Original Research Article

Invitro Evaluation of Anticancer Activity of the Methanolic and Aqueous Extracts of the Petals of *C.tinctorius l.*, (Safflower Florets)

Abstract

Free radicals are compounds that contain unpaired electrons in their outermost orbital, which are capable of reacting with any macromolecules. Antioxidants are compounds that scavenge these free radicals by accepting or donating a pair of electrons and make them less reactive. A redox balance between the free radicals and antioxidants should be maintained. If this is not maintained either due to decreased antioxidant content or excessive free radicals, oxidative stress condition prevails, which is involved in the pathogenesis of various diseases including cancer.

Cancer is a rapidly progressing disease, which is characterized by uncontrolled cell division and failure of apoptosis. Highly oxidative environment promotes the development of cancer. Hence, therapies have been formulated with antioxidant compounds, which scavenge the free radicals and prevent cancer progression.

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 changes in cell growth and apoptosis as a consequence of treatment with the standard chemotherapeutic drug, in the presence and the absence of the petals of safflower floral extracts, were assessed. The effects of the extracts were also compared with the standard chemotherapeutic drug commonly used for treatment, namely cisplatin for MCF-7 .

The anti-proliferative effect of the extracts was assessed using breast cancer cell lines. In each of the cell lines, the dose and time period was optimized. 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. Our finding showed that the extracts inhibited the proliferation of the cells in a dose and time dependent manner. In MCF-7 cell lines, the optimal dose of petals of safflower florets was found to be 150ug and the optimal exposure time was found to be 12h, 24h and48h MCF-7 cell lines respectively. Hence, the cells were treated with these optimal dose and time periods for further studies.

Key Words: C. tinctorius florets, secondary metabolites, Anticancer Activity, Cisplatin Drug.

INTRODUCTION

Since ancient times, people have been exploring the use of eczema, swelling and constipation [8]. Capparis nature particularly plants in search of new drugs. This zeylanica (Brassicaceae) is thorny stout climbing shrub resulting in the use of a large number of medicinal plants used as antidote to snake bite, to cure swelling of testicles, with curative properties to treat various diseases [1]. smallpox, boils, cholera, colic, hemorrhage, neuralgia, Nearly 80% of the world's population relies on traditional sores, pneumonic

and pleurisy [2]. Clematis gouriana medicines for primary health care, most of which involve (Ranunculaceae) climbing glabrous shrubs, Bruised the use of plant extracts [3]. In India, almost 95% of the leaves and stems are used for killing lice [4]. Cleome prescriptions were plant based in the traditional systems viscosa (Cleomaceae) is an erect viscous glandular herb of Unani, Ayurveda, Homeopathy and Siddha [5]. The Seed paste taken orally with hot water in anthelmintic study of plants continues principally for the discovery of liver complaints [6-7]. Cochlospermum religiosum novel secondary metabolites. Around 80% of products (Cochlospermaceae) are deciduous trees. The oils were of plant origin and their sales exceeded US $65 administration of gum powder about 20g mixed with ghee billion in 2003 [4].

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 [(9)]. 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 (10).

For many years herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Plants have been used in medicine for their natural antiseptic properties. Thus, research has developed into investigating the potential properties and uses of terrestrial plants extracts for the preparation of potential nonmaterial based drugs for diseases including cancer [3]. Many plant species are already being used to treat or prevent development of cancer. Multiple researchers have identified species of plants that have demonstrated anticancer properties with a lot of focus on those that have been used in herbal medicine in developing countries.(11-12)

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, the interest in this crop has been rekindled in the last few years as the medicinal use of these flowers in china, has become more widely known. China has a significant area under safflower plantation, but is grown almost exclusively for its flowers, which are harvested for use in traditional medicines. Safflower flowers are used in China for the treatment of many illnesses as well as in the preparation of "tonic tea".

Safflower is now mainly grown in India for its much-valued edible oil. Safflower produces oil rich in polyunsaturated fatty acids (linoleic acid 78 percent), which play an important role in reducing blood cholesterol .

