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IN VITRO SCREEENING OF PHYTOCHEMICALS AND ANTICANCER ACTIVITY OF ARGEMONE MEXICANA LEAF EXTRACT

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ABSTRACT

The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of *Argemone mexicana* in aqueous, ethanol and methanol extract of root, stem and leaf. Phytochemical analysis of the root, leaves and stem extracts showed the presence of alkaloids, phenol, sugar, terpenoids**,** glycosides, flavonoids and tannins. The flavonoids and tannin contents were present in maximum level in the aqueous extract of *A. mexicana* leaf followed by stem and root extracts. MCF- 7 Breast cancer cells were treated with different concentrations of the aqueous extract of leaf at two different time intervals. Cell viability and apoptosis induction, were investigated. *A.mexicana* extract treated MCF-7 cells exhibited a marked increase in apoptosis as compared to untreated group and the data showed that the activity of caspase-3, -8 and -9 were significantly enhanced by A. *mexicana* treatment. Aqueous extract of the leaf showed dose-dependent cytotoxic and apoptotic effect in MCF-7 breast cancer cells. The phytochemical analysis of *A.mexicana* fetches pharmaceutical companies in the production of the new drugs against cancer.

KEY WORDS: *A. mexicana*, cytotoxicity activity, aqueous extract, MCF-7 cells.

1. INTRODUCTION

Medicinal plants comprise the main health care resources and they act as sources to develop a variety of therapeutic agents. Currently, there is a renewed attention in traditional medicine and an increasing demand for more drugs from plant sources. This importance in plant derived drugs are mainly due to the current widespread belief that “green medicine” is non-

toxic and more liable than costly synthetic drugs, many of which have adverse side effects [1]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect them from various diseases [2]. *Argemone mexicana* L. belongs to family Papaveraceae. Its other name is Mexican poppy or prickly poppy and is used as medicinal herbs. It contains alkaloids, flavonoids, tannins, sterols and terpenes [3]. Phytoconstituents such as chelerytherine, sarguinarine, protopine, optisine and berberine are predominantly present in *A. mexicana* leaves ; moreover roots and seeds are traditionally used for skin-diseases, leprosy, bilious fever and inflammations [4]. The plant is known to possess antibacterial, antifungal and anti-malarial activities [5-7]. Leaves are useful in the treatment of ulcer, warts, cold sores, cough, wounds, cutaneous infections, skin diseases, itches etc [8]. The fresh juice of *A. mexican a*leaves is used externally as a disinfectant for open wounds and cuts [9, 10]. The aim of the present work was to evaluate the existence and concentration of phytochemicals in plant parts and to evaluate the anticancer potential of *A. mexicana* leaf extract against MCF-7 breast cancer cells.

2. MATERIALS AND METHODS

2.1. Plant Collection and Extract Preparation

Fresh and healthy *Argemone mexicana* L.. plants were collected from Coimbatore district,Tamil Nadu, India. It was identified (No.BSI/SRC/5/23/201314/Tech./1399) by Botanical Survey of India, Coimbatore. All the chemicals and solvents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemicals, India. Fresh plant materials such as leaves, stem and root were washed under running tap water, air dried for seven days in shadow and then homogenized to fine powder and stored in airtight bottles. 10 gram of fine powder was taken in clean sterile soxhlet apparatus and extracted with 150 ml of methanol, ethanol and water. After extraction the extracts were collected for further analysis.

2.2. Qualitative Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by using following standard methods [11,12].

2.3. Quantification of Flavonoids

The plant extract (leaf) (100 µl) was mixed with 20 % aluminium trichloride in methanol (100 µl) and a drop of acetic acid, and then diluted by methanol to 5 ml. The absorption at 415 nm was read after 40 minutes. Blank samples were made from 100 ml of plant extracts

and a drop of acetic acid, and then diluted to 5ml with methanol. The rutin solution (0.5 mg/ml) was used as standard. All experiments were carried out in triplicates [13].

