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Original Research Article

Anticancer potential of aqueous extract of *Mikania glomerata* against H292 human lung cancer cell line

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Abstract

Herbal medicine is the oldest form of healthcare known to the human race with a history of documentation for almost 4000 years. The *Mikania glomerata* Sprengel, popularly known as 'guaco', have a long standing record of application for the treatment of certain common ailments and at times of alarming emergency circumstances. In this study, *Mikania glomerata* was collected and processed against anticancer purposes. The aqueous extract of *Mikania glomerata* was challenged against H292 human lung cancer cell for anticancer activity. The results of cytotoxicity study using MTT assay on H292 human lung cancer cells in the presence of aqueous extract of *Mikania glomerata* proved that the plant extract inactivated the cancer cells. Exposure of cultures to the extract for 24hr in the concentrations 10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml had a mean OD of 0.83 ± 0.06, 0.893 ± 0.09, 0.816 ± 0.1 and 0.861 ± 0.05 correspondingly indicating no cytotoxicity in all the tested doses. Hence, this study confirmed that this plant may act as an alternative anticancer agent in near future.

Keywords: *Mikania glomerata*, H292 human lung cancer cell line, Anticancer activity, Aqueous extract.

1. Introduction

Traditional medicine has a long history of serving peoples all over the world. India is without doubt an herbal hub. Medicinal plants that are native to India and their use in various traditional systems of medicine are indeed awe-inspiring. The ethnobotany and ubiquitous plants provide a rich resource for Natural drug research and development. In recent years, the use of traditional medicine information on plant research received considerable interest. The medicinal plants contain several phytochemicals such as vitamins, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals etc [1][2]. These phytochemicals possess antioxidant activities, which prevent or can be used in the treatment of many diseases, including cancer. There are the several medicinal plants all over the world, especially in India, which are being used traditionally for the prevention and treatment of cancer. Medicinal herbs have been widely used for treatment of diseases in traditional way for several generations. Medicinal plants represent a vast potential resource for anticancer compounds. Out of an estimated 250000 higher plants, less than 1% have been screened pharmacologically [3]. Among the pharmacology important plants, very few plants only effect involve in the cancerous treatment. In cancerous treatment, one of the finest examples is *Mikania* sp. [4] The present study is focused to screen traditionally used medicinal plant of *Mikania* sp. for anticancer effect.

The species *Mikania glomerata* Sprengel and *Mikania laevigata* Schultz Bip. ex Baker, commonly known as guaco, are native plants in tropical areas that are widely employed for the treatment of several inflammatory and allergic conditions [5][6]. *Mikania glomerata* is a widely used plant material for several purposes. The flowering periods of *M. glomerata* is January/ winter period [7]. These herbs have long been used by rainforest inhabitants, who have an ancient tradition of using guaco for snake bites, fevers, stomach discomfort and rheumatism [2][7]. The guaco leaves have numerous uses because of their tonic, antipyretic, balsamic, anti-ophitic, stimulant, orexigenic, antispasmodic, expectorant, antimalarial, broncho-dilative, anti-ulcerogenic, hypo-allergenic, antispasmodic, anti-inflammatory, analgesic, anti-ophidian, anti-parasitic and monoamine oxidase inhibitor effects [1]-[7]. In the present study, the aqueous extract of *Mikania glomerata* has been successfully processed and was challenged against H292 human lung cancer cell line for anticancer activity.

2. Materials and methods

2.1 Collection of plant material

The plant *Mikania glomerata* (Figure 1) was collected from Central Research Institute for Siddha, Arumbakkam, Chennai, Tamil Nadu, India. The botanical identity of the species was authenticated at the Botanical Survey of India, Southern Regional Centre, Coimbatore.

Figure 1: *Mikania glomerata*

2.2 Preparation of leaf extracts (Aqueous extract)

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The most commonly used solvents for investigations in plants are methanol, ethanol, and water [8]. The leaves were collected individually from the plant, washed thoroughly thrice with distilled water; shade dried up to 5 days and ground into fine powder. The fine powder of the plant material was sterilized at 121 °C for 15 min and weighed. The ground plant material was sequentially extracted by exhaustive maceration at room temperature with de-ionized water. The supernatants were filtered and evaporated under vacuum to obtain the aqueous extracts. The aqueous extract was evaporated on a rotary evaporator and then reconstituted with sterile water (100 mg/mL) [9]. The extract was stored in a refrigerator at 4°C for further studies to avoid microbial contamination.

