. Significant result was not obtained, even in higher test dose (Table: 1; Figure: 1).

Development and optimization of *in vitro* assays provides for a simple, reliable, sensitive, reproducible, inexpensive and high-throughput method with which to assess the efficacy of novel chemotherapeutic pharmaceuticals on cell survival or proliferation early in the discovery process of drug development [12]. Anti-proliferative effect of the extract of the root of *Polygonum multiflorum* on MCF-7 human breast cancer cells and the possible mechanisms was demonstrated by Chen *et al* [13]. Methanol extracts of *Scrophularia* species, a medicinal plant widely used in folk medicine since ancient times exhibited strong anti-proliferative properties on HL-60 leukaemia cells leading to strong growth inhibition and high cell death rates [14][15]. Reagan-Shaw *et al* [16] in 2010 reported antiproliferative effects of apple peel extract against cancer cells. Similarly, hexane extract of *Raphanus sativus* root exerts potential chemopreventive efficacy and induces apoptosis in cancer cell lines through modulation of genes involved in apoptotic signaling pathway [17]. Inhibitory effect of bitter melon (*Momordica charantia*) on cell proliferation of adrenocortical cancer cells was illustrated by Brennan *et al* [18] in 2012. Aqueous extract of *Trametes robiniophila* induced inhibitory effect on both MCF-7 and MDA-MB-231 cells. In line with these authors cell proliferation assay was conducted on three human cancer cell lines using extracts of *M. glomerata*.

3.2 Cell cycle distribution analysis

Distribution of cells in various cell cycle phases of MCF7 human breast cancer cell line in the presence of ethanolic extracts of *M. glomerata* of no noteworthy changes in the number of cells in the quiescent state (PreG1 or G₀) in all the four test concentrations, compared to the control. no significant change in Gap 1 (G₁) phase in all the test doses of the extract. Near 50% reduction in cells in the synthesis phase (S) was observed in the distribution of cells only in 10 µg/ml (24 hr), when compared against the control (Table: 2; Figure: 2).

The cell cycle governs the transition from quiescence (G0) to proliferation while ensuring the fidelity of the genetic transcript. The phases associated with DNA synthesis (S phase) and mitosis (M phase) are separated by the gaps G1 and G2. Cell cycle checkpoints are control mechanisms that ensure the fidelity of cell division in eukaryotic cells. These checkpoints verify whether the processes at each phase of the cell cycle have been accurately completed before progression into the next phase. Multiple checkpoints have been identified in the cell cycle process [19]. The key parts of the cell cycle machinery are the cyclin-dependent kinases (cdks) and the regulatory proteins called cyclins. The cdks are rational targets for cancer therapy because their expression in cancer cells is often aberrant and their inhibition can induce cell death. Inhibitors of cdks can also block transcription [20]. *Momordica charantia* extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes [21].

3.3 Clonogenic survival assays

Apoptosis, or programmed cell death is a cascade of normal physiologic process for removal of unwanted cells. The occurrence of this mechanism was detected on MCF7 human breast cancer cell line using fluorochrome-labeled Annexin V after 24 hrs exposure of ethanolic extracts in graded concentrations. The Phosphatidylserine (PS) levels (RFUs) in cells from the untreated controls served as a baseline indicator for normal Phosphatidylserine levels. The cultures treated with the positive control, staurosporine, 0.1 µg/mL produced a significant increase in Relative Fluorescence Unit (RFU), indicating release of Phosphatidylserine to the cell surface and thereby demonstrating the initiation of apoptosis. The results showed no significant apoptosis activity following treatment with 10, 20, 30 and 40 µg/ml (24 hrs.) of ethanolic extract of *Mikania glomerata* on MCF7 cells (Table: 3; Figure: 3).

The clonogenic assay estimates the regenerative potential of the extract or drug treated cells. Any subtle metabolic effect rendered by drug may get reflected by reduced ability of the cells to proliferate. It can be detected by number and size of the colonies formed by cells after exposure to drug [22]. It is a routinely used assay to look at cell survival following radiation exposure. This is gold standard method for looking at long term cell growth and differentiation. Hudson *et al* [23] in 2000 reported that in the clonogenic assay, the brown rice bran extract decreased colony formation in SW 480 and MDA MB 468 cells (human-derived tumor cell lines) and further recorded that the effect was particularly potent against MDA MB 468 cell line. Combinatorial cytotoxic effects of *Curcuma longa* and *Zingiber officinale* on

the PC-3M prostate cancer cell line using clonogenic assay was investigated and the potent chemopreventive activity of the test substances against human cancer cell line was concluded by Kurapati *et al* [24] in 2012.

