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Cytotoxicity Effects of Formulated Mangosteen Rind and Grape Seed Extract Against Vero Cells, Breast Cancer and Colon Cancer

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Abstract

Globally, cancer causes millions of deaths in addition to significant social and economic consequences. Novel therapeutic strategies such as medications, biological molecules and immune therapies are now used to suppress the death rate and improve survival of metastatic cancer patients. Traditional medical practices rely heavily on plant-derived natural compositions to treat a variety of illnesses. When science-based "modern" medicine first appeared, it was carefully studied if there was any scientific basis for using natural remedies derived from plants to cure ailments. Scientists continued, however, to search for the scientific foundations of medical plants, herbal remedies, and functional foods. Finding therapeutic plant-based active principles has led to significant advancements in the identification and use of natural chemicals for the treatment of many ailments in recent decades. Mangosteen rind extract and grape seed extract, both high in antioxidants, were mixed as a formulation to explore the cytotoxic effects on breast and colon cancer. The cytotoxicity of MCF-7 and MDA-MB-231 (breast cancer) and HT-29 (colon cancer) was investigated and compared to the standard (Doxorubicin). The findings of the cytotoxicity assays showed that the addition of Mangosteen rind and grape seed extract to MTT assays gradually improved the inhibition of MCF-7, MDA-MB-231, and HT-29 cells. Mangosteen rind and grape seed extract treatment boosted the fraction of cells undergoing apoptosis in MCF-7, MDA-MB-231, and HT-29 cancer cell lines. Thus, the findings of invitro study results of naturally compounded mangosteen rind and grape seed extract revealed that they are potential andeffective anti-cancer,anti-fungal, anti-inflammatory,anti-bacterial, and antioxidant agents.

Keywords: *Antioxidant,Chemicals,Formulation,Mangosteen rind, Grape seeds.*

1. Introduction

Global burden of disease study offers a powerful resource to understand the changing health challenges facing people across the world in the 21st century. Worldwide Cancer is a leading cause of death, accounting for nearly 10 million deaths in 2020 (Ferlay*et al* 2021). World Health Organization and American Cancer Society provided recent information about the frequency, mortality and survival expectancy of the 15 leading types of cancers worldwide. Breast and lung cancers were the most common cancers contributing 12.5% and 12.2% of the total number of new cases diagnosed in 2020.

Colorectal cancer was the third most common cancer with 1.9 million new cases in 2020, contributing 10.7% of new cases (WHO, 2023).

Cancer has the highest clinical, social, and economic burden of any human disease in terms of cause-specific Disability-Adjusted Life Years (DALYs). The total risk of cancer for those aged 0 to 74 is 20.2%; for men, it is 22.4%, and for women, it is 18.2%. In terms of mortality, ischemic heart disease is the leading cause of death globally (8.97 million deaths), but cancer is expected to overtake it in 2060 (~18.63 million deaths) (Mattiuzzi and Lippi 2019).

The magnitude of the cancer burden and a good understanding of potential modifiable risk factors are critical for the development of effective prevention and therapy. The development of medications for cancer prevention and therapy is a top goal for the scientific community worldwide (www.thelancet.com). For many years, chemotherapeutic medicines' cytotoxic effects were attributed entirely to their ability to cause genotoxic death.

As a result, there is an urgent need to create alternative therapeutic approaches against this lethal disease, such as the use of biological and natural products. Cancer chemoprevention using foods and medicinal herbs rich in antioxidants and micronutrients has emerged as one of the most noticeable areas of cancer management.

Medicinal plants continue to play an essential role in the healthcare systems of a large proportion of the world's population. Several medicinal plants are commonly employed in traditional systems of medicine for the prevention and treatment of diseases such as cancer. Several plant-derived chemicals have been discovered to be important in the creation of clinically relevant anticancer medicines. The use of complementary medicines has grown in recent years. Furthermore, natural compounds account for approximately 50% of all modern clinical medications. Some plants contain antitumor chemicals, and these plant-derived compounds can be exploited to build cancer-fighting chemopreventive medicines.

Plant compounds continue to be a valuable source of pharmaceuticals for the global population, with several plant-based medications in widespread clinical use. Cancer is currently treated with agents capable of limiting cell proliferation, including apoptosis modifying signal transmission.

