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Antioxidant and Anticancer Activity of Edible Flowers

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ABSTRACT

Cancer is the second major cause of deaths after cardiovascular diseases due to exposure to various carcinogens in the food we eat, the water we drink and the air we breathe. Excessive free radicals produced during cellular metabolism can attack the deoxyribose, DNA backbone and bases, which leads to cytotoxicity or mutation and finally it causes cancer. There is a necessity for research to search of new compounds with cytotoxic activity, as the treatment of cancer. The available anticancer drug is often unsatisfactory due to the problem, which cause cytotoxicity to the normal cells along with cancer cells. Plants are considered as the valuable sources of bioactive compounds with antioxidant activity, which produce certain substances that have effects on living animal cells like apoptosis of cancer cells. In recent days emphasis has been on the use of edible sources for maintenance of the health. Therefore edible flowers which are now extensively used in preparation of foods is gaining much more attention for their medicinal value. The aim of the present study is to evaluate the in vitro antioxidant and antitumor activity of aqueous and ethanol extracts of Rose and Pea flowers.

Keywords: Edible flowers, Blue pea flower, Rose, Anticancer, Antioxidant

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INTRODUCTION

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism) [1]. Elevated levels of ROS have been detected in almost all cancers where they promote many aspects of tumor development and progression. When there is an imbalance between oxidative stress and antioxidants then there is a chance that diseases such as cancer, autoimmune disorders, ageing, cardiovascular, neurodegenerative diseases and other ROS induced multiple diseases may develop[2, 3]. While undergoing conventional treatment such as chemotherapy, radiotherapy, hormone therapy cancer cells may acquire further mutations and become resistant or refractory to that therapy leading to progression or recurrence of cancer. The body will defend itself by endogenous antioxidants. If percentage of free radicals outrage endogenous antioxidants exogenous antioxidants from diet has to be taken. Therefore herbal formulations tame aggressive cancer cells by repairing damaged DNA inhibiting mutation in genes and pathways blocking various cancer promoting enzymes and hormone thereby reviving the process of apoptosis, inhibiting tumors angiogenesis and boosts immune system of the body. There are various anticancer herbs having DNA repairing, antimutagenic, antitumourangiogenic, proapoptotic, immunoenhancing, radioprotective and chemoprotective properties. The primary objective of this work is to explore the biochemical components, antioxidant activities and anticancer activity in edible flowers which are locally available. In view of this and the present understanding about ROS induced multiple diseases, screening of the antioxidant activity and anticancer activity was carried out. The Indian culinary world might have embraced the concept of edible flowers fairly recently, but they have been a part of traditional Indian cuisines since the very beginning. Edible flowers which are now extensively used in preparation of foods is gaining much more attention for their medicinal value. Flowers are not new to the plate. In many forms, they are routinely consumed, whether as vegetables (cauliflower and banana inflorescence) or as colouring and flavouring agents (rose, saffron, jasmine) and even as spices (cloves are dried buds). The original rose jam, gulkand, has been around for 700 years. Then there is also a very old recipe of gulmohar ghosht from Rajasthan. They use dianthus flowers in rasmalai and malai johar chaat[4]. Clitoria ternatea, commonly known as blue pea is a plant species belonging to the Fabaceae family. In traditional

Ayurvedic medicine, it is ascribed various qualities including memory enhancing, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative properties[5]. Rosa Macdub (Rose) is a perennial plant of the genus *Rosa*, within the family *Rosaceae*. Rose petals work as a diuretic and flush toxins from the body. They are known to relieve bronchial and chest congestion and also provide relief from sore throat. Rose water because of its antiseptic property has been used as eyewash and is also used to moisturize the skin.

MATERIALS AND METHODS

The flowers were obtained from local gardens. The petals separated from flowers were cleaned and washed in distilled water for the assay of various biochemical components and antioxidant activities. 1g of petals of two flowers were taken and ground with 10ml of distilled water and ethanol using pestle and mortar and centrifuged at 10,000xg for 10 minutes at 4°C in a cold centrifuge and filtered through Whatmann No.1 filter paper. The filtrate was evaporated to dryness and stored at 4°C.

