Free Radical Scavenging and Cytotoxic Potential of *Celosia argentea*

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ABSTRACT

Introduction: Oxidative stress due to reactive oxygen species often leads to pathogenesis of chronic diseases such as cancer. Research states that a diet rich in polyphenols renders many health benefits by scavenging such harmful reactive species. *Celosia argentea* (Amaranthaceae), a common weed in India has been reported as a potential source of cheap, natural antioxidants due to its phenolic abundance. In this research work efforts were made to identify and screen the phenolic rich fractions of *Celosia argentea* for their antioxidant and anticancer potential. **Materials and Methods:** Various solvent fractions with increasing polarity were subjected to total phenolic content, followed by antioxidant assays- DPPH, ABTS and anti proliferative assays- Brine shrimp Bioassay, Antimitotic and MTT assays. **Results:** IC_{50} value of methanolic fraction for DPPH assay was statistically significant (26.25; ***P<0.001) when compared with ascorbic acid (12.50; ***P<0.001). Also TEAC values for methanolic fraction and BHT (standard) for ABTS assay were similar (2.1; ***P<0.001) Methanolicfraction at 400 μg/ml exhibited strong cytotoxicity (9.0 ± 0.81; ***P<0.001) against brine shrimps comparable to Methotrexate at 50 μg/ml(10; ***P<0.001) and significantly reduced mitotic index from 96.8 to 38.0 (***P<0.001) which was further confirmed by MTT assay where IC_{50} value of methanolic fraction for SiHa and MCF-7 cells was found to be 28 μg/ml with no cytotoxicity to normal cells proving its anticancer potential. **Conclusion:** This research proves antioxidant and anticancer potential of phenolic rich fraction of *Celosia argentea* and suggests it to be useful in cancer management as antifroliferative, chemo preventive and in cancer chemotherapy induced immune suppression and oxidative stress.

Key words: Antioxidant, Brine shrimp, Mitotic index, MTT, Phenolics.

INTRODUCTION

Imbalanced metabolism and excess reactive oxygen species (ROS) generation end into development of oxidative stress leading to range of disorders such as cancer, diabetes, atherosclerosis, cardiovascular diseases, Alzheimer's, Parkinson's disease, aging and many other neural disorders. Toxicity of free radicals contributes to proteins and DNA injury, inflammation, tissue damage and subsequent cellular apoptosis. Antioxidants are now being looked upon as persuasive therapeutic as they have capability to combat by neutralizing free radicals.[1]

As the natural antioxidant defense mechanism becomes inefficient, dietary intake of antioxidant is important to replenish and regenerate antioxidants that are responsible for removal of free radicals, scavenging ROS or their precursors, and binding metal ions needed for catalysis of ROS generation.[2] Synthetic antioxidants like BHT and BHA have their accompanied unavoidable side effects like radio-sensitization, toxicity of other chemicals, mutagenicity, and tumor formation from chemical carcinogens.[3] Hence, there is a need to explore the nature and screen the medicinal plants as potent antioxidants. It is found that dietary polyphenols obtained from various herbs, spices, fruits and vegetables are found to possess anticancer effects via