2.4. Quantification of Tannins

Quantification of tannins was carried out according to Van-Buren and Robinson (1981) method [13]. 50 mg of plant extract was suspended in 100 ml of distilled water, shaken for 1 h in a mechanical shaker and filtered. 5 ml of each was combined with 2 ml ferric chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferro-cyanide. The optical density at 120 nm was recorded within 10 min. Tannins contents were expressed as percentage of the dried fraction.

2.5. Anticancer Analysis

2.5.1. Maintenance of Cell Cultures

Human breast MCF-7 cell culture was obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were maintained and propagated in Eagles Minimum Essential Medium (EMEM) supplemented with Fetal Bovine Serum (10%, v/v) at 37 ˚C in a CO_2 incubator (5% CO_2, 95% air and 100% relative humidity). MCF-7 cells were harvested after trypsinization.

2.5.2. Trypan Blue Dye Exclusion Assay

Trypan blue dye exclusion assay was used to assess the impact of aqueous extract of *Argemone* leaf on the viability of MCF-7 cells. The cells (0.5×10^5 cells/ml) were seeded in six well plates in complete medium. The MCF-7 cells were mixed with 6.25, 12.5, 25, 50 and 100 µg/mL concentrations of aqueous extract of *A. mexicana* leaf and incubated for 24 and 48 h. After incubation period, the cultures were collected and washed. Then it was resuspended with PBS (0.4% trypan blue) and the cells were calculated using hemocytometer. Each experiment was carried out with three replications.

2.5.3. MTT Assay

In order to investigate cytotoxicity of aqueous extract of *A. mexicana* leaf, MCF-7 cells were harvested in the exponential phase of growth, seeded into 96-well tissue culture plates (15,000cells/well) and allowed to adhere for 48 h. Then, different concentrations (6.25, 12.5, 25, 50, 100 µg/ml) of aqueous extract of *A. mexicana* were added to the preferred wells and incubated for 48 h. A 20 µl of EMEM medium having MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5 mg/mL) was added to each well after incubation and

again it was incubated at 37 ˚C for 4 h. Consequently, the medium was changed with 100 µL of DMSO, and optical densities were measured at 570 nm. All studies were performed in triplicates and the data were expressed as the mean ± standard error.

2.5.4. Morphological Changes

MCF-7 cells (5×10^4cells/well) were seeded in triplicate into 6-well plates and treated with various concentrations of the aqueous extract of *A. mexicana* leaf. For qualitative analysis and images were taken randomly using an inverted phase-contrast microscope.

2.5.5. Caspase Activities

MCF-7 cells were plated in 6-well plates and treated with *A. mexicana* aqueous extract for 24h. Then the cells were harvested and caspase -3 -8 and -9 activity was measured by using colorimetric assay kits from Calbiochem. This method is based on colorimetric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labelled substrate DEVD-pNA (caspase 3) IETD-pNA (caspase 8) and LEHD-pNA (caspase 9). The free pNA was quantified using a spectrophotometer at 405nm. Comparison of the absorbance of pNA from an apoptotic sample with an untreated control allows determination of the fold increase in caspase activity.

2.5.6. Statistical Analysis

All experiments were carried out three times. Results are represented as mean ± standard deviation (S.D). Student t- test was performed and $p<0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Screening of Phytochemicals

The percentage yield of different extracts of different parts of *Argemone mexicana* L is shown in Table 1 and higher yield was observed in aqueous extract than other extracts. Phytochemical screening of methanol, ethanol and aqueous extracts of root, stem and leaf of *A. mexicana* is summarized in Table 2. It was observed that sugar and tannin were present in the all extracts of root, stem and leaf. Saponins were absent in all extracts of root, stem and leaf. The aqueous extract of leaf had significant amount of phytochemicals like sterol, reducing sugar, sugar, flavonoids, tannin, amino acids, glycosides and terpenoids when compared to other extracts. Methanol extract had lowest level of phytochemicals. The

previous studies reveals that alkaloids, amino acids, phenolics and fatty acids as major phytochemical groups in *A.Mexicana* [14-17].