2.3 H292 human lung cancer cell line

The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment. The H292 lung cancer cell line was obtained from the American Type Culture Collection (Rockville, MD). This is a human cell line with near-diploid chromosome counts. The rate of cells with hyperploids count was 3.9%. Twelve markers were common to most cells. Among them were del(1) (q32.1), der (5)t(5;13) (p15.33;q11), i(5p), der(1)t(1;?) (p34.3;?) and der (6)t(6;7) (p25.3;q21.2). All markers were present in single copy per cell. Normal N1 and N6 were absent. The cells were isolated in a chemically defined medium HITES (Hydrocortisone, Insulin, Transferrin, Estradiol, Selenium) and later adapted to growth in media supplemented with serum. The cells retained their mucoepidermoid characteristics in culture as determined by their ultrastructure and expression of multiple markers of squamous differentiation.

The base medium for this cell line was ATCC-formulated RPMI-1640 Medium (Roswell Park Memorial Institue Medium), Catalog No. 30-2001. To make the complete growth medium, the following components were added to the base medium: fetal bovine serum to a final concentration of 10%. Temperature: 37°C; Atmosphere: air, 95%; carbon dioxide (CO2), 5%. The culture medium was removed and discarded. 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 effects

All cells were grown as described earlier. 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 and cell counts were taken.

2.5 Assessment of cell density and evaluation of viable cells

Determination of cell concentration is one of the mandatory procedures for estimating cell culture. Hence, cell density studies were done by evaluating the cell suspension using spectrophotometer. Optical density is applied in spectrophotometer to measure any turbid solution. It is an indirect method for any estimation since, only emitted light is measured at the end of the experiment.

2.6 Counting of cells using hemocytometer

Assessment of cell viability and distinction of cell types was carried using Neubauer counting chamber.

2.7 Fluorescence assay

Flow cytometry provides a rapid and reliable method to quantify viable cells in a cell suspension. One method to assess cell viability is through the use of dye exclusion. Live cells have intact membranes that exclude a variety of dyes that easily penetrate the damaged, permeable membranes of non-viable cells. Propidium iodide (PI) is a membrane impermeant dye that is generally excluded from viable cells. It binds to double stranded DNA by intercalating between base pairs

2.8 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. Clonogenic assay serves as a useful tool to test whether a given cancer therapy can reduce the clonogenic survival of tumor cells. 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 [10]. The stock flask of cells containing the cells that have to be tested is trypsinized. The cells should be in single-cell suspension to 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 and shaken gently to distribute the cells evenly. The flasks were then incubated for 12-14 days at 37ºC. The flasks were then stained with Gentian violet and scored for number of colonies.

2.9 Apoptosis

Apoptosis induction was determined by measuring Annexin V activity using Annexin V apoptosis kit. 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. Stauroporine 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. A aliquot of 100 µL of contents from each well was transferred to white-walled 96 well plate. The light emitted was measured by Packard lumicount microplate luminometer and measurement was recorded using THERMOmax^{TM} plate reader linked to a computer using SoftMax Pro software [11].

2.10 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 spectrophotomerically [12][13]. 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 [14]. Qualitative and quantitative evaluations of viability of cells were determined using MTT assay. Cells were seeded at the density of approximately $1x10^5$ 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 [15].

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^{TM} 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) CO_2 in air for approximately 24 hours. After incubation period, the cells on the plate were examined microscopically to assess any morphological alterations indicative of toxicity.

3. Result 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, flavonoids and many other compounds [16].

3.1 Cell proliferation assays

An investigation to evaluate the inhibitory effect of aqueous extract of *Mikania glomerata* on the proliferation of H292 human lung cancer cell line was done (Table 1). Initial cell counts were made in all the three culture vessels and 24hrs counts were made to ensure optimal conditions for cell proliferation in the taken test cell line. Control cell lines devoid of test extract were set to evaluate the inhibitory effect. 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 [17]. Aqueous extract of *Mikania glomerata* on the H292 human lung cancer cell line had no significant impact on cell growth in the higher dose of the test substance, but a near 20% reduction in cell proliferation was found in 20 µg/ml concentrations. Cell cycle distribution pattern of H292 human lung cancer cell line in the presence of aqueous extracts of *M. glomerata* was observed (Table 2). No noteworthy changes in the number of cells in the quiescent state (PreG1 or Go) 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. A marginal increase in synthesis phase (S) was observed in the distribution of cells in 10 µg/ml (24 hr), 20 µg/ml (24 hr) and 40 µg/ml (24 hr), when judged against the control. A slight and insignificant fall in the number of cells in Gap 2/ Mitosis (G2/M) phase was observed in all the test doses of the extract.