Protein estimation

Protein content of aqueous samples was determined by the method of Lowry et al., [6] using BSA as protein standard.

Estimation of total sugars

Total sugars were estimated according to the procedure of phenol-sulphuric acid method[7]. Total sugars were expressed in terms mg of glucose/g of flower. Glucose (0-25µg) was used as reference standard.

Determination of total phenolics by Folin-Ciocalteau assay

The concentration of total phenolics in all the extracts was determined by the Folin-Ciocalteu assay. It involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium[8]. In this study Gallic acid was used as spectrophotometric standard. The phenolic contents of the extracts were determined from calibration curve and were expressed in mg of Gallic acid equivalents/ g sample.

Estimation of total flavonoids

Aluminium chloride colorimetric method[9] was used for flavonoids determination. The absorbance of the reaction mixture was measured at 420 nm with UV visible spectrophotometer. The content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution in distilled water. The concentration of flavonoid was expressed in terms of mg/g of sample.

Antioxidant activity by DPPH method

Determination of antioxidant activity by the DPPH method[10] was done for all the extracts. Diphenyl-1-picryl hydrazyl (DPPH·) was used as a stable radical for assessing antioxidant activity. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorption at 517nm. Thus the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Percentage of radical scavenging activity was calculated for all the extracts.

Determination of reducing power

Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form Potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm.The reducing power of all the extracts was evaluated according to the method of Oyaizu[11]. The absorbance was then measured at 700 nm against blank sample.

Antioxidant activity by TBA method

Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532nm as per Halliwell and Gutteridge[12]. The egg yolk was used for the study of invitro lipid per oxidation. The percentage of anti- lipid per oxidative activity (%ALP) is calculated for all extracts.

Collection of cell lines

Human peripheral B- lymphoblast DAUDI cells were purchased from national Center for Cell Sciences (NCCS, Autonomous Institute of Department of Biotechnology, Government of India), Pune, India. DAUDI cells are Epstein Barr-Virus positive Burkitt's lymphoma cells which have been extensively used to understand the molecular mechanism leukemogenesis. DAUDI cells were cultured in RPMI cell culture media with 10% FBS along with 1X Penicillin streptomycin antibiotics. Cells were maintained under humidified atmosphere with 5% CO_2 / 95% Incubator.

Tryphan blue assay (Cell viability assay)

The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as Tryphan blue, Eosin or propidium, whereas dead cells do not. In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye[13, 14]. The inhibitory effects of rose and pea flower aqueous extracts (5, 10, 20 40, 50 µg/ml) on leukemia cells were performed.

% of viable cells= Live cells/ total number of cell X 100

MTT Assay (Bioassay for anticancer activity)

The tetrazolium based MTT assay has long been regarded as the gold standard of cytotoxicity assay it is highly sensitive and has been miniaturized for use as a high throughput screening assay. This assay measures cell viability in terms of reductive activity as enzymatic conversion of tetrazolium compound to water insoluble formazan crystals by dehydrogenase occurring in the mitochondria of living cells[15]. The percentage of growth inhibition was calculated, and concentration of extracts needed to inhibit cell growth by 50% (IC_{50}) was obtained from the dose response curves.

RESULTS AND DISCUSSION

The percentage of yield, concentration of protein, carbohydrate and phytochemicals, antioxidant activity and anticancer activity is depicted in table 1. The yield was found to be greater in ethanol extract of Rose flower followed by aqueous extract of Pea, ethanol extract of Pea, aqueous extract of Rose respectively. The concentration of protein and carbohydrates results are depicted in the figures 1 and 2. The protein and total sugar content content was maximum in aqueous Clitoria ternatea flower extract with 1100 mg/g of sample and 11.5mg/g of sample. Aqueous extract of Rosa Macdub exhibited the maximum amount of phenolic content among the extracts, i.e., 72 mg/g of material followed by 34 mg/g of sample in aqueous extract of Clitoria ternatea, 18.45 mg/g of sample in ethanol extract of Clitoria ternatea and 11.3 mg/g of sample in ethanol Rosa