Table 1. The percentage yield of different extracts of different parts of *Argemone Mexicana* L.

Solvent	Stem	Leaf	Root
Hexane	10%	13%	10%
Ethanol	12%	17%	12%
Ethyl acetate	11%	14%	8%
Methanol	12%	19%	11%
Water	13%	25%	16%

Table 2. Preliminary phytochemical analysis of *Argemone mexicana* L. plant materials

S.No	Phytochemicals	Ethanol extract			Methanol extract			Aqueous extract		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
1	Sterols	-	-	-	-	-	-	-	-	+
2	Reducing sugar	+	+	+	+	+	+	-	+	+
3	Sugar	+	+	+	+	+	+	+	+	+
4	Alkaloids	-	+	+	-	-	+	-	-	-
5	Flavonoids	+	+	+	+	+	+	-	+	+
6	Tannin	+	+	+	+	+	+	+	+	+
7	Saponins	-	-	-	-	-	-	-	-	-
8	Amino acids	-	-	+	-	-	-	-	-	+
9	Glycosides	-	-	-	-	-	-	-	-	+
10	Terpenoids	-	-	+	-	+	+	-	+	+

+ means present; - means absent.

Table.3. Quantitative phytochemicals of aqueous extract of *Argemone mexicana* L. leaf.

S. No	Phytochemicals	Ethanol extract	Methanol extract	Aqueous extract
1	Flavonoid	10 ± 0.7 mg	22 ± 1.2 mg	35 ± 1.3 mg*
2	Tannin	6 ± 1 mg	10 ± 1.5 mg	20 ± 1.5 mg*

Values are mean ± S.D.*p < 0.005

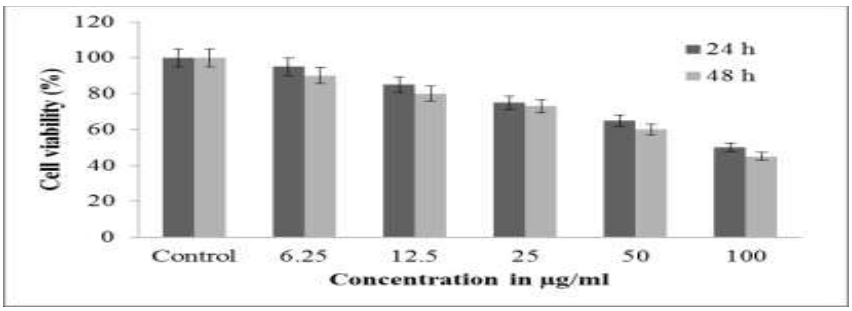

Fig. 1: MCF-7 cells were treated with various concentrations of aqueous extract of *Argemone mexicana* L. leaf for 24 h and 48 h, and the viable cells were determined by the trypan blue-dye exclusion method.

