Asian Journal of Biotechnology and Genetic Engineering

Volume 6, Issue 2, Page 82-86, 2023; Article no.AJBGE.101450

In vitro Evaluation of Anticancer Activity of the Methanolic and Aqueous Extracts of the Petals of *C. tinctorius* L. (Safflower Florets)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/101450

Original Research Article

Received: 14/04/2023
Accepted: 18/06/2023
Published: 26/06/2023

ABSTRACT

The anticancer activity of the methanolic and aqueous extracts of the petals of safflower florets were analyzed using breast cancer cell lines namely MCF-7. The, MCF-7 cells were incubated with increasing concentrations of the methanolic and aqueous extract of the petals ranging from 5-150ug/ml for different time periods (6, 12, 18, 24, 36, 48, 72) and the cell viability was analyzed using MTT assay, aqueous extract of safflower florets Manjira and SSf-658 was found to be potent cytotoxic (IC50 value 34.17,36.96µg/ml) as similar to standard drug cisplatin. In addition to methanol extract pbns-12 was found to be potent cytotoxic with IC50 value 47.401±3.991 respectively.

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Keywords: C. tinctorius florets; secondary metabolites; anticancer activity; cisplatin drug.

1. INTRODUCTION

"Cancer has been a constant battle globally with a lot of development in cures and preventative therapies. The disease is characterized by cells in the human body continually multiplying with the inability to be controlled or stopped. Consequently, forming tumors of malignant cells with the potential to be metastatic" [1]. "Current treatments include chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer" [2]. The plants derived compounds are favourable for the treatment of cancer.

Safflower, *Carthamus tinctorius L.* is a thistle herb belonging to the family Asteraceae.

Safflower Plants are 30-150 cm tall with globular flower heads (capitula) and, commonly, brilliant yellow, orange or red flowers. It is one of humanity's oldest crops cultivated in india mainly for oil from the seeds and a dyes from the flowers..Though, safflower flowers have been used in preparations of ayurvedic medicines in india and also merit mention in European and Japanese pharmacopoeias.

In India, flowers of safflower are regarded as stimulant, sedative and as a promoter of menstrual discharge. In large doses, they are laxative. The main active ingredients in safflower florets are safflower yellow (carthamidin), which is water-soluble, and safflower Carthamin (red pigment) used in some preparations [3-8].

2. MATERIALS AND METHODS

DMEM (Dulbecco's modified Eagle's medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from eppendorf India.

2.1 Maintenance of Cell Line

The MCF-7 breast adenocarcinoma cancer cell line were purchased from NCCS, Pune and the cells were maintained in MEM supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37^0C.

2.2 Preparation of Test Compound

For MTT assay, Each Test compound was weighed separately and dissolved in aqueous and methanol. With media make up the final concentration to 1 mg/ ml and the cells were treated with a series of concentrations from 10 to 100 µg/ ml.

2.3 MCF-7 Cell Viability by MTT Assay

2.3.1 Method

100 µl media in 96 well plate culture medium and incubated overnight at 37^0C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compounds in representative wells. After 48 hrs., Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37^0C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % values is generated from the dose-response curves for each cell line using with origin software.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

3. RESULTS

3.1 *In vitro* Cytotoxic Analysis by MTT Assay

Among all the prepared extracts tested for the in-vitro anticancer activity using MTT assay MCF-7 cells, aqueous extract of safflower florets Manjira and SSf-658 was found to be potent cytotoxic (IC50 value 34.17,36.96µg/ml) as similar to standard drug cisplatin (Table 2). In addition to methanol extract pbns-12 was found to be potent cytotoxic with IC50 value 47.401±3.991 respectively, whereas aqueous extract of Nari-6, methanolic extract of SSF-658,A1 and Manjira showed the moderate anticancer activity against MCF-7 cell lines n (Table 1).

Table 1. Result for carthamin extracts

S. No	Sample Name	IC50 (µg/ml)
1	SSF-658	120.483±4.253
2	pbns-12	47.401±3.991
3	A1	328.079±9.909
4	Manjira	114.74±4.041
5	Nari-6	ND
6	CO-1	258.061±7.297
7	Cisplatin	4.653±0.330

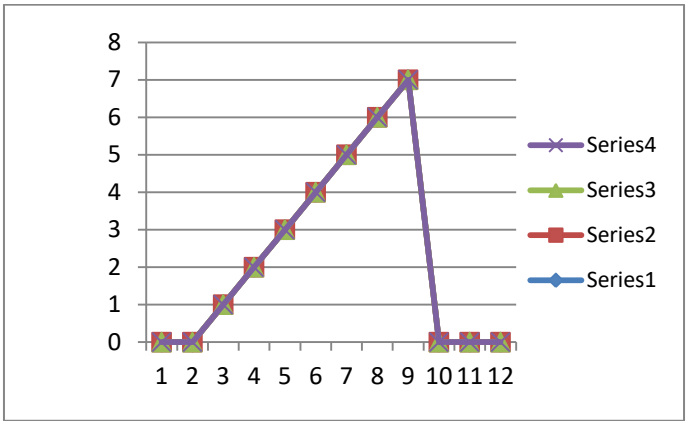

Fig. 1. *In vitro* cytotoxicity

Table 2. Anti-cancer activity of carthamidin extract

Sl. No	Compound in MCF7	IC50 (µg/ml)
1	SSF 6 58	34.967±4.506
2	Manjira	34.873±3.112
3	PBNS -12	ND
4	Nari – 6	393.407±8.774
5	Cisplatin	4.653±0.330
6	A1	ND
7	CO-1	ND