Macdub extract respectively. The total flavonoid content of edible flowers of aqueous and ethanol extracts of Rosa Macdub and Clitoria ternatea extracts is found to be 1600, 984, 300, 123 mg/g of sample respectively as depicted in figure 4 . Aqueous Rosa Macdub flower extract was found to contain high amount of flavonoids. The involvement of free radicals in tumors is well documented. Antioxidants, on interaction with DPPH, transfer either an electron or hydrogen atom to DPPH, thus neutralizing its free radical character. The colour changed from purple to yellow and the absorbance at wavelength 517 nm decreased. DPPH assay is based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenolics. The bleaching of DPPH solution increases linearly with increasing amount of antioxidants in a given extract. The experimental data revealed that the extracts are likely to have the properties of scavenging free radicals with aqueous extract of Rosa Macdub showing higher antioxidant capacity with 99.53% of radical scavenging activity with high amount of phenolics and flavonoids followed by ethanol Clitoria ternatea flower extract with 90.29%, aqueous Clitoria ternatea flower extract with 88.94% and ethanol Rosa Macdub flower extract with 79.30% of radical scavenging activity respectively. The reducing power activity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 6 shows the reducing power activity of different edible flower extracts at 100 µg concentration using the potassium ferricyanide reduction method. At 100 µg concentration, the reducing power activity of edible flowers of aqueous and ethanol extracts of Rosa Macdub and Clitoria ternatea extracts showed absorbances of 1.24, 0.73, 0.5 and 0.28 respectively at 700 nm. Thus, the good reducing activity was observed in aqueous Rosa Macdub flower extract. The reducing power activity is due to the presence of reductones (Phenolics) which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Lipids and proteins are more susceptile to oxidative damage. The results of the effect of various edible flower extracts at 100 µg concentration to prevent lipid peroxidation are shown in figure 7. At 100 µg concentration, the edible flowers of aqueous and ethanol extracts of Rosa Macdub and Clitoria ternatea extracts showed 56.25%, 34.48%, 12.5% and 31.25% of inhibition of lipid peroxide generation by this method. Thus the antilipid peroxidation, DPPH radical scavenging activity and reducing power activity of Rosa Macdub extract correlated with its phenolics and flavonoids content. The anticancer activity of edible flowers, Rosa Macdub and Clitoria ternatea extracts was studied. The anticancer activity was evaluated by MTT assay and Trypan blue dye exclusion test. The results of trypan blue exclusion assay of aqueous extracts of Rose and Pea on Human peripheral B- lymphoblast DAUDI cells, cancerous cell lines were represented in Graphs 8 and 9 and table 2 and 3 respectively. The plant drug may disturb the membrane integrity and caused the cell death, which is one of the hall marks of apoptosis. The aqueous extracts of Rose showed 50% of cytotoxicity at 20µg of extracts against Human peripheral B- lymphoblast DAUDI cancerous cell lines (Table 2 and 3). The plant extract encounters the cancer cells by inducing the apoptosis. The result of MTT assays revealed that the aqueous extracts of Rose flower decreased the percent viability of the cancerous cells. The extract was found to induce more cytotoxicity towards cancer cell lines (Figure 10 and 12). These results revealed morphological changes and shrinkage of cells leading to cell death induced by the extracts in the cancer cell lines (Figures 11 and 13). Among 5, 10, 20, 40 µg/mL concentrations, 18.5µg/mL of aqueous extract of Rose was the most effective (IC50) in producing percentage growth inhibition (Table 4). The aqueous extract of Rose showed greater effect than other extract. The MTT assay results were in correlation with tryphan blue dye assay method. Antitumor activity of these antioxidants is either through induction of apoptosis or by inhibition of angiogenesis.

Table 1: Biochemical, phytochemical and antioxidant activity of aqueous and ethanol edible flower extracts.