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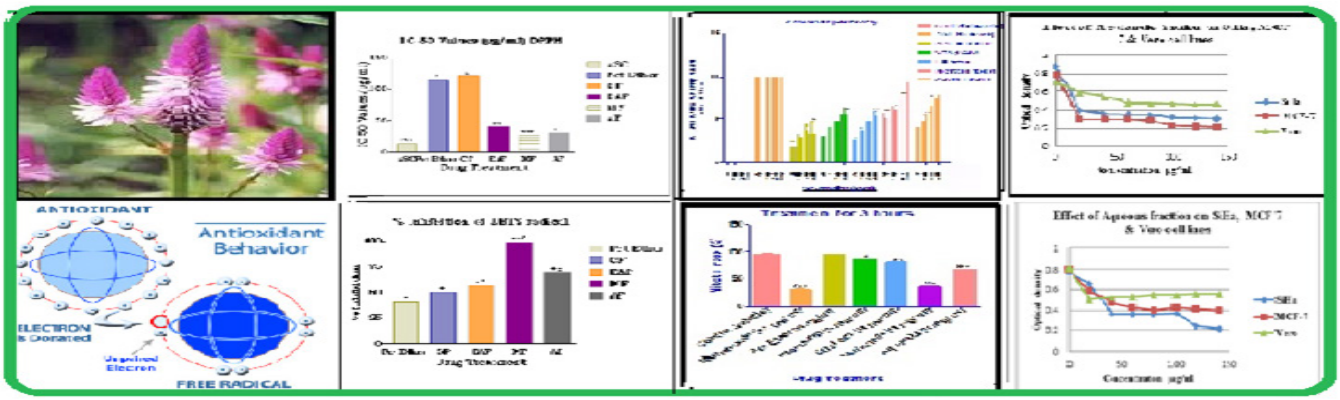

Graphical Abstract

a variety of mechanisms such as removal of carcinogenic agents, modulation of cancer cell signaling and antioxidant enzymatic activities, and induction of apoptosis and cell cycle arrest.[4]

Celosia argentea Linn. Belonging to family Amaranthaceae is a common weed in India found in the bajara fields widely cultivated as an ornamental plant, especially in Southern Europe

The entire plant is used in treatment of ulcers, piles, bleeding nose, inflammation, gynecologic disorders, mouth sores, eye diseases, glandular swellings, eczema, constipation and as an aphrodisiac.[5,6] The seeds are used in the treatment of blood diseases, diarrhea and the roots are well known for their anti-diabetic activity.[7] The *in vitro* and *in vivo* antioxidant activity of the plant is reported to be due to abundance of phenolics making *Celosiaargentea* as a potential source of cheap, natural antioxidants.[8]

Thus, the main objective of research work was to identify and separate the polyphenolic rich fraction using various solvents and subject these fractions to screen its antioxidant and anticancer potential using different assays.

MATERIAL AND METHODS

Plant Material

Fresh whole plant material of *Celosia argentea* Linn was collected from Bhor region, district- Pune, Maharashtra and was authenticated at the Botanical Survey of India (BSI), Pune; (Registration number-BSI/ WC/ Tech./2007) (BSI/ WRC/ Tech./2011).

Extraction

The separated, cleaned and powdered aerial parts were subjected to defatting using n-hexane followed by exhaustive successive solvent extraction using solvents with ascending order of polarity. The extracts obtained were concentrated by using rotary vacuum evaporator and preserved in vacuum desiccator for further pharmacological screening.[9]

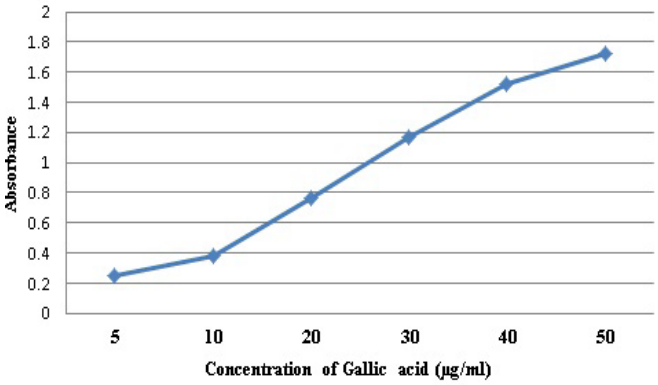

Figure 1: Concentration response curve for gallic acid at 765 nm

Estimation of Total phenolic content

Samples were measured for total phenolic content colorimetrically using the Follin C method with modification. A 100 µl of extract was mixed with 0.5 ml Follin C reagent (diluted 10 times with distilled water). The solution was added with 7 ml of distilled water and allowed to stand at room temperature for 5 minutes. Then, 1.5 ml sodium bicarbonate (60 mg/ml) solution was added to the mixture and left at room temperature in dark place for 2 hours. Absorbance was read at 765 nm against blank using UV-Visible spectrophotometer (Perkin Elmer Lambda 35, USA). A calibration curve was prepared, using a standard solution of gallic acid (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) (Figure 1). Results were expressed as gallic acid equivalents mg (GAE)/100 g.[10]

