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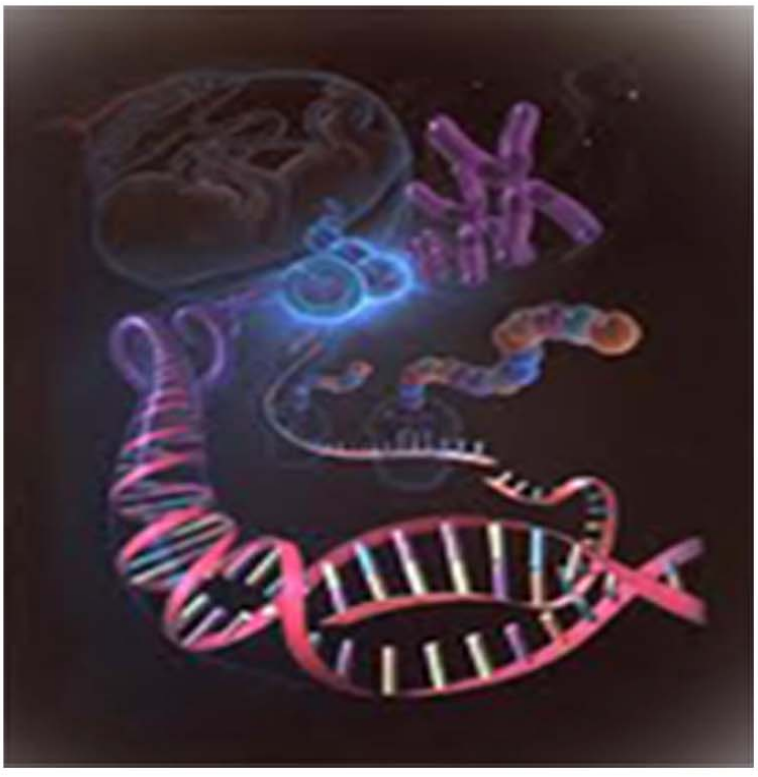

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Research Paper

EXTRACTION AND CYTOTOXICITY POTENTIAL OF MARIGOLD FLOWER (*CALENDULA OFFICINALIS*) AGAINST BREAST CANCER CELL LINE (MCF 7)

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The major source for lutein is marigold flower (*Calendula officinalis*). Lutein is a form of diesters and they are used as food colorant. They have many medicinal values and pharmacological activity. The present study deals with the extraction of lutein from the flower and the purification of compounds by thin layer chromatography and gas chromatography. The cell viability assay from the flower extract obtained was treated against breast cancer cell line (MCF 7). Thin layer chromatography was done and green color spot was observed and it was identified as lutein. GC-MS analysis was performed and these contains compounds were identified as diesters of lutein. Acetone extract was treated for cytotoxicity study against breast cancer cell line and the IC_{50} value is 79.5µg/ml.

Keywords: *Calendula officinalis*, Lutein, diesters, Breast cancer, Cytotoxicity, IC_{50}

INTRODUCTION

Marigold is a common flower native to India and some more countries. It has great potential uses, especially as traditional medicine and pharmaceutical drugs. It was used in traditional medicine, especially for wound healing, jaundice and blood purification. This plant is rich in many pharmaceutical active ingredients like carotenoids, flavonoids, glycosides, steroids and sterols quinines, volatile oil, and amino acid. The major compound present in marigold flower is lutein which is used as a coloring compound. Marigolds are also characterized by the presence of some important polycarbohydrates (Neukiron *et al*., 2004), which are soluble in water. Lutein (3R,3'R,6'R-$\beta\varepsilon$-carotene-3, 3'-diol) is a member of a group of pigments known as xanthophylls and has no provitamin A activity. Lutein is used as a pigment , role in human eyes as macular degeneration, cataracts, and photophobia.