The pericarp of mangosteen includes medicinal and health-promoting qualities. Mangosteen pericarp is high in bioactive chemicals that could be used as medicinal agents or as a nutraceutical. Mangosteen is also a rich source of polyphenols known as Xanthones. Several studies have found that Xanthones isolated from mangosteen pericarp have antioxidant, antitumoral, anti-inflammatory, anti-allergy, anti-bacterial, antifungal, and anti-viral effects (Ashton *et al* 2019).

Grape seed extract *(Vitisvinifera)* belongs to family *Vitaceae.* Polyphenols, flavonoids, EFA, vitamin E, and gallic acid are all abundant in grape seeds. The most significant flavonoid antioxidant ingredient found in grape seeds, oligomericproanthocyanin, lowers blood pressure, delays the aging of the skin, and may prevent cancer. Additionally, it appears to have antioxidant, antiviral, and antifungal qualities (Gupta *et al* 2019).

To understand their antiproliferative action, cytotoxic and antimutagenic activities must be assessed. Several plant-derived chemicals have been proposed as anticancer agents in recent studies. The current study compares the cytotoxicity of mangosteen rind and grape seed extracts, a mixture of natural plant extracts, against breast cancer and colon cancer to vero cells.

2. Materials and Methods

Formulation of nutraceutical

On the market, there are several herbal nutraceutical combinations with various therapeutic effects. In accordance with the Ayurvedic pharmacopoeia Draksha and the Indian MateriaMedicaGarciniamangostana has numerous medicinal properties. Recent research on oligomericproanthocyanidin from grape seed extract reveals a positive antioxidant effect on cancer (Okuno et al 2022; Nie et al 2023) and xanthones extracted from mangosteen has a powerful antioxidant effect.Combining thesetwonutraceuticals, should help inhibit proliferation of cancer cells. The composition of the final product including the percentage of each constituent was arrived at after a thorough search of current literature.

Table 1 - Dosage of formulated nutraceutical supplement

Extracts	Percentage	Dosage
Mangosteen rind Extract	10 percent Xanthones	400 mg
Grape seed Extract	90 percent Proanthocyanidin	100 mg

Dosage of formulated nutraceutical supplement

Methods and materials

A) Reagents

- 25mm $NaHCo_3$
- TrypsinPhosphateVerseneGlucose
- 10 µg/ml StreptomycinSulphate
- 100µg/mlPenicillin G
- MTT salt
- Doxorubicin

B)Media

- Dulbecco'sModifiedEagleMedium (DMEM)
- Foetal bovine Serum (FBS)
- New Born CalfSerum (NBCS)
- Modified Eagle's Medium (MEM)
- Modified Eagle's Medium withoutphenol red

C)Glassware

- 96- wellmicro titre plate
- Tissue culture flasks
- Reagent tubes
- Pipettes

D)Equipment's

- Incubator
- Haemocytometer
- Microplate Reader

E) Cytotoxicity Screening

Cell Line used: The National Centre for Cell Science (NCCS) in Pune, India provided Human Cervical Cancer cell lines, Vero cell (Monkey fibroblast), MCF-7, MDA-MB-231 (Human Breast Cancer), and HT 29 (colon cancer). The cell lines were cultured in Dulbecco's Modified Eagle's Medium, which contained 10% Foetal Bovine Serum, 25mm NaHCO3, 10 g/ml Streptomycin Sulphate, and 100 g/ml. Penicillin G was used in an incubator atmosphere with 5% CO2 and 95% air at a temperature of 37ºC.

Passaging the Cells (Freshney's I.R, 2005)

Procedure

Before use, all reagents were brought to 37ºC. In tissue culture canted 25 cm^2 flasks, a sufficient amount of Trypsin phosphate- Versene -glucose (TPVG) Solution was applied to cover the monolayer, washed, and discarded. Fresh TPVG solution was added and left to stand for 2-3 minutes at room temperature. The TPVG solution was discarded, and the flask holding the monolayer was incubated at 370°C for 3-5 minutes before being gently tapped to release the cells off the surface. To break up the cell clumps, 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) with 10% serum was pipetted into the flask. A haemocytometer was used to count the total number of cells. Determine the total number of cells.The medium was added based on the cell population required.According to the cell count, the appropriate amount of medium containing the requisite number of cells (0.5-1.0 x 105 cells/ml) was placed into bottles, and the volume was made up with medium and the required amount of New born calf serum (NBCS) serum (10% growth medium and 2% maintenance medium) was added. The flasks were incubated at 37ºC in a CO2 incubator, and the cells were monitored for morphological changes and contamination on a regular basis. The cells were then used when they had formed a monolayer.