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)which is alcohol soluble, which are used in some preparations. Many clinical and laboratory studies support the use of safflower medicines for Cardiovascular disease and pain and swelling associated with trauma(8).

MATERIAL AND METHOD

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.

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 ^{0}C.

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.

MCF-7 cell viability by MTT Assay:

Principle:

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reducetetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purplecoloured formazan crystals. The cells are then solubilized with an DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

Method

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. MCF- 7 cells were trypsinized and performed the trypan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated

overnight at 37 ^{0} C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compounds in representative wells in 96 plates. 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 ^{0} C 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.

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

Result

In vitro cytotoxic analysis by MTT assay

To study the anticancer effects, the methanolic and aqueous extracts of safflower florets was tested for its effect on inhibition of cell growth against cell line i.e., MCF-7 over a concentration range of 5-100 µg/ml to determine their potency (IC50 ie,50% inhibition of cell growth) by comparing with standard .Assay was performed in vitro on exponentially growing cells. The activity was evaluated by measuring the levels of surviving cells after incubation with the test samples, using the MTT assay and is represented in below Figures.

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(Table-1). This anticancer activity may be due to the various phytoconstituents present in the plant, indicating the presence of flavonoids and triterpenoids ,fatty acids of aqueous and methanol extract which may attribute the anticancer activity.