3.4 Apoptosis using Annexin V

Apoptosis, or programmed cell death is a cascade of normal physiologic process for removal of unwanted cells. The occurrence of this mechanism was detected on MCF7 human breast cancer cell line using fluorochrome-labeled Annexin V after 24 hr exposure of alcoholic extracts in graded concentrations. The Phosphatidylserine (PS) levels (RFUs) in cells from the untreated controls served as a baseline indicator for normal Phosphatidylserine levels. The cultures treated with the positive control, staurosporine, 0.1 µg/mL produced a significant increase in Relative Fluorescence Unit (RFU), indicating release of Phosphatidylserine to the cell surface and thereby demonstrating the initiation of apoptosis. The results showed no significant apoptosis activity following treatment with 10, 20, 30 and 40 µg/ml (24 hr) of alcoholic extract of *Mikania glomerata* on MCF7 cells (Table: 4; Figure: 4).

The binding of Annexin V to phosphatidylserine is a common assay used as a measure of drug toxicity as it reflects translocation from the inner to the outer leaflet of the plasma membrane, which is one of the earliest features of cellular death via apoptosis [25][26]. Different researchers have documented the effect of plant extracts on cancer cell lines by using Annexin V for the reading of apoptosis. According to Mukherjee *et al* [27], ethanolic leaf extract of *Thuja occidentalis* blocks proliferation of A549 cells (lung carcinoma cells) and induces apoptosis in vitro. Anti-cancer potential study of plant extracts adopting Annexin V on *Ziziphus mauritiana* in vitro against HL-60, Molt-4, and HeLa cancer cell lines[28], algal extract on the AML cell lines HL-60 and MV-4-11 [29], *Sutherlandia frutescens* (cancer bush) extract on SNO oesophageal cancer cell line [30], *Chaetoceros calcitrans* extract on human breast cell line (MCF-7) [31], *Mikania hirsutissima* leaf extract fraction on U87 human glioblastoma cell line [32] and *Melilotus officinalis* (sweet clover) extract fraction on human TK6 lymphoblastoid cell line [33] were studied and their apoptotic property has been recorded.

3.5 Cytotoxicity using MTT assay

The optical density (OD) mean value calculated for untreated cells was 0.82 ± 0.08 . The positive control (phenol 1%) computed mean OD value was 0.192 ± 0.07 indicating the validity of the experiment. Introduction of cultures to the extract for 24hrs in the graded concentrations of 10 µg/ml, 20

µg/ml, 30 µg/ml and 40 µg/ml was done to assess the cytotoxic effect. The mean OD values obtained were in the order of 0.843 ± 0.08 , 0.844 ± 0.08 , 0.906 ± 0.1 and 0.518 ± 0.09 . No significant cytotoxicity was noticed in all the tested doses. The results, thus confirmed the non-cytotoxic nature of *M. glomerata* extract in ethanolic form to MCF7 cells. (Table: 5; Figure: 5). The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living

cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell line [34][35]. Extracts of *Petunia punctata*, *Alternanthera sessilis* and *Amoora chittagonga* when tested using the MTT assay were found to induce over 70% cell death in three human pancreatic carcinoma cell lines; Panc-1, MIA PaCa-2 (MIA and Capan-1) [36][37].

Table: 1 Cell proliferation assays in ethanolic extract of *Mikania glomerata* on MCF7 cells

Conc.	Cell counts per flask				
	Culture 1	Culture 2	Culture 3	Mean	SD
Initial cell count	20654	20764	20913	20777	130
24 hrs. counts	54462	49441	48838	50914	3088
Control	49246	48018	54067	50444	3197
10 µg/ml	49743	49005	38914	45887	6050
20 µg/ml	57245	41502	53980	50909	8309
30 µg/ml	43641	45965	52271	47292	4465
40 µg/ml	58330	47840	57511	54560	5834

Table: 2 Cell cycle distribution analysis of ethanolic extract of *Mikania glomerata* on MCF7 cells

Conc. (24 h)	Distribution of cells in various cell cycle phase			
	Pre G1	G1	S	G2/M
Distribution of seeding	1.47	70.85	7.81	19.88
Control	1.19	74.44	8.33	16.04
10 µg/ml	1.50	77.17	4.22	17.11
20 µg/ml	1.94	79.66	9.26	9.14
30 µg/ml	1.32	75.24	5.01	18.43
40 µg/ml	1.53	70.64	7.60	20.24