Sl No	Name of the Flowers	Yield %	Total phenolics (mg/g of sample)	Total flavonoids (mg/g of sample)	Total sugars (mg/g of sample)	Protein (mg/g of sample)	DPPH assay (% radical scavenging activity)	Reducing power assay (OD at 700nm)	TBA assay (% Anti lipid peroxidation)
1	Rosa macdub (aqueous)	4	72	1600	7.0	960	99.53	1.24	56.25
2	Rosa macdub (ethanol)	12.3	11.3	984	11.3	------	79.30	0.73	34.48
3	Clitoria ternatea (aqueous)	5.0	34	300	11.5	1100	88.94	0.5	12.5
4	Clitoria ternatea (ethanol)	4.1	18.45	123	8.2	-------	90.29	0.28	31.25

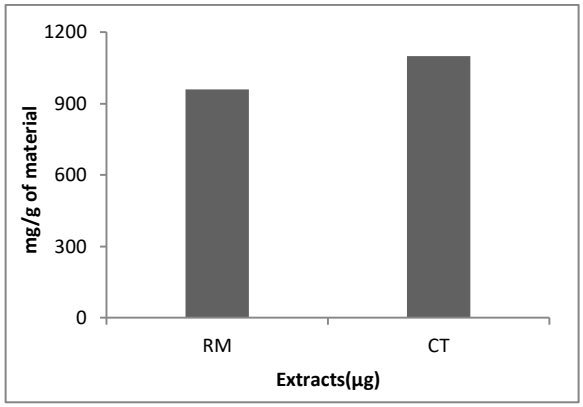


Figure 1: Total protein in edible flower extracts.

RM (Rosa Macdub) CT Clitoria ternatea

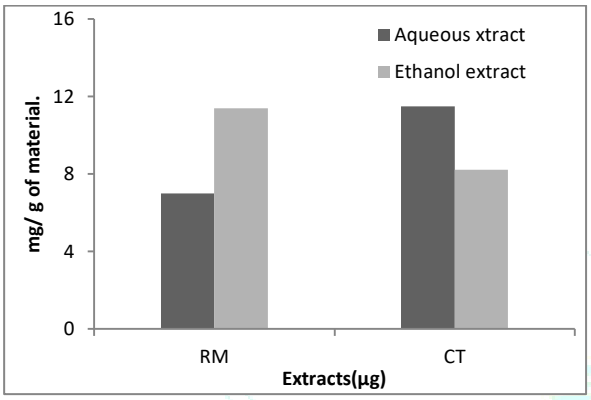


Figure 2: Total carbohydrate in edible flower extract

RM (Rosa Macdub), CT (Clitoria ternatea).

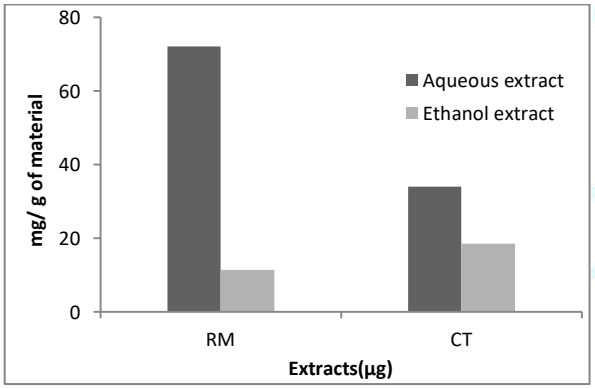


Figure 3: Total phenolics in edible flower extracts.

RM (Rosa Macdub), CT (Clitoria ternatea)

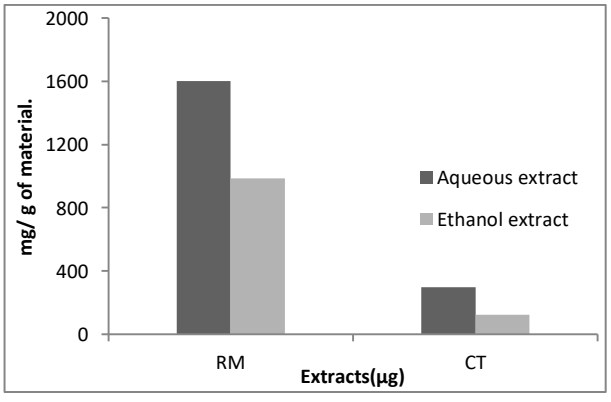


Figure 4: Total flavanoid in edible flower extracts.

