

# Evaluation of Cytotoxic Potential of Ethanolic and Acetone Extract of *Abelmoschus moschatus* on Hep G2 Cell Lines

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## ABSTRACT

This study aims to highlight the cytotoxic effects of *Abelmoschus moschatus* on Hep G2 cell lines by methylthiazolotetrazolium (MTT)-cleavage assay. The yellow tetrazolium salt MTT is converted by mitochondrial dehydrogenases of metabolically active cells to an insoluble purple formazan product, which was then solubilized with dimethylsulfoxide. The optical density of this homogeneous solution was suitable for a precise spectrophotometric measurement by a plate reader at a wavelength of 570 nm. The cytotoxic effect of acetone extract was shown at 92.01 µg/ml and ethanol extract was shown at 72.16 µg/ml respectively when compared with the standard Doxorubicin. The results showed that both the extracts possess significant cytotoxic activity. The studies reveal that natural plant extract has the ability to work as a defense by enhancing the immune system against cancer.

**Key words:** MTT assay, Cytotoxicity, Anticancer activity, Hep G2 cell lines, *Abelmoschus moschatus*, IC<sub>50</sub>

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## INTRODUCTION

Cancer is one of the major causes of death worldwide and there is a steady growth in the number of cancer cases. Current treatment options for cancer have many pitfalls, the major one being the emergence of drug resistance. Currently a molecular approach based on cancer cell pathology is used for treating cancer cases and to reduce the mortality rates. Cancer chemoprevention refers to the use of agents for the inhibition, delay, or reversal of carcinogenesis before invasion. A diet rich in

vegetables and fruits appears to convey more protection than individual micronutrients.<sup>[1]</sup>

Plant natural products are promising resource for cancer chemoprevention.<sup>[2]</sup> Historically medicinal botanicals, as part of complementary medicine in the USA, have provided a source of inspiration for discovering novel drug agents, and more than half of currently available drugs are natural products or are related to them.<sup>[3]</sup> Of particular interest, considerable epidemiological evidence has been accumulated indicating that over 50% of the approved anticancer agents like retinoid and podophyllotoxin are either natural compounds or natural product derivatives derived from herbal medicine. Numerous secondary metabolites from plants like phenolics, flavonoids, alkaloids, carotenoids, gingerols, as well as organosulfur compounds have the capacity to suppress early and late stages of

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carcinogenesis in various *in vivo* and *in vitro* models.<sup>[4]</sup> Therefore discovery of newer natural anticancer agents from natural products is of considerable importance in anticancer research.

### Plant profile

The genus *Abelmoschus* belongs to Malvaceae family and comprise of about 15 species of flowering plants. It is an aromatic and medicinal oil yielding plant commonly grown in some parts of our country.<sup>[5]</sup> *Abelmoschus moschatus* (subspecies: *tuberosa*) has lots of medicinal properties and is called as Kattukasthuri or musk mallow in different areas. The plant is widely used in folk medicine for its therapeutic properties. Almost every part of musk mallow has medicinal properties. The plant has a long record of use in conventional health care systems. Many races think this plant as a remedy for various diseases. In India, roots, leaves, and seeds of this plant are used for therapeutic purpose.<sup>[6]</sup> Mucilage prepared from root and leaves of the plant is advocated in sexually transmitted bacterial infections. The poultice of leaves and roots is used in cystitis, fever, headache, rheumatism, and for varicose veins and boils. Seeds are used to alleviate gastric disorders. Different types of ayurvedic formulations from seeds are used for nervous weakness, hysteria and other neurological conditions. Traditionally seeds have considerable role in the therapy of progressive loss of neurological functions. Seeds rubbed to a paste with milk are used to cure itch.<sup>[7]</sup>

Flower infusion is contraceptive. The mucilaginous seeds are emollient and demulcent. Seed oil has the ability to relieve abdominal cramps, circulatory insufficiency and joint pain. Ambrette oil has insecticidal properties and increase lust.<sup>[8,9]</sup> Although the plant has enormous use in traditional medicine, most of the biological activities are not scientifically validated. *Abelmoschus moschatus* is a perennial plant. Stems are solid and are about 30cm tall. Leaves have variable size and shape and some are sagittate. Leaf margins are lobed. Leaf apex is obtuse. Flowers have a diameter of 4 – 5cm. They are solitary. The flowers have 5 petals with colours ranging from white, pale yellow to dark pink. The flowers last for only one day and their flowering depends on weather conditions. Seeds are held in hairy tough, papery capsules. The seeds have a sweet, flowery, heavy fragrance similar to that of musk.<sup>[10]</sup>

### Synonyms

*Abelmoschus ficulneus*, *Hibiscus abelmoschus*, *Hibiscus moschatus*, *Abelmoschus sagittifolius*.