Table: 3 Clonogenic survival assays of ethanolic extract of *Mikania glomerata* on MCF7 cells

Conc. (24 h)	Colonies per plate				
	Culture 1	Culture 2	Culture 3	Mean	SD
Control	92	63	67	74	15.7
10 µg/ml	94	57	70	74	18.8
20 µg/ml	45	75	42	54	18.2
30 µg/ml	54	70	80	68	13.1
40 µg/ml	81	92	66	80	13.1

Table: 4 Apoptosis using Annexin V levels in the presence of ethanolic extract of *M. glomerata* on MCF7 cells

Conc. (24 h)	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Mean	SD
Untreated	1991	1998	2034	2100	2290	2530	2157	214
10 µg/ml	1953	2182	2149	2089	1958	2536	2145	214
20 µg/ml	2131	2558	2004	2181	2471	2588	2322	247
30 µg/ml	2595	2299	2550	2368	2360	2573	2458	129
40 µg/ml	3518	2548	2309	4020	3336	3443	3196	643
Staurosporine 0.1 µg/ml	4876	4866	4887	4579	5307	4533	4841	277

Table: 5 Cytotoxicity using MTT assay in the presence of ethanolic extract of *M. glomerata* on MCF7 cells

Conc. (24 h)	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Mean	SD
Untreated	0.8122	0.9732	0.7988	0.8196	0.804	0.7097	0.82	0.085
10 µg/ml	0.7392	0.9811	0.8428	0.8257	0.8608	0.8072	0.843	0.08
20 µg/ml	0.7295	0.8129	0.9711	0.8258	0.7684	0.956	0.844	0.099
30 µg/ml	0.9872	0.9212	0.8928	0.9916	0.9239	0.7179	0.906	0.1
40 µg/ml	0.5066	0.6384	0.4847	0.6094	0.5039	0.3621	0.518	0.099
Phenol 1%	0.2096	0.1038	0.1038	0.2936	0.2202	0.2206	0.192	0.075

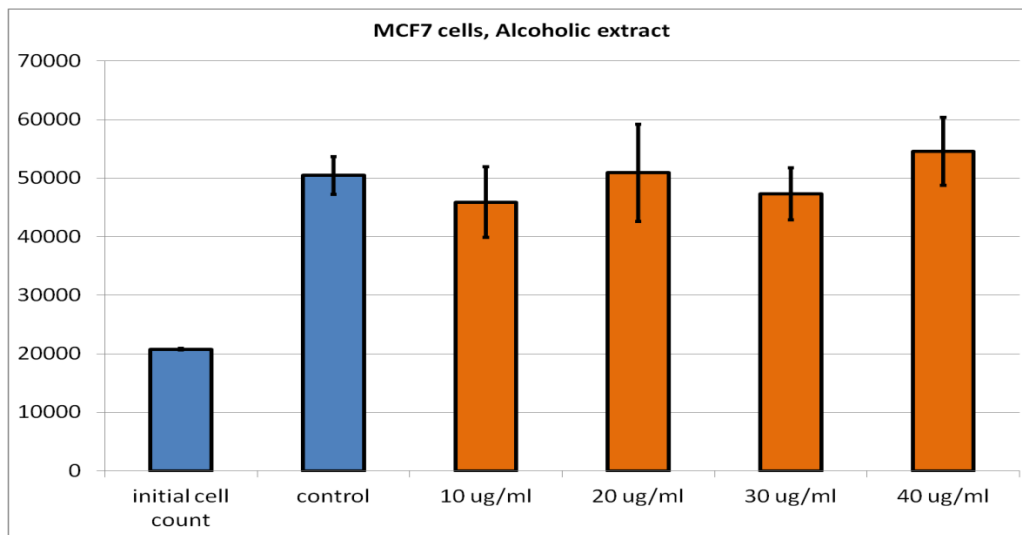


Figure 1: Cell proliferation assays of ethanolic extract of *M. glomerata* on MCF7 cells