RM (RosaMacdub), CT (Clitoria ternatea)

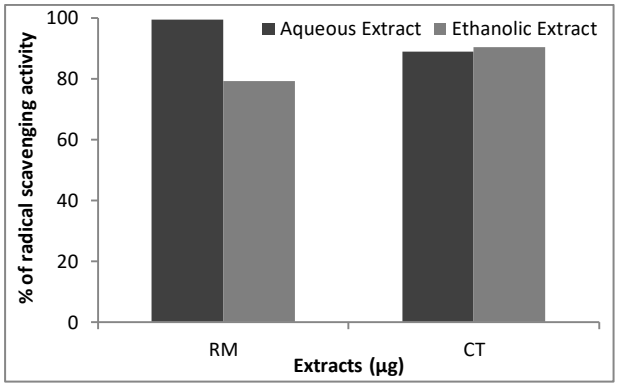


Figure 5: Percentage of radical scavenging activity in edible flower extracts at 100µg concentration.

RM (Rosa Macdub), CT (Clitoria ternatea)

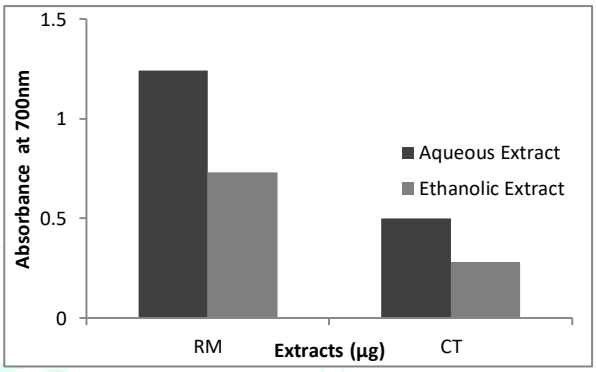


Figure 6: Reducing power activity in edible flower extracts at 100µgconcentration.

RM (Rosa Macdub), CT (Clitoria ternatea)

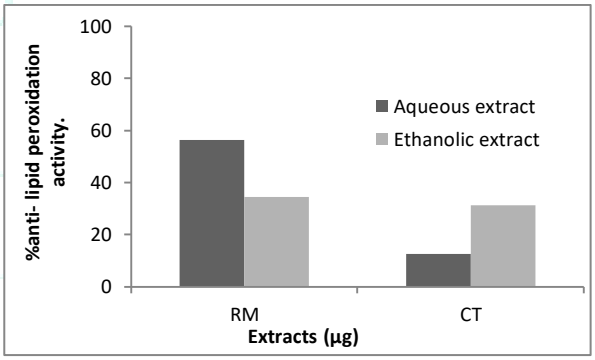


Figure 7: Percentage of anti- lipid per oxidation activity in edible flower extracts at 100µg concentration.

RM (Rosa Macdub), CT (Clitoria ternatea)

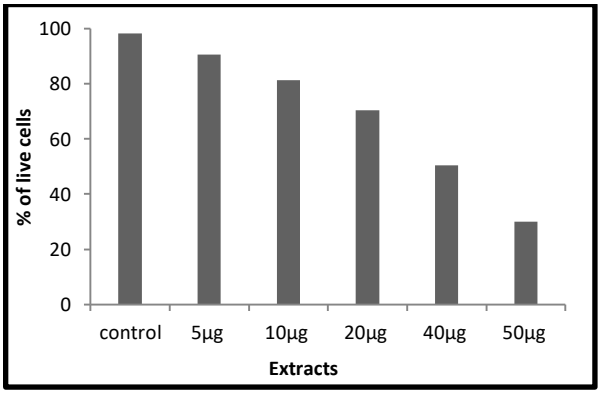


Figure 8: Cell viability assay (Cell viability using Tryphan Blue staining after 48 hours) with aqueous Rose flower extract