Marigold flower extracts have high lutein content and it has anticancer activity. Some of the studies have been showed that this lutein has reducing risk of certain type of cancer such as

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breast and lung cancer. The incidence of breast cancer in India is on the rise and it is rapidly becoming the number one cancer in females, pushing cervical cancer to the second spot. It has been reported that about 10.4% of women have breast cancer. The data from the National Cancer Registry Program shows that in all the urban areas of India, breast cancer has now surpassed cervical cancer as the most frequently diagnosed cancer in women. As in other developing regions, the mortality rates for breast cancer in India are high in comparison to its incidence rates. The objective of the study deals with the identification of compound by thin layer chromatography, GC-MS analysis and the cytotoxicity study against the breast cancer cell line.

MATERIALS AND METHODS

Sample Collection

The marigold flowers were collected from Salem districts of Tamil Nadu, India. The flower was dried and used for extraction purpose.

Extraction

For the extraction purpose two methods have been followed. One was by boiling method and another was by soxhalet extraction. In soxhalet extraction the dried flower was packed in the filter paper and placed inside the soxhalet extractor flask. The solvent was taken in a round bottom flask. The Condenser was placed on top of the soxhalet by connecting inlet and outlet. The round bottom flask was placed in heating mantle. The solvent was used based on their polarity in increase order like benzene, ethyl acetate, acetone, ethanol and water, respectively.

Thin Layer Chromatography

TLC analysis carried out for compound isolation. TLC analysis was performed with the mixture of hexane: ethyl acetate in ratio of 7:3. Silica gel was used as a stationary phase. Silica gel was prepared, plated and dried. Then the extracts were spotted in the plate and placed in solvent mixtures. After few hours the separation of compound takes place. Retention factor was calculated using the formula

$$Rf = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

GC – MS Analysis

GC-MS analysis was conducted on a thermo GC - trace ultra VER: 5.0, thermo MS DSQ II. DB 35 - MS capillary standard non - polar column was used and Helium gas was used as inlet flow with 1 ml/ min. The temperature was raised from 80 ºC to 250 ºC at 6º C/ min. 1 µl of the extract was injected in and the peak was determined.

In Vitro Cytotoxicity Assay

Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density

Table 1: Retention Factor for Thin Layer Chromatography

Sample	Distance Moved by Solute	Distance Moved by Solvent	Retention Factor
Acetone	1.3	6.1	0.21
Ethyl acetate	0.7	6.9	0.10

Table 2: List of Compounds Obtained from the Extract

S. No.	Rt Value	Compound Name	Molecular Formula	Molecular Weight	Area %
11	36.16	Cyclotrisiloxane, hexaphenyl-	C36H30O3Si3	594	3.30
12	36.40	Lanosta-7,9(11),20-triene-3á,18-diol, diacetate	C34H52O4	524	1.98
13	36.62	Difuro[2',3':5,6:3'',2'':7,8] perylo[1,12-def][1,3]dioxepin-8, 15-dione, 10,11,12,13-tetrahydro-1, 7-dihydroxy-10,13-dimethyl-	C27H18O8	470	2.23
14	37.90	Stigmasterol	C29H48O	412	17.22
15	38.82	n-Tetracosanol-1	C24H50O	354	3.00

of $1x10^5$ cells/ml. 100 µl per well of cell suspension were seeded into 96- well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

The MTT cell proliferation assay is based on the metabolic activities of viable cells. In this assay, the yellow water soluble tetrazolium salt (3,4,5-dimethyl-2-thiazol-2,5-diphenyl-2,4-tetrazolium bromide) used is cleaved by the enzyme, succinate dehydrogenase, present in the mitochondria of metabolically active cells in to water insoluble dark blue formazon crystals. These intracellular formazon crystals can be solubilized using isopropanol or other organic solvents. After it is solubilized, the formazan formed can be easily and rapidly be spectrophotometrically quantified at 570 nm.

This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion is directly related to the number of viable cells. When the amount of purple formazon produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of the cells can be deduced, through the production of adose-response curve (Mosmann, 1983 and Monks, 1991).

MTT Assay Procedure

After 48 h of incubation, 15 µl of MTT (5 ml/ml) in Phosphate Buffered Saline (PBS) was added to each well and incubated at 37 ºC for 4 h.

The medium with MTT will be then flicked off

and the formed formazan crystals were solubilized in 100 µl of DMSO and then the absorbance was measured at 570 nm using micro plate reader.