Table 1: Cell proliferation (H292 cell line) assays in the presence of aqueous extracts of *M. glomerata*

	Cell counts per flask				
	Culture 1	**Culture 2**	**Culture 3**	**Mean**	**SD**
Initial cell count	20401	20410	20333	20381	42
24 hr counts	54635	52391	50816	52614	1919
control	51919	54860	48557	51779	3154
10 µg/ml	37953	58914	34424	43764	13239
20 µg/ml	39937	31390	54752	42026	11820
30 µg/ml	51916	57038	37492	48815	10135
40 µg/ml	57267	49733	40194	49065	8556

Table 2: Cell cycle distribution (H292 cell line) analysis in the presence of aqueous extracts of *M. glomerata*

	Distribution of cells in various cell cycle phases			
	Pre G1	**G1**	**S**	**G2/M**
Distribution at seeding	1.05	70.89	4.30	23.76
control (24 hr)	1.91	72.02	5.05	21.02
10 µg/ml (24 hr)	1.79	71.45	9.68	17.08
20 µg/ml (24 hr)	1.14	73.95	7.02	17.89
30 µg/ml (24 hr)	1.63	77.45	4.15	16.77
40 µg/ml (24 hr)	1.29	72.36	8.36	17.99

3.2 Clonogenic survival assays

The clonogenic cell survival assay was performed to examine the effects of a botanical agent with potential applications in the clinic. Hudson *et al*., (2000)[18] 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. In the present study, different concentrations of aqueous extract of *Mikania glomerata* was used to test whether it can reduce the clonogenic survival of tumor cells (Figure 2). The test was conducted in triplicate culture plates with H292 human lung cancer cell line and the results were observed after 24hrs of insult in graded concentrations (Table 3). A replica of culture plates without the extract was prepared to serve as control for the purpose of comparison (Mean value of 69). The results indicated that the test plant material had no significant impact on colony or clone formation of H292 cells. Even the higher dose (40 µg/ml (24 hr)) did not produce the desired effect (Mean value of 85).

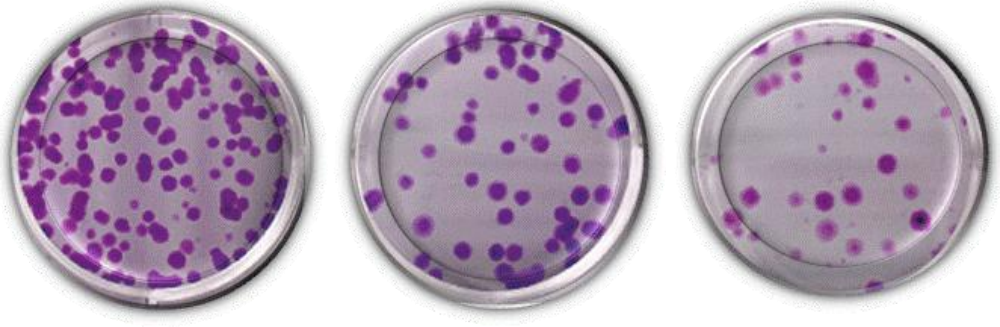

Figure 2: Clonogenic survival assays in the presence of extracts of *M. glomerata* - culture plate showing colonies

Table 3: Clonogenic survival (H292 cell line) assays in the presence of extracts of *M. glomerata*

	Colonies per plate				
	Culture 1	**Culture 2**	**Culture 3**	**Mean**	**SD**
control (24 hr)	58	51	99	69	25.9
10 µg/ml (24 hr)	57	62	49	56	6.6
20 µg/ml (24 hr)	97	64	87	83	16.9
30 µg/ml (24 hr)	89	76	99	88	11.5
40 µg/ml (24 hr)	61	93	100	85	20.8

3.3 Apoptosis assay

Apoptosis, or programmed cell death detection was done on H292 human lung cancer cell line using fluorochrome-labeled Annexin V after 24 hr exposure of aqueous extracts in 10, 20, 30 and 40 µg/ml concentrations. The Phosphatidyserine (PS) levels (RFUs) in cells from the untreated controls served as a baseline indicator for normal Phosphatidyserine levels. 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 (Bechelli *et al.*, 2011). The cultures treated with the positive control, stauroporine, 0.1 µg/mL produced a significant increase in Relative Fluorescence Unit (RFU), indicating release of Phosphatidyserine to the cell surface and thereby demonstrating the initiation of apoptosis. The results exhibited no apoptosis activity following treatment with 10, 20, 30 and 40 µg/ml (24 hr) of aqueous extract of *Mikania glomerata* indicating that the investigated material in aqueous form did not have the capacity to induce apoptosis in H292 cells (Table 4). According to Mukherjee *et al.*, (2012)[19], ethanolic leaf extract of *Thuja occidentalis* blocks proliferation of A549 cells (lung carcinoma cells) and induces apoptosis *in vitro*.