Table 2: Cell viability assay (Cell viability using Tryphan Blue staining after 48 hours)

Aqueous Rose Extract Concentration (µg/ml)	Cell Viability Mean± SD
Control	94.67±3.05
5µg	94.00±3.05
10µg	76.67±8.96
20µg	**54.67±1.52******
40µg	36.67±3.51
50µg	31.33±2.08

Table 3: Cell viability assay (Cell viability using Tryphan Blue staining after 48 hours)

Aqueous Pea Flower Extract Concentration (µg/ml)	Cell Viability Mean± SD
Control	98.33±1.15
5µg	90.67±1.52
10µg	81.33±6.11
20µg	70.33±5.85
40µg	**50.33±1.52******
50µg	30.003±2.00

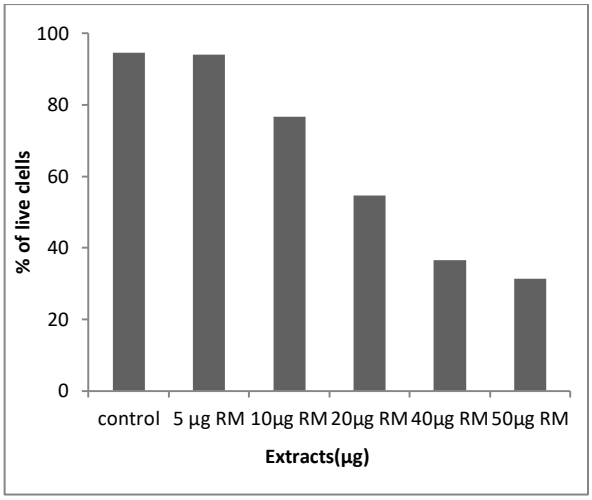


Figure 9: Cell viability assay (Cell viability using Tryphan Blue staining after 48 hours) aqueous Pea flower extract

Table 4: Cell viability assay (Cell viability using MTT staining after 48 hours)

% of Live cells (MTT assay)		
Concentration of extract (µg)	Pea (IC50-40 µg)	Rose (IC50-18.5µg)
Control (cells not treated with extract)	73.8	83.8
5	62.6	67.9
10	56.2	56.1
20	43.2	38.9
40	36.8	21.1

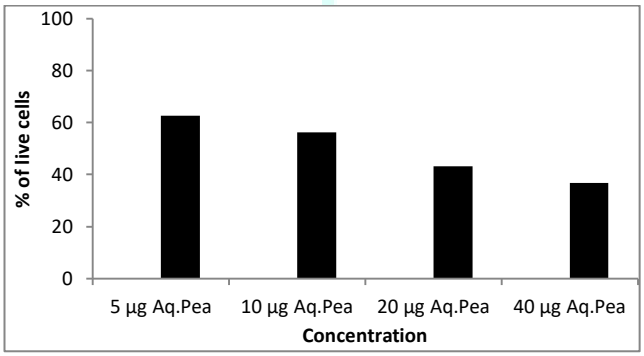
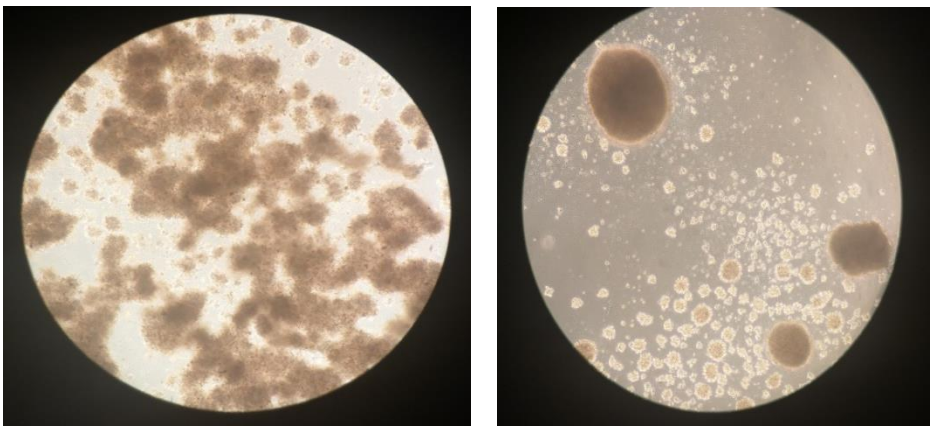


Figure 10: Dose dependent MTT assay of aqueous pea flower extracts



Control **Aqueous Pea flower extract 40 µg**

Figure 11: Light microscopic evaluation of DAUDI cells. DAUDI cells were treated with or without aqueous pea flower extract and the apoptotic cells were captured and documented.