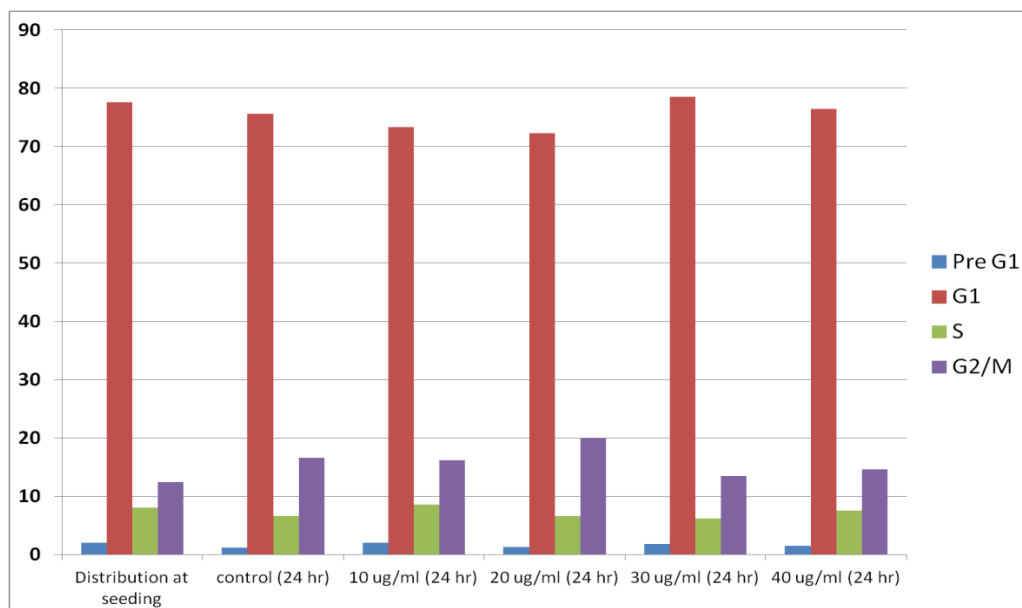


Figure 2: Cell cycle distribution analysis of ethanolic extract of *M. glomerata* on MCF7 cells