Results

Table-1. Result for Carthamin extracts

S. No	Sample	IC50 (µg/ml)

	Name	
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**

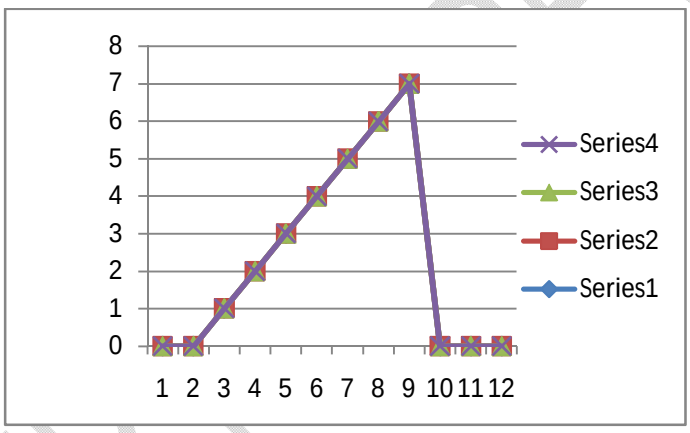

Fig 1. In Vitro Cytotoxicity

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**

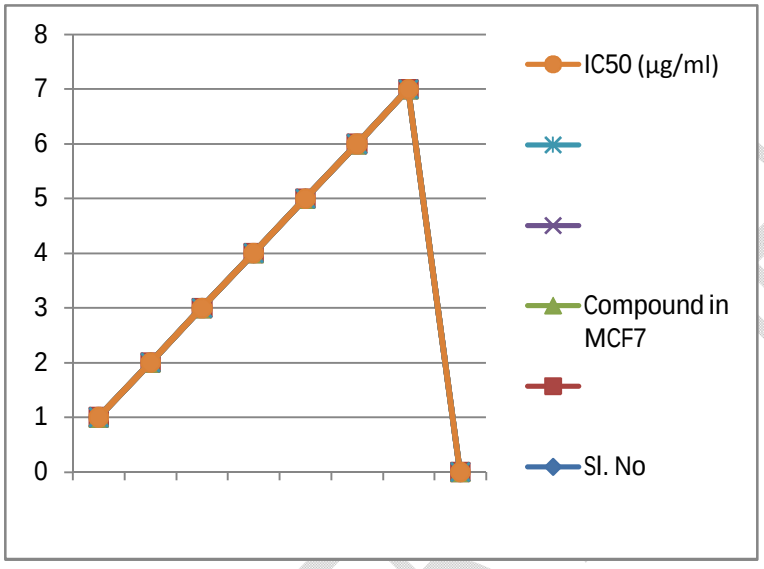

Fig 2. Cyt otoxic effec t of the sa mple in M CF 7 Cell line

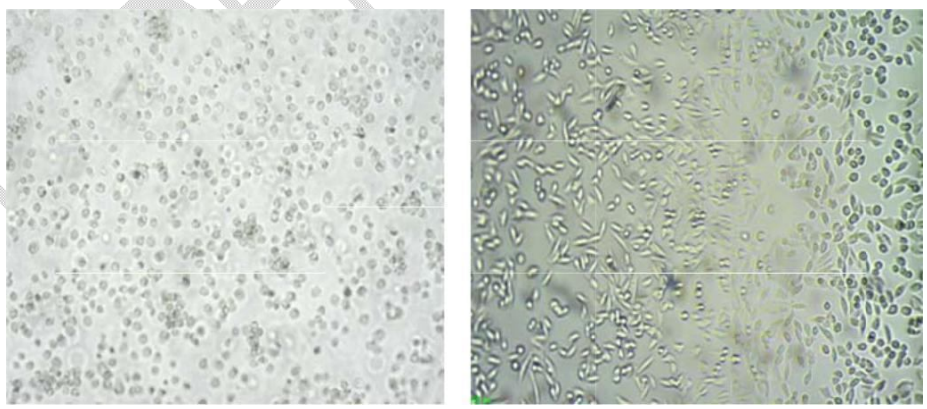

Plate 1. MCF-7 cell lines with media and sample MCf-7 cell lines with media and sample +M TT

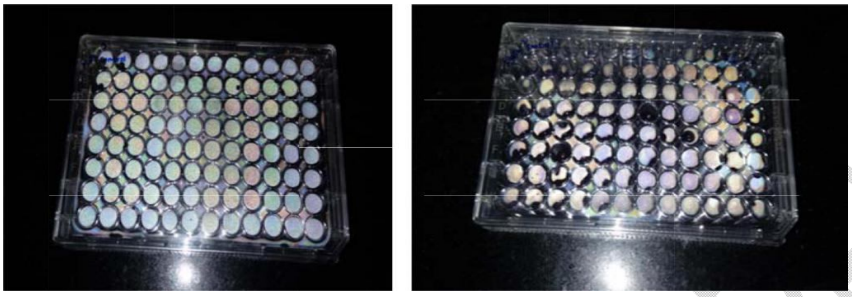

Plate 2. EL ISA plate s with MC F-7 cell lin es along with sample and MTT reagent.

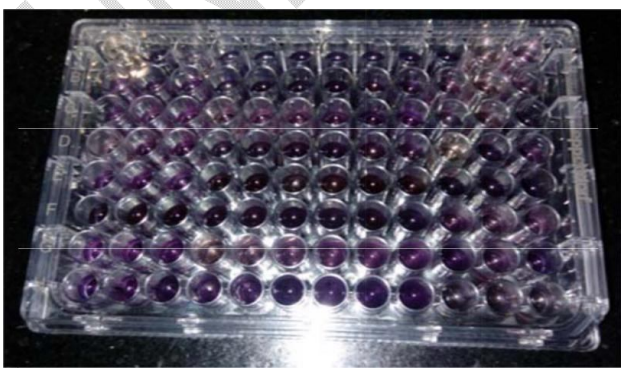

Plate 3. ELISA plate showing positive reaction

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(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. This anticancer activity may be due to the various phytoconstituents present in the plant, indicating the presence of flavonoids and triterpenoids ,fatty acids of aqueous and methanol extract which may attribute the anticancer activity.

CONCLUSION

The present study concludes that aqueous extract of safflower florets carries potent activity specifically against breast cancer cells MCF-7 cell lines. The results also revealed that the extract also significantly inhibited the colony formation and also the migration property of cancer cells in a dose-dependent manner. This unique mechanism can qualify the C.tinctorius aqueousextract to become a better treatment option for Breast cancer patients as appropriate.

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