The % cell inhibition will be determined using the following formula.

$$\% \text{ cell inhibition} = \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Nonlinear regression graph was plotted between % cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph pad Prism software.

RESULTS AND DISCUSSION

Collection of Extracts

The extracts were obtained by using soxhalet apparatus. The solvents used for extraction was based on polarity such as benzene, ethyl acetate, acetone, ethanol, water, respectively.

Thin Layer Chromatography

The mobile phase used was hexane: ethyl acetate in volume ratio 7:3. The silica gel was used as stationary phase. The TLC is performed with different extracts such as benzene, ethyl acetate, acetone, ethanol, water. The green color obtained was identified as lutein from the reference of Panatpong boonnoun *et.al*. (2012). Acetone and ethyl acetate have the green spot and by comparing to reference it was confirmed to be the lutein compound. The retention factor is calculated for acetone and the value is 0.21 and the green color spot was identified to be the lutein. The retention factor is calculated for ethyl acetate also and the value is 0.10 and it may be lutein (Figure 1 and Table 1).

GC – MS Analysis

The GC-MS analysis was performed to identify the compounds present in the sample. Many compounds have been reported which has been recorded in peaks. The peak was high at 37.90 min and the compound was found to be Stigmasterol has the highest probability. The peak at 36.62 min indicates the presence of Difuro[2',3':5,6:3'',2'':7,8] perylo[1,12-def][1,3] dioxepin-8,15-dione, 10,11,12,13-tetrahydro-1,7-dihydroxy-10,13-dimethyl- compound and another peak at 38.82 min the compound present was n-Tetracosanol-1 (Figure 2 and Table 2).

Cell Viability Test

MTT Assay

The acetone extract was treated with the breast cancer cell lines (MCF 7) with varying concentration as mentioned below and was incubated as per the protocol mentioned. At various concentration the cell death occurred and

Table 3: Calculation for IC_{50} Value

Conc (μg)	% Cell Inhibition	IC_{50}	79.5 μg/ml
6.25	1.191567		
12.5	1.833181		
25	6.507791	R^2	0.9953
50	19.61503		
100	66.36114		