Table 4: Apoptosis (H292 cell line) using Annexin V levels in the presence of aqueous extracts of *M. Glomerata*

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Mean	SD
Untreated	2390	2397	2472	2160	2505	2306	2372	125
10 µg/ml (24 hr)	1976	2347	2600	2533	2194	2305	2326	227
20 µg/ml (24 hr)	1927	2150	1984	1912	2001	1980	1992	85
30 µg/ml (24 hr)	2303	2533	1994	2104	2530	2496	2327	234
40 µg/ml (24 hr)	2480	2040	2421	2260	2573	2393	2361	188
Stauroporine 0.1 µg/mL	5421	4632	3994	4743	4331	4450	4595	481

3.4 MTT assay

The results of cytotoxicity study using MTT assay on H292 human lung cancer cells in the presence of aqueous extract of *Mikania glomerata* have been tabulated in Table 5. The optical density (OD) of untreated cells had a mean value of 0.886 ± 0.09. The positive control (phenol 1%) had a mean OD value of 0.238 ± 0.05 indicating the cogency of the experiment. Exposure of cultures to the extract for 24hrs in the concentrations 10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml had a mean OD of 0.83 ± 0.06, 0.893 ± 0.09, 0.816 ± 0.1 and 0.861 ± 0.05 correspondingly indicating no cytotoxicity in all the tested doses. The results demonstrated the non-cytotoxic nature of *M. glomerata* extract in aqueous form to H292 cells. Different concentrations of the methanolic extract of leaves of the plant *Orthosiphon thymiflorus* were subjected to cytotoxic activity study against Dalton Lymphoma Ascites (DLA) cells using the MTT assay and reported that cell viability was inhibited to diverse extents by different concentrations of the extract [18].

Table 5: Cytotoxicity (H292 cell line) using MTT assay in the presence of aqueous extracts of *M. Glomerata*

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Mean	SD
Untreated	0.9205	0.8132	0.9621	0.7376	0.9564	0.9248	0.886	0.09
10 µg/ml (24 hr)	0.8107	0.7429	0.8821	0.7991	0.8049	0.9384	0.83	0.069
20 µg/ml (24 hr)	0.9753	0.7637	0.8368	0.9528	0.9986	0.83	0.893	0.095
30 µg/ml (24 hr)	0.8664	0.7295	0.7096	0.9178	0.9292	0.7445	0.816	0.1
40 µg/ml (24 hr)	0.8662	0.9075	0.8639	0.9361	0.7893	0.8054	0.861	0.057
Phenol 1%	0.2687	0.237	0.2869	0.227	0.138	0.2702	0.238	0.054

4. Conclusion

Among the medicinal species used in India, *M. glomerata* is especially important due to their relevant therapeutic properties. In popular medicine, this species have a long history of use, and they are still employed especially for the treatment of many diseases. Regarding the safety of the extracts, phytomedicines and isolated compounds, guaco species did not present significant toxic and genotoxic effects in humans. Based on these anticancer studies, *M. glomerata* have potential for development of anticancer agents against for H292 Human lung cancer cell line.

References

[1] Ruppelt BM., Pereira EF, Goncalves LC, Pereira NA. Pharmacological screening of plants recommended by folk medicine as anti-snake venom: I. Analgesic and anti-inflammatory activities. *Mem. Inst. Oswaldo Cruz.* 1991; 86:203-205.

[2] Pereira NA., Pereira BMR, Nascimento MC, Parente JP, Mors WB. Pharmacological screening of plants recommended by folk medicine as anti-snake venom; IV. Protection against jararaca venom by isolated constituints. *Planta Med.* 1994; 60:99-100.

[3] Savita Dixit and Huma Ali. Anticancer activity of Medicinal plant extract-A review. *Journal of Chemistry and Chemical Sciences.* 2010; 1 (1): 79-85.