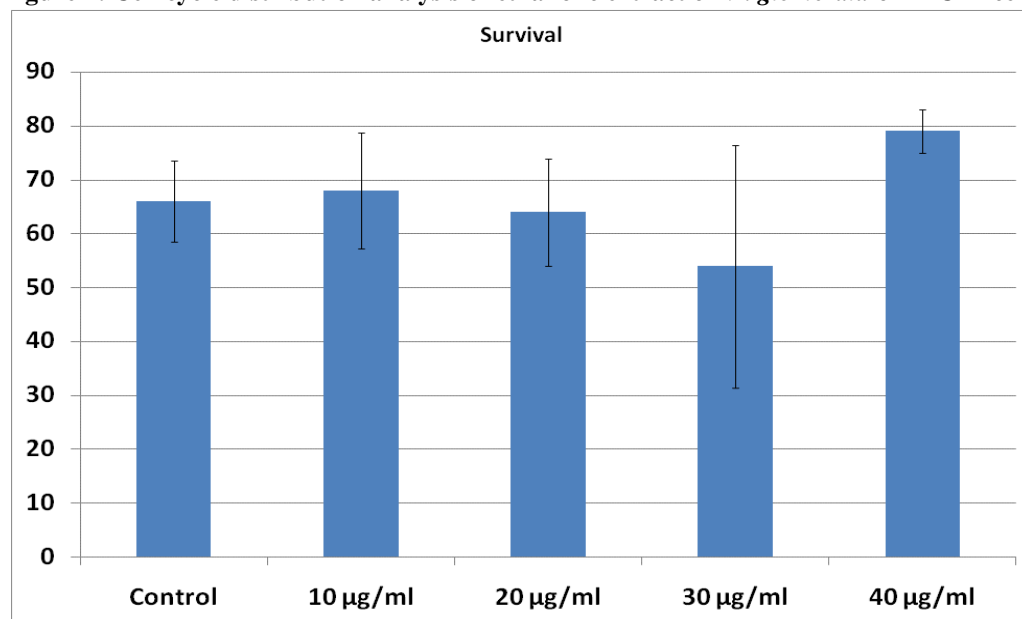
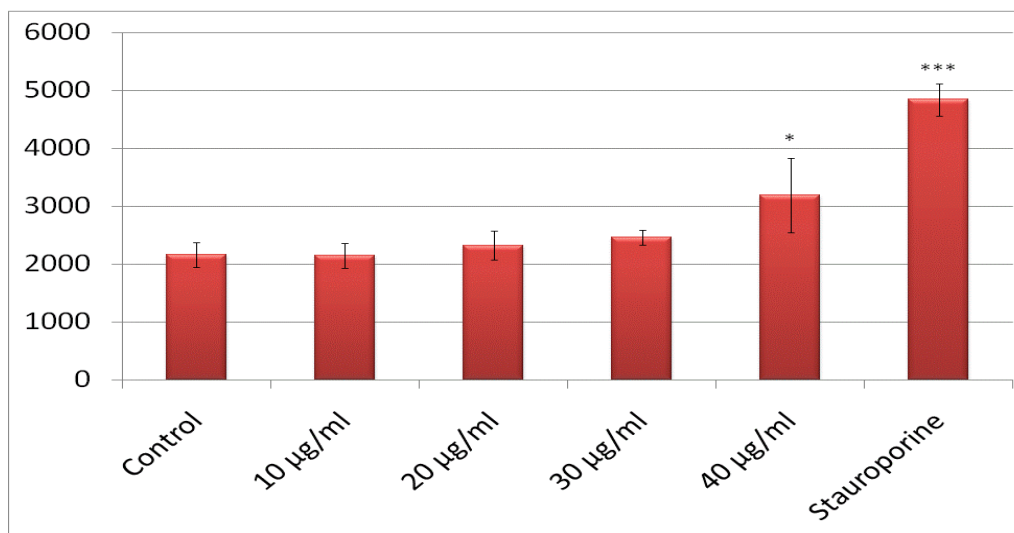
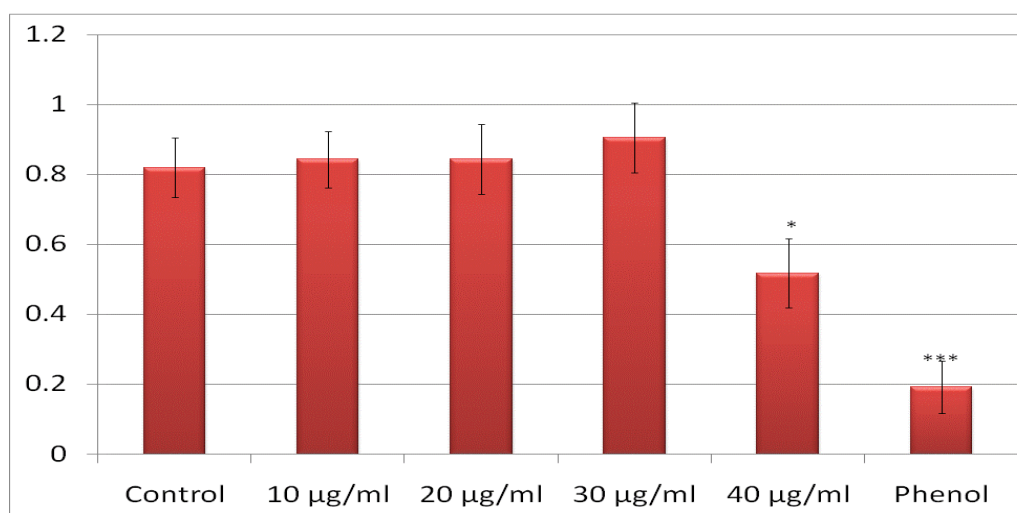


Figure 3: Clonogenic survival assays of ethanolic extract of *M. glomerata* on MCF7 cells



*** P<0.001; * P<0.05 on one way anova compared to controls

Figure 4: Apoptosis using Annexin V levels in the presence of ethanolic extract of *M. glomerata* on MCF7 cells



*** P<0.001; * P<0.05 on one way anova compared to controls

Figure 5: Cytotoxicity using MTT assay in the presence of ethanolic extract of *M. glomerata* on MCF7 cells

4. Conclusion

The anti-cancer activities of *Mikania glomerata* components were recognized thousands of years ago but proper scientific research with this important traditional medicine is a very recent story. More research works should be emphasized behind this because it is a safe and promising anticancer agent. Specially, researchers should investigate the active ingredients more broadly, because, there is very few authentic reports about the chemical composition of seeds or oil of *M. glomerata* exist. Also, the exact molecular mechanisms of components on different cancers should be investigated with more emphasis because current understandings are mostly unclear. For example, it is reported that *M. glomerata* oil can protect cells from radiation, but the molecular mechanisms behind this is not properly understood. Currently, in some parts of the world, there is a

renaissance of interest in traditional remedies. Many investigators now believe that traditional medicine is a promising source of new therapeutics against cancer. Extensive research with *M. glomerata* may contribute to the discovery of new anticancer strategies.

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