Cytotoxicity assay

Determination of Mitochondrial Synthesis by MTT Assay

Most cytotoxicity tests have relied on cells' ability to withstand a toxic shock. This assay assumes that dead cells or their products do not reduce tetrazolium. The assay is affected by the number of cells present as well as the mitochondrial activity per cell. The cleavage of MTT by living cells to a blue formazan derivative is clearly a very successful mechanism on which the assay is based.

Principle

The mitochondrial enzyme succinate dehydrogenase cleaves the tetrazolium salt MTT (3-(4,5 dimethyl thiazole-2 yl)- 2,5-diphenyl tetrazolium bromide) producing a blue-colored product (formazan).The amount of formazan produced by the cells was found to be related to the number of cells used (Denizot and Lang 1986).

Procedure

Using MEM/DMEM medium with 10% FBS/NBCS, the monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml. 10µl of the diluted cell suspension (about 10,000 cells/well) was added to each well of a 96 well microtitre plate. When a partial monolayer formed after 24 hours, the supernatant was flicked off, the monolayer was washed once with medium, and the cells were treated with Mangosteen rind + Grape seed extract with Doxorubicin (positive control) at 10-100 g/ml concentrations produced in maintenance medium and added per well to the partial monolayer in microtitre plates. The plates were then incubated at 37ºC in a 5% CO2 environment for 48 hours, with microscopic examination and observations recorded every 24 hours. After 48 hours, the sample solutions in the wells were removed, and each well received 20µl of MTT (2mg/ml) in MEM-PR (MEM without phenol red). The plates were gently shaken and incubated at 37ºC in a 5% CO2 environment for 3 hours. The supernatant was withdrawn, and 100µl of propanol was added before gently shaking the plates to dissolve the produced formazan. At 540nm, the absorbance was

measured using a microplate reader. All measurements were performed in three replicates, and at least three unique experiments were conducted. To quantify the percentage of cell growth inhibition, untreated cells with 100% viability were used as controls.

The percentage growth inhibition was determined using the following formula, and the concentrations of drug or test samples required to inhibit cell growth by 50% were estimated using the dose-response curves for each cell line.

Calculation

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} X100$$

3.Results and Discussion

The cytotoxicity effect of mangosteen rind and grape seed extract were determined using concentrations ranged from 10-100µg/ml for 48 hours. After 48 hours of exposure, mangosteen rind and grape seed extract induced concentration- dependent cytotoxicity effects in MCF-7 and MDA-MB-231 with IC 50 were 41.52±0.84 and 39.24±0.88 respectively using MTT methods and 47.54±0.20 for colon cancer by MTT method. Thus, the data on cytotoxicity effects of mangosteen rind and grape seed extract using two human cell lines in vitro revealed that the sample extracts are showing better cytotoxicity effects for MCF-7 and DA-MB-231 (breast cancer) and HT-29 (colon cancer).The conventional Doxorubin medicines have IC 50 values of 310.46 g/ml in normal Vero cell lines, proving to be less hazardous to normal non-cancerous cell lines.

Similarly, the formulated mangosteen rind and grape seed extract demonstrated an IC 50 value of 261.22 g/ml, demonstrating that the formulation was equally non-toxic to vero cell lines in comparison to the standard Doxorubicin standard drugs, which demonstrated a cytotoxic effect on breast cancer cell lines. Similarly, the formulated mangosteen rind and grape seed extract demonstrated a toxicity value IC 50 of 41.52 g/ml, demonstrating that the supplement was cytotoxic to MCF-7 breast cancer cell lines.

Table 2: Cytotoxic effect of formulated mangosteen rind and grape seed extract against normal and cancer cells

S.No	Sample	IC 50 (µg/ml)			
		Vero cells	MCF-7	MDA-MB-231	HT-29
		Normal	Breast		Colon
1	Doxorubicin Std	310.46 ± 0.84	11.85 ± 0.48	14.12 ± 0.28	19.63 ± 0.82
2	Mangosteen rind + Grape seed extract	261.22 ± 0.66	41.52 ± 0.84	39.24 ± 0.88	47.54 ± 0.20