Figure 1: Thin Layer Chromatography

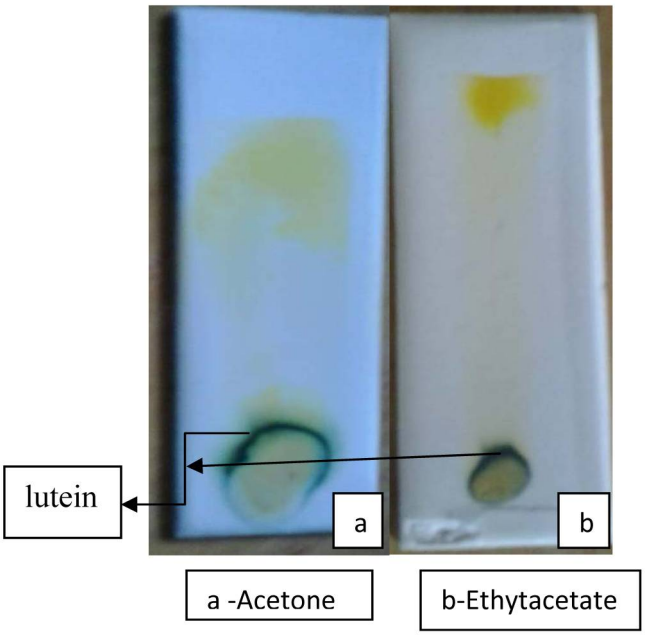


Figure 2: GC – MS Analysis for Acetone Extract

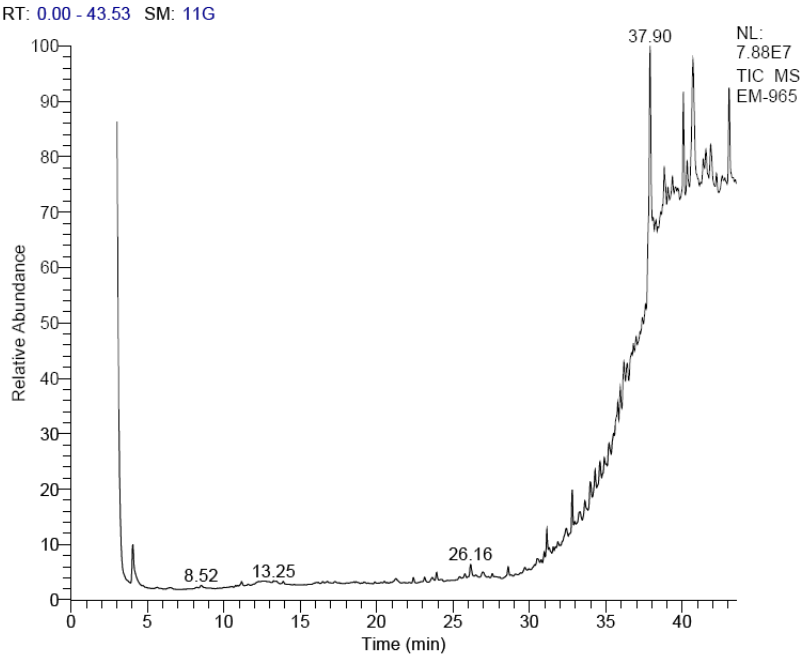
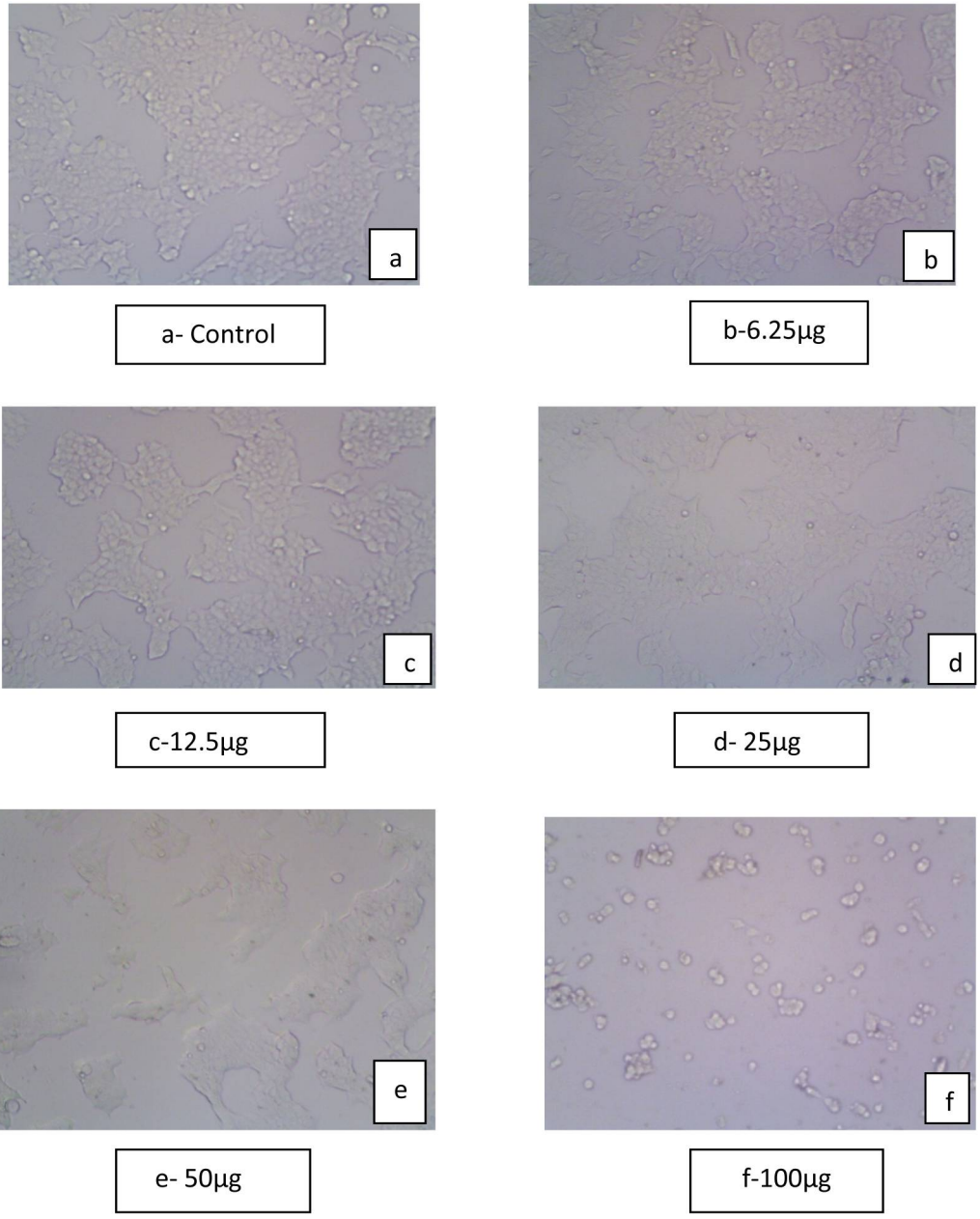
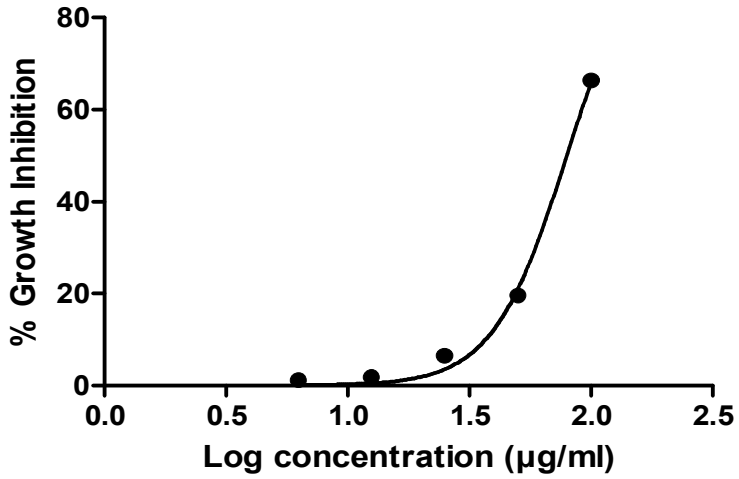