Pharmacological Investigations of Extracts

In vitro Antioxidant Activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay[11]

A solution of 3.3 mg DPPH in 100 ml methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02 - 0.1 mg of the extract. The reaction mixture was vortexes thoroughly and left in the dark at 25°C for 30 min. The antioxidant oninteraction with DPPH, both transfer electron or hydrogen atom to DPPH, thus neutralizing thefree radical character and convert it to 1, 1-diphenyl-2-picryl-hydrazine. The absorbance ofthe mixture was measured at 517 nm. BHT was used as reference. The ability to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ Radical Scavanging Activity} = Abs_{control} - Abs_{sample} / \times 100$$

$Abs_{control}$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol;

Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) scavenging activity

The stock solutions included 7 mM ABTS solution and 2.4 mM potassium per sulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantitiesand allowing them to react for 12 hours at room temperature in the dark. The solution was thendiluted by mixing 1 ml ABTS + solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS+ scavenging capacity of the extract was compared with that of BHT and percentage inhibition was calculated using the same equation as that for DPPH method.[12]

In vitro anticancer activity

Cytotoxic Evaluation by Brine shrimp bioassay[9]

In this test, Brine Shrimp *(Artemiasalina)* eggs were hatched in artificial sea water (3.8 g sea salt /liter of water). Generally the samples of test drugs for the experiment are prepared in methanol solution, which acts as a control vehicle. The sample tubes were previously prepared by dissolving specific concentration of test drug to prepare different dilutions followed by evaporation of methanol. After 48 hours of incubation, 10 Brine Shrimps were transferred to each sample tube using Pasteur pipette and artificial sea water was added to makeup the final volume up to 5 ml. Survivors were counted after 12 and 24 hours. Control vials were prepared using methanol only. Three replicates were prepared for each concentration of test drug.

Antimitotic activity[13]

The antimitotic activity was evaluated using the meristematic cells of onion *(Allium cepa)* root tips. The *Allium cepa* roots were sprouted in tap water at room tempatature. When the roots were about 5 mm long,the onion bulbs (five each) were paced on beakers containing the pet ether, chlorofor, ethyl acetae, methanolic and aqueous fractions (10 mg/ml) such that the roots were immersed in the extract. Distilled water and Methotrexate (0.1 mg/ml) were used as control and standard respectively. One hour latter the root tips were cut and transferred to fixing solution acetic acid(45%): ethanol (95%) in the ratio of 1:3v/v (10-12 hrs) followed by warming the root tips in 1 N hydrochoric acid in oven at 50^0C for 15 min. The root tips thus treated were then stained with carmine red stain were observed after a duration of one and three hours.

The slide was then scanned under the microscope to record the number of cells in each stage of cell-division and the mitotic index was caculated using the following formula as per the standard procedure. MI was determined by examination of 500 cells per slide, using 3-5 root tips from each bulb. The slides were examined from right to left, up end down, and the first 500 cells were scored for MI frequency

Standard formula

$$\text{Mitotic index} = \frac{\text{No of dividing cells}}{\text{Total no of cells}} \times 100$$

MTT Assay

The MTT-assay is based on the conversion of the tetrazolium salt MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide) to blue formazan which depicts cell viability. Color development in terms of optical density was measured spectrophotometrically at 540 nm and documented by ELISA reader at IRSHA (Bharati

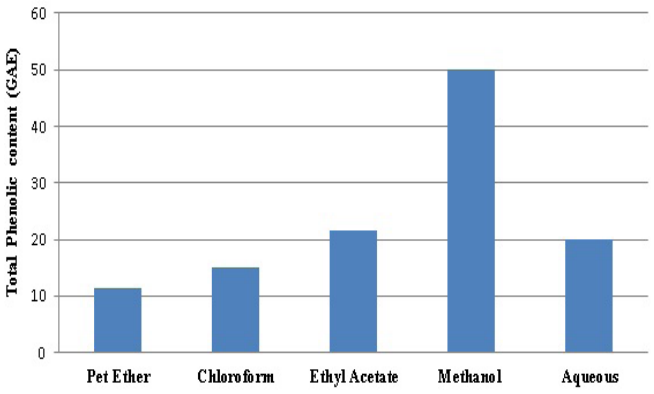

Figure 2: Total phenolic content of various fractions (GAE)