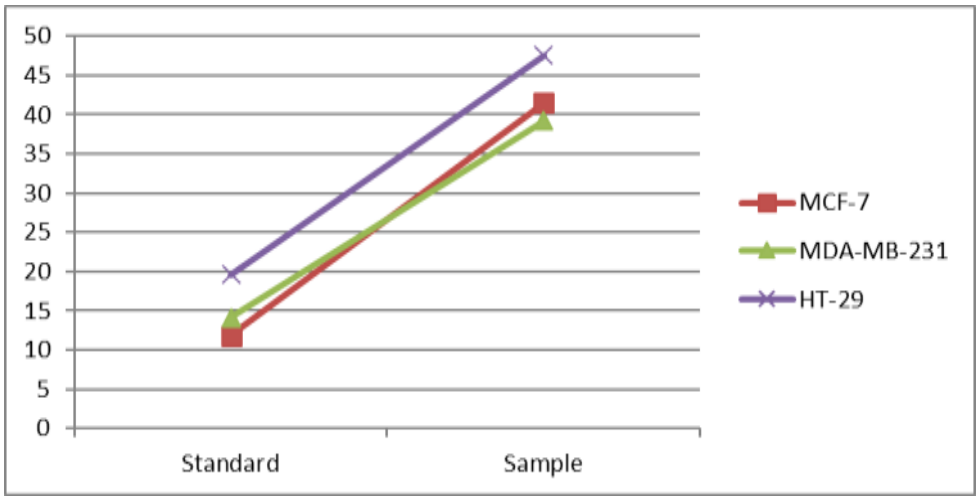
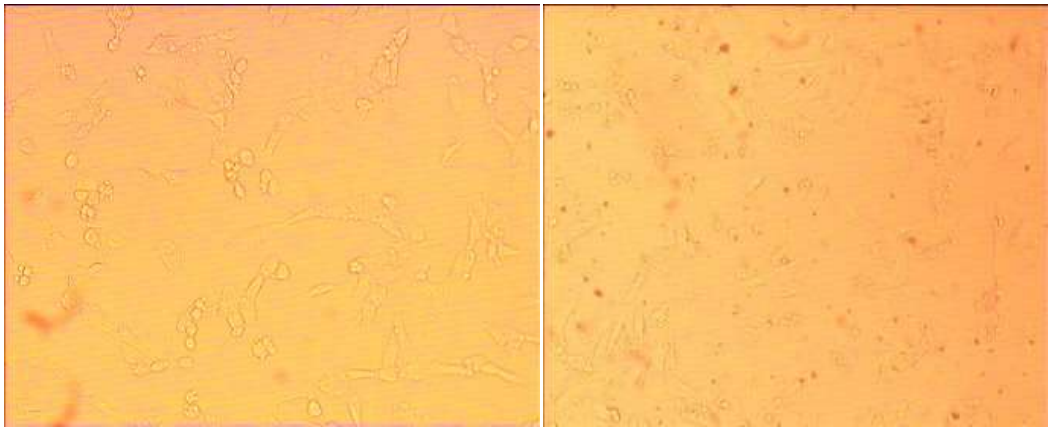


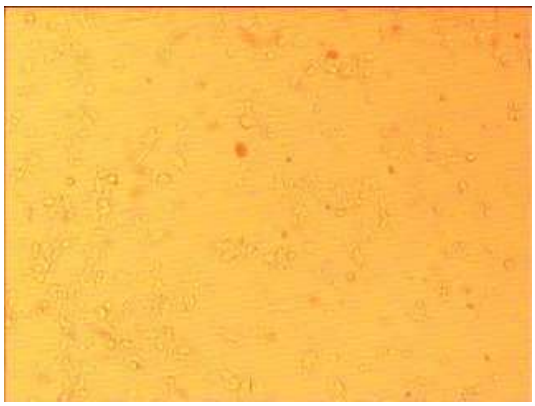
Figure 1: Comparative Cytotoxicity study of different assays: standard v/s samples

Doxorubicin standard medicines have a cytotoxic impact on colon cancer cell lines with an IC 50 value of 19.63 g/ml. Similarly, the formulated mangosteen rind and grape seed extract demonstrated a toxicity value IC 50 of 47.54g/ml, demonstrating that the supplement had a cytotoxic effect on MCF-7 breast cancer cell lines. Thus, results on the cytotoxicity effects of mangosteen rind and grape seed extracts in vitro employing two human cell lines revealed that the sample extracts have stronger cytotoxicity effects for MCF-7 and MDA-MB-231 (breast cancer) and HT-29 (colon cancer) as compared to standard (Doxorubicin).In conclusion, the inclusion of phytochemicals such as xanthones, proanthocyanidin, and other phenolic compounds in the formulation demonstrated anti-cancer activity against cancer cell lines. The findings of the cytotoxicity assays show that the addition of mangosteen rind and grape seed extract to MTT assays gradually improved the inhibition of MCF-7, MDA-MB-231, and HT-29 cells. Apoptosis is a natural process of cell elimination, and one of the hallmarks of cell apoptosis is DNA fragmentation. Mangosteen rind and grape seed extract treatment boosted the fraction of cells undergoing apoptosis in MCF-7, MDA-MB-231, and HT-29 cancer cell lines. Thus, studies on mangosteen rind and grape seed extracts revealed that they are effective anti-cancer and antioxidant agents.