[4] Freitas TP., Silveira PC, Rocha LG, Rezin GT, Rocha J, Citadini-Zanette V, Romao PT, Dal-Pizzol F, Pinho RA, Andrade VM, Streck EL. Effects of *Mikania glomerata* Spreng. and *Mikania laevigata* Schultz Bip. ex Baker (*Asteraceae*) extracts on pulmonary inflammation and

oxidative stress caused by acute coal dust exposure. *J. Med. Food* 2008; 11:761-7666.

[5] Block LC., Scheidt C, Quintao NL, Santos AR, Cechinel-Filho V. Phytochemical and pharmacological analysis of different parts of Wedelia paludosa DC. (Compositae). *Pharmazie* 1998; 53:716-718.

[6] Duke JA., Godwin MB, Ottesen AR. Duke´s handbook of medicinal plants of Latin America. CCR Press, Boca Raton. 2009; P 449.

[7] Napimoga MH., Yatsuda R. Scientific evidence for *Mikania laevigata* and *Mikania glomerata* as a pharmacological tool. *J. Pharm. Pharmacol.* 2010; 62:809-820.

[8] Rojas, JJ, Ochoa VJ, Ocampo SA and Monoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in treatment of onnosocomial infections. *BMC Complementary and Alternative Medicine*, 2006; 6: 2.

[9] Sá, RCS., Leite MN, Peters VM, Guerra MO, Almeida RN. Absence of mutagenic effect of Mikania glomerata hydroalcoholic extract on adult wistar rats *in vivo*. *Brazilian Archives of Biology and Technology*, 2006; 49(4): 599-604.

[10] Nicolaas, FAP., Hans RM, Jan S, Jaap H, Chris VB. Clonogenic assay of cells *in vitro*. *Nature Protocols*., 2006; 1(5): 2315–2319.

[11] Muñoz-Alonso, MJ., González-Santiago L, Zarich N, Martínez T, Alvarez E, Rojas JM, Muñoz A (2008) Plitidepsin has a dual effect inhibiting cell cycle and inducing apoptosis via Rac1/c-Jun NH2-Terminal Kinase Activation in Human melanoma cells. *Journal of Pharmacology and Experimental Therapeutics* 2008; 324: 1093-1101.

[12] Lakshmi Praba J., Arunachalam S, Riyazuddin R, Divya R, Vignesh S, Akbarsha A, Arthur James R. DNA/ RNA binding and anticancer/ antimicrobial activities of polymer-copper (II) complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013; 109: 23-31.

[13] Vignesh G., Pradeep I, Arunachalam S, Vignesh S, Arthur James R, Arun R, Premkumar K. Biological and protein-binding studies of newly synthesized polymer–cobalt (III) complexes. *Luminescence*. 2015; DOI 10.1002/bio.2992.

[14] Loosdrecht AAV., Beelen RHJ, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MMAC. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *Journal of Immunological Methods*., 1994; 174: 311–320.

[15] Vignesh G., Sugumar K, Arunachalam S, Vignesh S, Arthur James R, Arun R \Premkumar K. Studies on the synthesis, characterization, human serum albumin binding and biological activity of single chain surfactant–cobalt (III) complexes. *Luminescence*. 2015; DOI 10.1002/bio.2991.

[16] Gasparetto JC, Campos FR, Budel JM, Pontarolo R. Mikania glomerata Spreng. M. laevigata Sch. Bip. ex Baker, Asteraceae: agronomic, genetic, anatomical, chemical, pharmacological, toxicological studies and its use in herbal therapy programs in Brazil. *Revista Brasileira de Farmacognosia* 2010; 20 (4): 627-640.

[17] Vega-Avila E., Pugsley MK. An Overview of Colorimetric Assay Methods Used to Assess Survival or Proliferation of Mammalian Cells. *Proceedings of the Western Pharmacology Society*. 2011; 54: 10-14.

[18] Hudson EA., Dinh PA, Kokubun T, Simmonds MSJ, Gescher A. Characterization of Potentially Chemopreventive Phenols in Extracts of Brown Rice That Inhibit the Growth of Human Breast and Colon Cancer Cells1. *Cancer Epidemiology Biomarkers and Prevention*, 2000; 9(11): 1163–1170.

[19] Mukherjee A., Sikdar S, Bishayee K, Paul A, Ghosh S, Boujedaini N, Khuda-Bukhsh AR. Ethanolic extract of *Thuja occidentalis* blocks proliferation of A549 cells and induces apoptosis *in vitro*. *Journal of Chinese Integrative Medicine*, 2012; 10(12): 1451-1459.