Vidyapeeth University, Pune).[14] Optical density values were converted to % cell viability to get the respective IC_{50} values for SiHa, HCT-15, MCF-7 and Normal monkey kidney Vero cells.[15]

Statistical Analysis

All the results were analyzed using one way ANOVA followed by Dennett's t test.

RESULTS AND DISCUSSION

Total Phenolic Content

The total phenolic content of various fractions of *Celosia argentea*was found in the range of 12-50 µg/ml (GAE/g) with methanolic fraction showing the highest (50 µg/ml;GAE/g) phenolic content (Figure 2).

DPPH Assay

The methanolic fraction of C*elosia argentea* was able to significantly reduce the stable DPPH radical to the yellow-colored diphenyl picryl hydrazine complex indicating potential free radical scavenging activity. The scavenging effect of various fractions and ascorbic acid, measured in terms of IC_{50} value (µg/ml) was observed in the following order: Ascorbic acid (12.50) (***P<0.001) > Methanolic fraction (26.25) (***P<0.001) > Aqueous fraction (30.00) (**P<0.01) > Ethyl acetate fraction (40.00) (**P<0.01) > Chloroform fraction (120.52) (*P<0.1) > Pet Ether fraction (115.00) (*P<0.1) fraction (Figure 3).

ABTS Assay

The TEAC value reflects the relative ability of hydrogen or electron-donating antioxidants to scavenge the ABTS radical cation compared with that of Trolox. The standard curve was linear between 0.5 and 2.0 mM of Trolox (Figure 4) As depicted in (Figure 5), Methanolicfraction showed maximum percentage inhibition of ABTS radical (99.17 %), with a TEAC value of 2.1 (***P<0.001) comparable with a standard antioxidant; BHT (2.1) (***P<0.001) (Figure 5).

Both the antioxidant assays clearly indicate that the Methanolicfraction of *Celosia argentea* possesses significant and concentration dependent free radical scavenging activity equipotent to the standard antioxidants such as ascorbic acid and BHT.

Brine Shrimp Bioassay

The number of brine shrimps dead after 12 hours with chloroform, methanolic and aqueous fractions of Celosia (400 mg/ml) was found to be 5.2, 7.0 and 5.0 respectively. The number was insignificant for pet. Ether and ethyl acetate fractions. Whereas the number of brine shrimps dead after 24 hrs was found to be 3.0, 5.5, 5.2, 9.0 and 7.0 for the pet. Ether, chloroform, ethyl acetate, methanolic and aqueous fractions respectively.