Pictorial representation of assayed cells



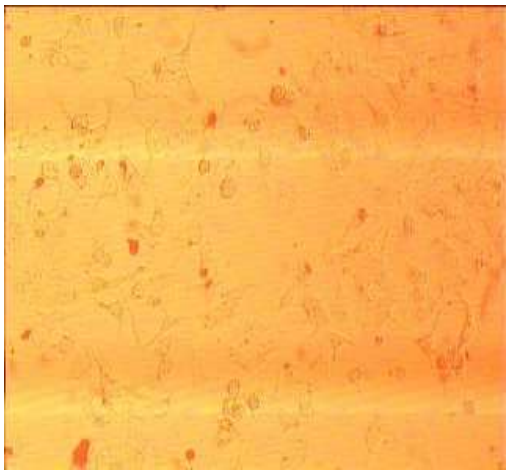
a) Control b) Mangosteen + Grape ext 10µg/ml



c) Mangosteen + Grape ext 100µg/ml

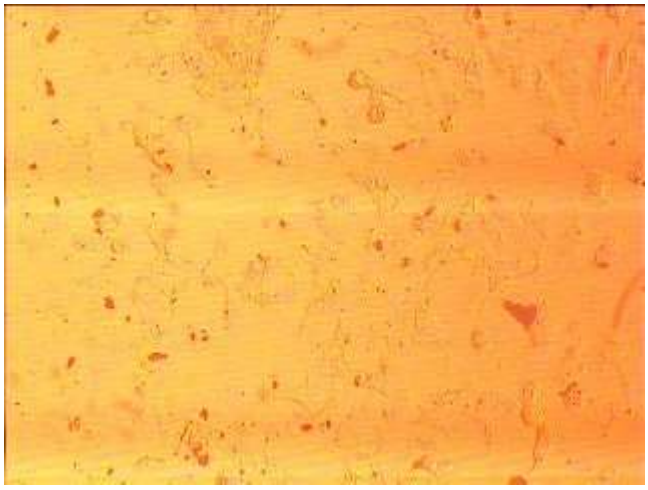
Figure 2: Vera cells (Normal) against Mangosteen rind and grape seed extract (10µg/ml and 100 µg/ml)