Figure 3: Cell line Inhibition of Extract at Different Concentration



at 100 µg concentration the cleavage was clear and the activity was seen. The control was taken for comparison. The IC_{50} value has been calculated to test the cytotoxicity of the extract. The IC_{50} value was calculated to be 79.5 µg/ml. The graph have been plotted for the cell growth inhibition. The acetone extract have the good activity and it can be treated against breast cancer cell line. It has been reported that breast cancer is one of the most common breast cancer spreading widely throughout the world. Mostly women get affected by breast cancer. So the

Figure 4: Graph for % Growth Inhibition



extract can be formulated and it can be treated against the breast cancer. The IC_{50} value is not too low or high so the extract can be treated.

CONCLUSION

From the present study the extracts were obtained from the marigold flower. TLC was performed to identify the compound present in the flower extract and the green color spot was developed in TLC plate and the compound was lutein .GC-MS analysis was performed to identify the compound present in the acetone extract based on peak obtained. The cell line study was performed for acetone extract against breast cancer cell line and the IC_{50} value was 79.5 µg/ml .Thus it was concluded that acetone extract of marigold flower have anticancer activity. The statistical analysis showed that the breast cancer occurred in female was increasing and it was first common disease in India in recent days. The acetone extract have good cytotoxic activity so it can be treated against breast cancer.

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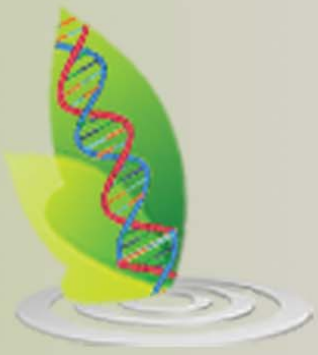

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