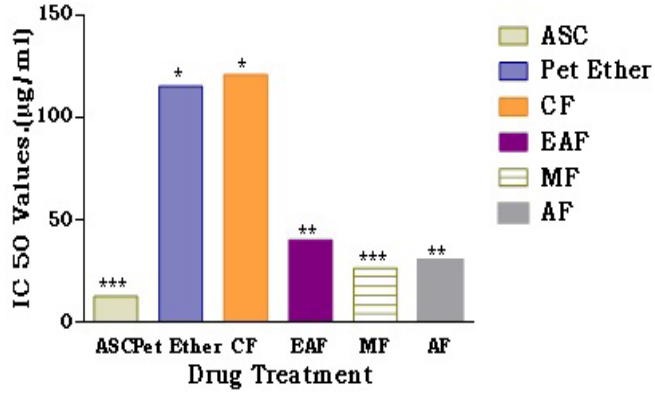

Figure 3: IC_{50} values of various extracts for DPPH Assay

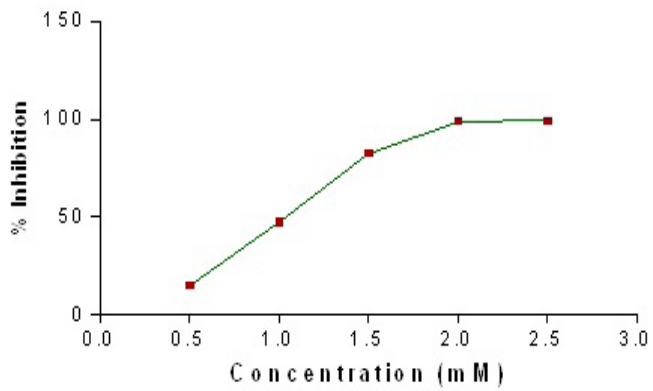

Figure 4: Concentration response curve of Trolox for ABTS at the absorbance at 734 nm

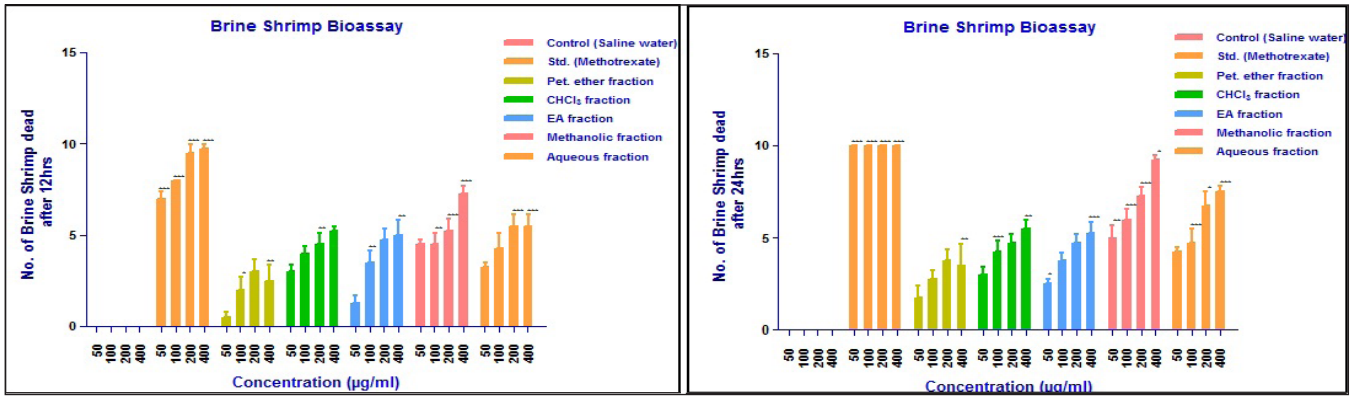

Figure 5: % Inhibition of ABTS radical by various fractions

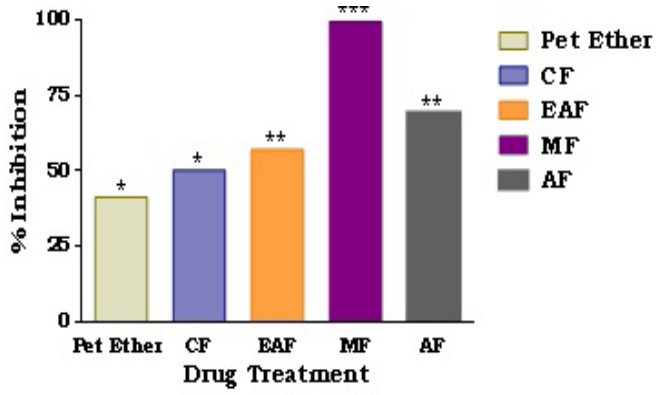

Figure 6: Number of Brine Shrimps dead after 12 and 24 hours.