MCF-7



a) Control

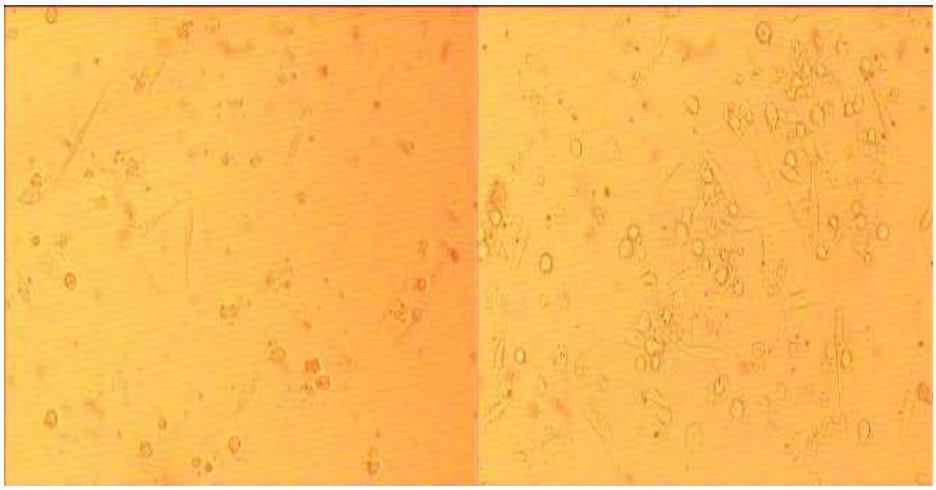
b)Mangosteen + Grape ext 10 µl/ml



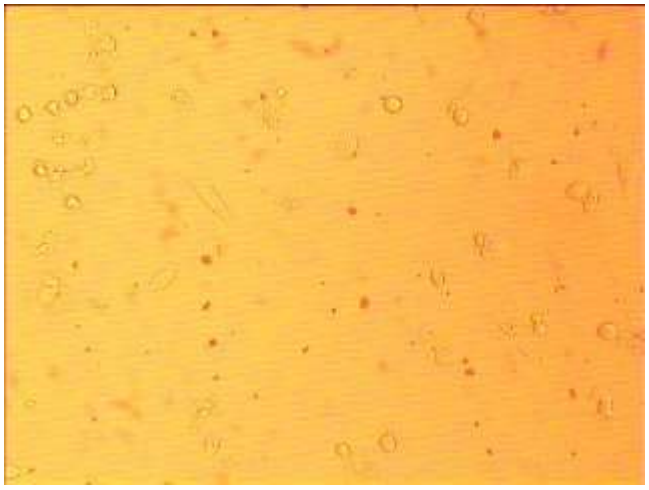
c)Mangosteen + Grape ext 100µg/ml

Figure 3: MCF-7 (Human Breast Cancer Cells) against Mangosteen rind and grape seed extract (10µg/ml and 100 µg/ml)

MDA-MB-231



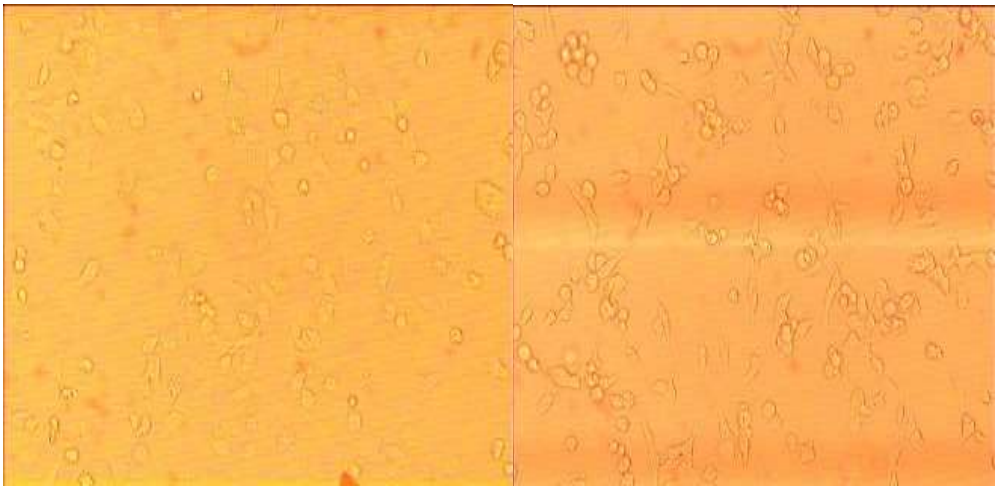
a) Control b)Mangosteen + Grape ext 10 µg/ml



c)Mangosteen + Grape ext 100 µg/ml

Figure 4: MDA-MB-231 (Human Breast Cancer Cells) against Mangosteen rind and Grape seed extract (10µg/ml and 100 µg/ml)

HT 29 cells



a) Control b) Mangosteen + Grape ext 10 µg/ml

c)Mangosteen + Grape ext 100 µg/ml

Figure 5: HT-29 (Colon Cancer Cells) against Mangosteen rind and Grape seed extract (10µg/ml and 100 µg/ml)

4.Conclusion

Cellular proliferation is influenced by the rates of cell division and death, hence many anticancer medications have been developed to limit cancer cell division in order to inhibit cancer cell growth. In vitro cytotoxicity assays can be used to predict human toxicity as well as for general chemical screening (Chang, Cheung, and Shah 2023). It has previously been observed that different cytotoxicity assays can produce varying results based from the test agent and cytotoxicity assay utilized (Jablonska et al 2021). The findings of the cytotoxicity assays show that the addition of Mangosteen rind and grape seed extract to MTT assays gradually improved the inhibition of MCF-7, MDA-MB-231, and HT-29 cells. Apoptosis is a natural process of cell elimination, and one of the hallmarks of cell apoptosis is DNA fragmentation. Mangosteen rind and Grape seed extract treatment boosted the fraction of cells undergoing apoptosis in MCF-7, MDA-MB-231, and HT-29 cancer cell lines. Thus, the invitro study results of compounded mangosteen rind and grape seed extract revealed that they are effective anti-cancer, anti-fungal, anti-bacterial, and antioxidant agents.

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