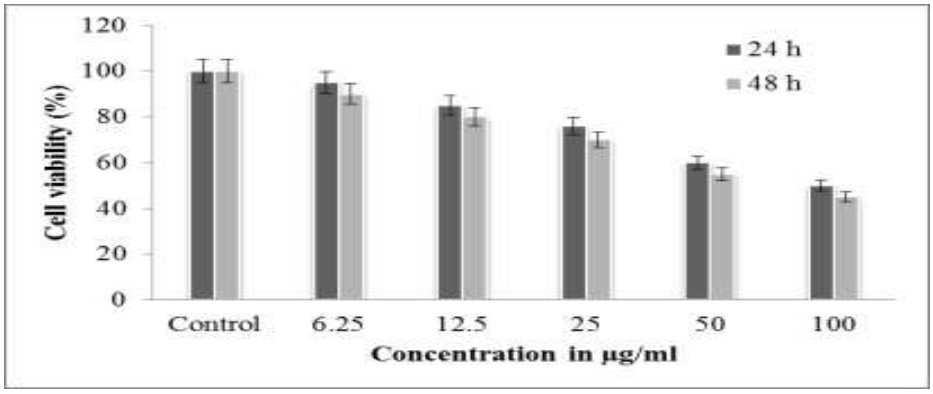

Fig. 2: Effect of various concentrations of aqueous extract of *Argemone mexicana* L. leaf for 24 h and 48 h on viability of MCF-7 cells as measured by MTT assay

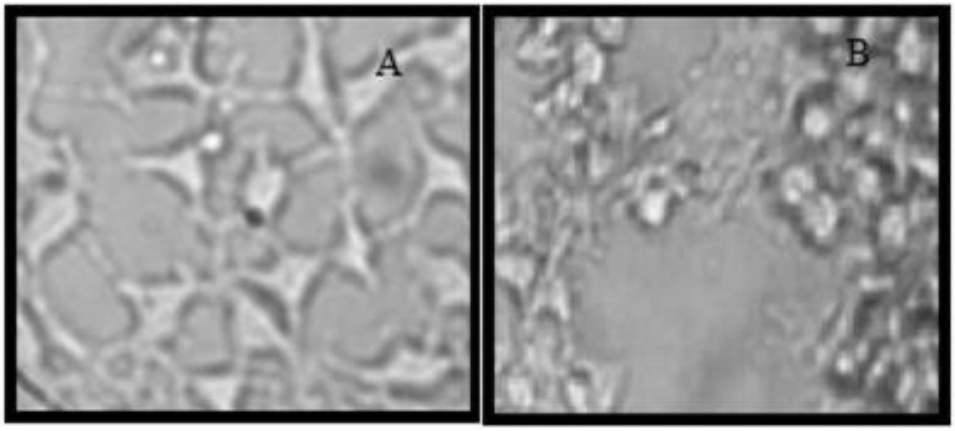

Fig. 3: Morphological changes A. Control MCF-7 cells and B. 100 µg/ml of aqueous extract of *Argemone mexicana* L. leaf treated MCF-7 cells

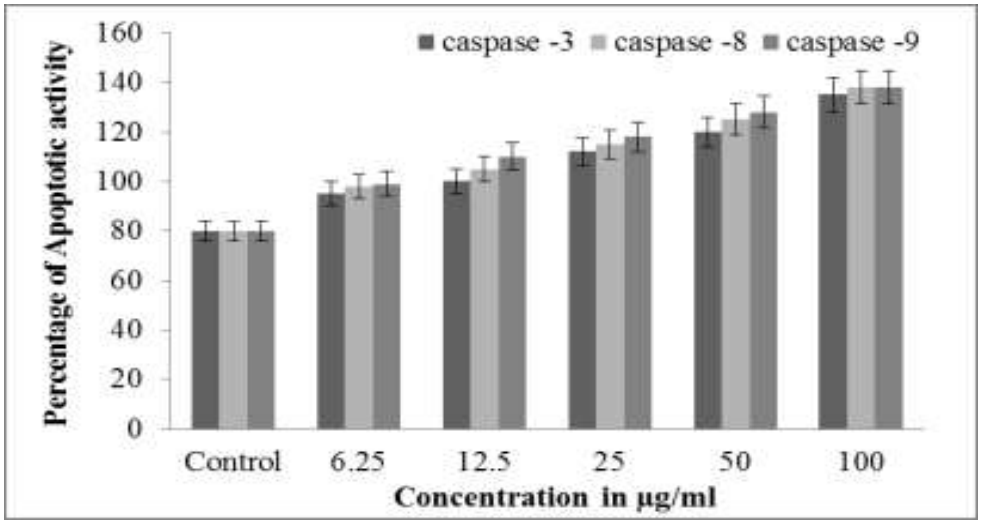

Fig. 4: Caspase -3,-8 and -9 activity in MCF-7 cells treated with different concentrations of aqueous extract of *Argemone mexicana* L. Leaf at 48 h.