Further, all the brine shrimps survived in saline water (control) and all the Methotrexate (50 mg/ml) treated Brine Shrimps were found dead (positive control) before 24 hours indicating its potent cytotoxic effect (Figure 6). The above findings indicate that the methanolic fraction at 400 mg/ml showed significant cytotoxic activity (9.0 ± 0.81; ***P<0.001) comparable to Methotrexate (10; ***P<0.001); a standard anticancer drug.

Antimitotic activity

The mitosis in the control group continued to be normal even after 3 hrs. (Figure 7) shows that mitotic indices of all extracts at two different durations were lower than the mitotic index of the control. The methanolic fraction of *Celosia argentea* (10 mg/ml) exhibited significant antimitotic activity in terms of reduction in mitotic index from 97.2 to 43.6 (1 hr treatment) and from 96.8 to 38.0 (3 hr treatment). The results obtained were comparable to that of Methotrexate with Mitotic indices of 46.2 (1 hr treatment) and 32.8 (3 hr treatment) (***P<0.001). Pet. ether, chloroform, ethyl acetate and aqueous fractions were found to be insignificant.

MTT Assay

The potential cytotoxic effect of various fractions of *Celosia argentea* was investigated on the viability of SiHa and MCF-7 cell lines by MTT assay. The results summarized in (Figure 8) indicate that methanolic fraction showed potent anticancer activity against both the cell lines with inhibitory concentration of 28 µg/ml (***P<0.001) with no toxicity towards the normal cells than the investigated