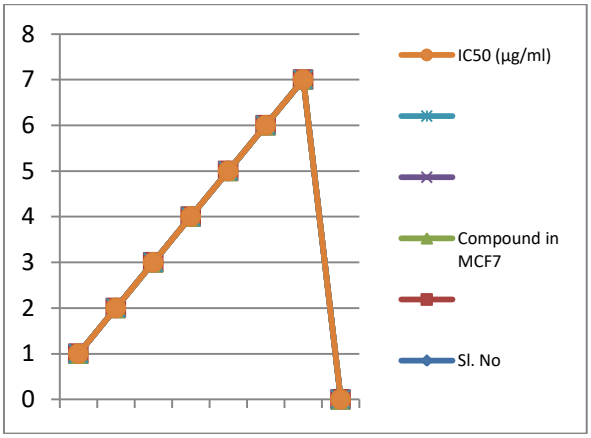

Fig. 2. Cyt otoxic effec t of the sample in M CF 7 cell line

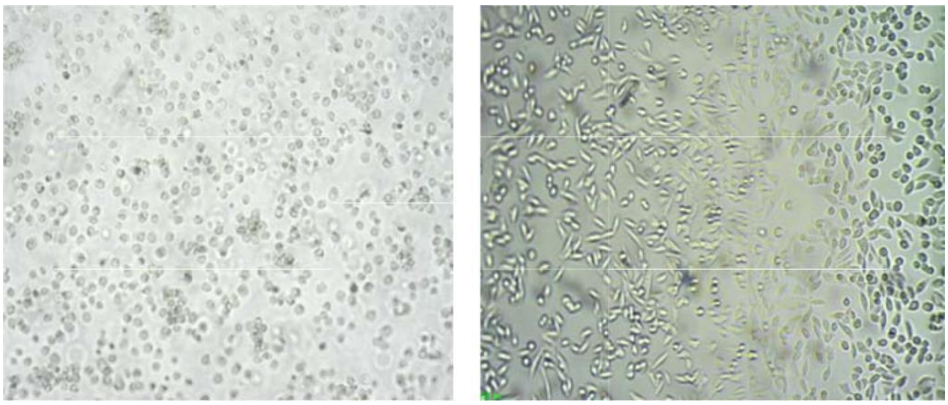

Plate 1. MCF-7 cell lines with media and sample MCf-7 cell lines with media and sample +M TT

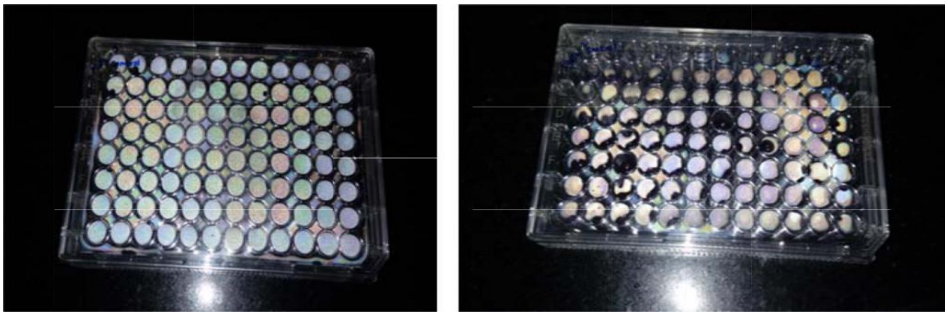

Plate 2. EL ISA plate s with MC F-7 cell lin es along with sample and MTT reagent

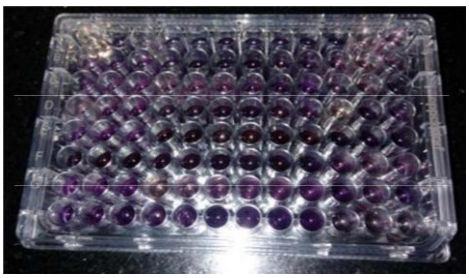

Plate 3. ELISA plate showing positive reaction

4. DISCUSSION

Among all the prepared extracts tested for the in-vitro anticancer activity using MTT assay MCF-7 cells, aqueous extract of safflower florets Manjira and SSf-658 was found to be potent cytotoxic (IC50 value 34.17,36.96µg/ml) as similar to standard drug cisplatin.This anticancer activity may be due to the various phytoconstituents present in the plant [9-12].

5. CONCLUSION

Based on the results of this study ,it is possible to conclude that safflower florets processes various biological activities including antioxidant, antidiabetic and antimicrobial activities as well as anticancer activity. The aqueous extracts of safflower florets traditionally used in ayurvedic medicines was screened for cytotoxic activity. However further studies are suggested to investigate these effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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