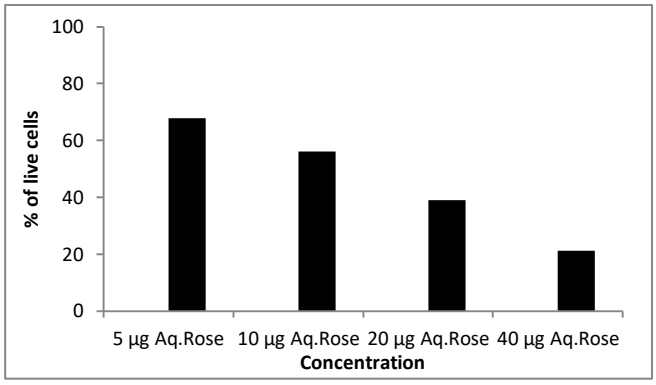
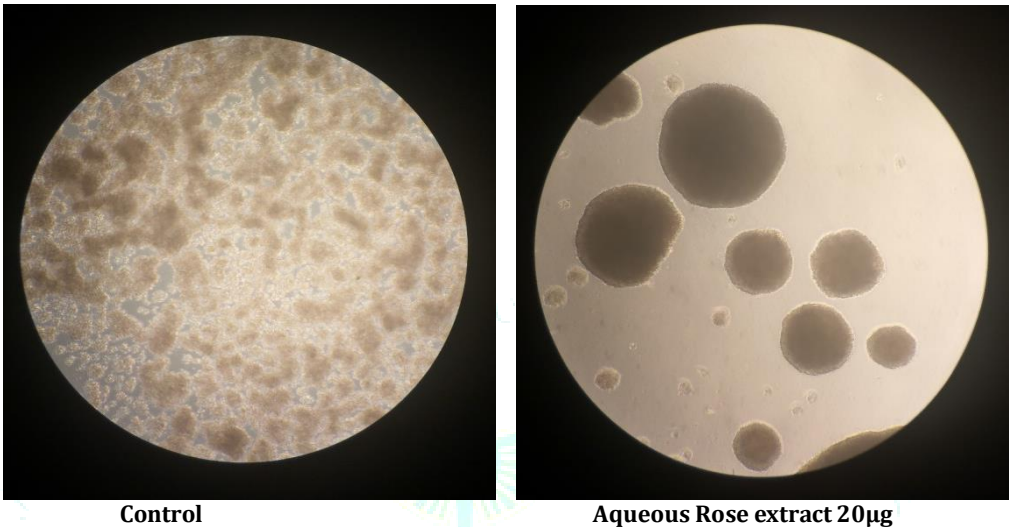


Figure 12: Dose dependent MTT assay of aqueous Rose flower extracts



Control **Aqueous Rose extract 20µg**

Figure 13: Light microscopic evaluation of DAUDI cells. DAUDI cells were treated with or without Aqueous Rose flower extract and the apoptotic cells were captured and documented

CONCLUSIONS

The present work confirms that Rose flower showed strong antioxidant activity as studied by DPPH assay, Reducing power assay and TBA assay and potent inhibitive effects on cancer cell lines which was studied by performing MTT based cytotoxic assay and trypan blue dye exclusion assay. These results provide promising baseline information for the potential use of aqueous extract of Rose and Pea in the treatment of cancer. Further studies to investigate the lead compounds present in the flower, evaluate its anticancer potential on *in vivo* animal models and put forward an attempt to carry out trails on human beings.

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