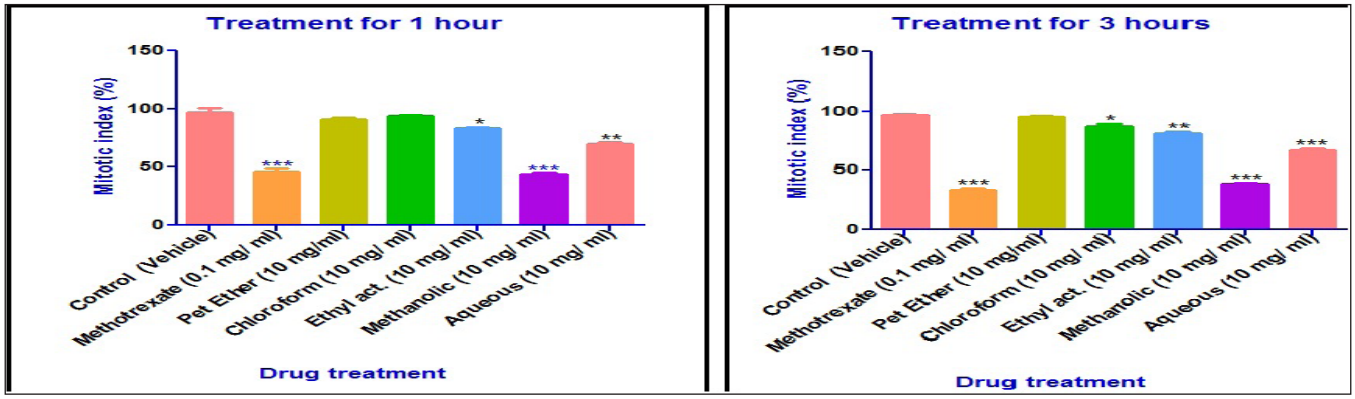

Figure 7: % reduction in Mitotic Index after treatment for 1 and 3 hours

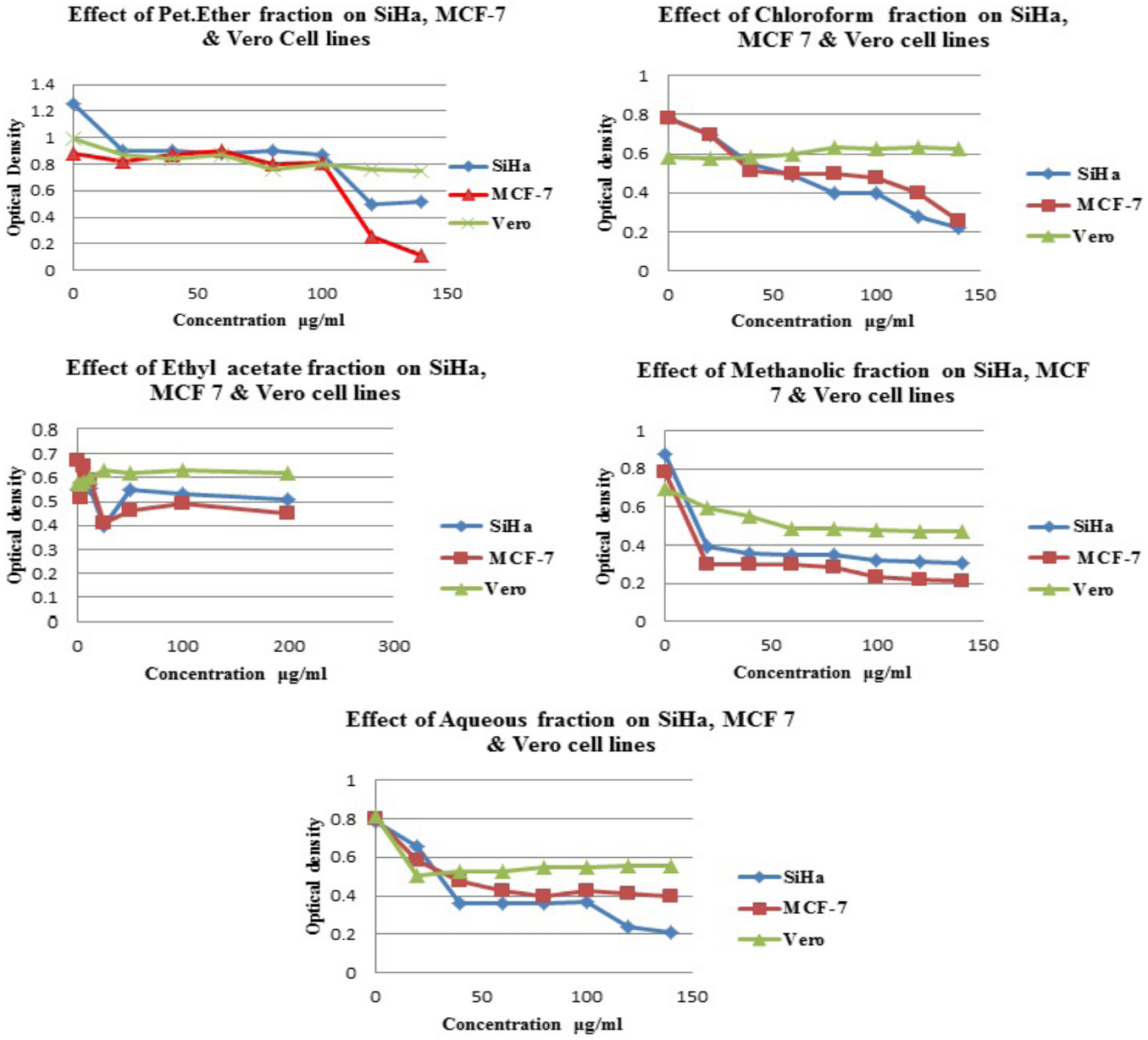

Figure 8: MTT Assay of various fractions

cancer cell lines.

CONCLUSION

The antioxidant potential of methanolic fraction makes it useful in reducing the oxidative stress generated due to reactive oxygen species for combating cancer and cancer chemotherapy induced immunosuppression. Thus, the phenolic rich methanolic fraction of *Celosia argentea* can is used either individually or as a natural adjuvant in cancer therapy along with other chemotherapeutic agents.

ABBREVIATIONS

ROS :Reactive oxygen species
BHA :Butylated hydroxyl anisole
BHT :Butylated hydroxyl toluene
DPPH :2,2-Diphenyl-1-picrylhydrazyl
GAE :Gallic acid equivalent
TEAC :Trolox equivalent antioxidant capacity
ABTS :2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid
BST :Brine Shrimp test
MTT :(3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide)
ELISA :Enzyme-linked immunosorbent assay

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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