3.2. Quantification of Flavonoids and Tannin

Total flavonoids and tannin content in all the extracts of *A. mexicana* leaf were presented in Table 3. Maximum levels of flavonoids were present in the aqueous extract of leaf when compared to hexane and ethanolic extract. The minimum level of flavonoid content was present in hexane extract. Highest tannin content was observed in aqueous extract of leaf. And lowest tannin content was noted in ethanol extract. Similarly, flavonoids were observed earlier in seeds. Gunstone *et al*. reported that highest amount of flavonoids were quantified in the selected plant species [18-20].

3.3. Anticancer Activity

3.3.1. Trypan Blue Dye Exclusion Assay

The cytotoxic effect aqueous extract of *A. mexicana* leaf on human breast cancer cell line MCF-7 was investigated for their viability with trypan blue dye exclusion method. The cell viability was calculated at 24 and 48 h of treatment with trypan blue staining. After 48 h, treatment exhibited a significant increase in cell death than 24 h treatment period. The concentrations of 50 and 100 µg/ml resulted in increased cell death whereas at concentration of 6.5 µg/ml had lowest cell death. Figure 1 clearly shows enhanced cytotoxicity in human breast cancer cells in a dose and time dependent manner, which reflected the loss of viability. Comparable results were observed by Kiranmayi*et al.*[21]in other cancer cell line with methanol extract of *A. Mexicana*.

3.3.2. MTT Assay

The anticancer activity of the *A. mexicana* aqueous extract was assessed against MCF-7 breast cancer cell lines at 6.5 to 100 µg/ml concentrations. The IC50 value of A. *mexicana* leaf aqueous extract was found to be 66.42 µg/ml (Figure 2). Maximum concentration of the plant extract (100µg/ml) effectively inhibited the growth of cell by more than 97%.Satish Kumar *et al.* [22] reported that 83% inhibition of growth in A-549 cell line and ethanol extract of *A. mexicana* was reported for anticancer activity against some cancer cell lines.

3.3.3. Morphological Assay

A. mexicana leaf extract treated cells showed the apoptotic features and which were observed microscopically. The survival rate of the cells after treatment of leaf extract was graphically plotted against time and dose. After 48 h, leaf extracts treated MCF-7 cells showed the typical apoptotic features such as chromatin condensation, membrane blebbing and cell shrinkage. 65% of apoptotic changes were detected after 72 h. The [Figure 3] showed and

confirmed that the apoptosis was caused by the mediated aqueous extract. Kiranmayi *et al*.[21] reported that apoptosis of cancer cells was induced by methanol extract.

3.3.4. Caspase Activities

The data presented a time and dose-dependent inhibition of MCF-7 cell proliferation by *A. mexicana extract*. There are many mechanisms through which apoptosis can be induced incells. The mitochondrial apoptotic pathways and death receptor pathways are the two majorpathways that have been characterized in mammalian cells. The effector caspase, caspase-3,and the initiator caspases, caspase-8 and -9, are the main executors of apoptosis [23]. Theresults revealed that *A. mexicana* aqueous extract exhibit cytotoxic effects via blocking themultiplication of MCF-7 cells and inducing apoptosis by controlling the expression of caspase-3, -8, and -9 levels (Figure 4). Equally, despite the absence of Caspase-3 expression, MCF-7cells undergo morphological apoptosis in response to a variety of agents [24, 25].

4. CONCLUSION

The present study demonstrates that *A. mexicana* leaf extract significantly inhibited the growth of MCF-7 human breast cancer cells *in vitro* through stimulation of apoptosis via inhibition growth and proliferation. Further studies are necessary to isolate and purify the active components of the plant to assess its mode of action.

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