### Plant Profile

Botanical Name: *Abelmoschus moschatus* subsp. *tuberosus*

Family: Malvaceae

Vernacular Names English: Musk mallow, Ambrette

Malayalam: Kasthurivenda Tamil: Varttilaikasturi

## MATERIALS AND METHODS

### Collection of the plant

The plant *Abelmoschus moschatus* (subspecies *tuberosa*) was collected from Pala, Kottayam district, Kerala. The plant was authenticated at Botanical Survey of India, and deposited in the herbarium with voucher No. BSI/SRC/5/23/2020/Tech/65.

### Chemicals and Reagents

All the chemicals and reagents were procured from certified suppliers and of analytical reagent grade.

### Preparation of the extracts

The plant extracts were prepared following the procedure of Gopalsatheeskumar by Soxhlet extraction method.<sup>[11]</sup> The aerial parts of the plant were dried under shade and were ground to a powder using an electrical blender. Extraction was carried out by continuous hot percolation method by using the following solvents in order i.e., Petroleum ether, chloroform, acetone and ethanol. The extracts were then concentrated using a rotary evaporator and kept at 4°C until used.

### Phytochemical screening

Phytochemical analysis of crude extracts of aerial parts of *Abelmoschus moschatus* (subspecies: *tuberosa*) was carried out as per standard procedures.<sup>[12,13]</sup>

### GC MS analysis

GC – MS analysis was done on all the four extracts of aerial parts of *Abelmoschus moschatus* (subspecies: *tuberosa*). Shimadzu GC – MS Model Number: QP2010S instrument with GC – MS solutions software was used for analysis. The oven temperature is maintained at 280°C at a rate of 5°C/min. Elite - 5MS column of 30m length, 0.25mmID and 0.25micrometer thickness was used. Since ethanol and acetone extracts of *Abelmoschus moschatus* contained more phytoconstituents, these extracts were selected for further studies. The ethanolic extract of *Abelmoschus moschatus* was designated as AMWE (*Abelmoschus moschatus* whole plant ethanol extract) and the acetone extract was designated as

AMWA (*Abelmoschus moschatus* whole plant acetone extract)

### In vitro cytotoxicity determination by MTT assay

#### Cell lines and maintenance

HepG2 (human hepatoma) cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India.

#### Cell culture media and maintenance

The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). The cell containing TC flasks (25cm<sup>2</sup>) were incubated at 37°C at 5% CO<sub>2</sub> environment with humidity in a cell culture incubator (Galaxy170 Eppendorf, Germany).

#### MTT assay

The MTT assay is used to measure cellular metabolic activity as an indicator of cell proliferation, viability and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H dependent oxidoreductase enzymes which reduce the MTT to formazan.<sup>[14]</sup> The insoluble formazan crystals are dissolved using a solubilizing solution (100% DMSO) and the resulting purple colored solution is quantified by measuring absorbance at 570 nm using an ELISA plate reader.

Procedure: Hep G2 cells (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37°C and 5% CO<sub>2</sub> environment in the incubator for 24 hrs. The test samples were prepared in DMEM media (100 mg/ml) and filter sterilized using 0.2 µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100 µg/ml respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 hrs. After incubation period, the media from the wells were aspirated and discarded. 100 µl of 0.5 mg/ml MTT solution in PBS was added to the wells. The plates were further incubated for 2 hrs for the development of formazan crystals. The supernatant was removed and 100µl DMSO (100%) were added per well. The

absorbance at 570nm was measured with micro plate reader. Two wells per plate without cells served as blank. All experiments were done in triplicates.<sup>[15]</sup>

The cell viability was expressed using the following formula:

$$\text{Percentage cell viability} = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of control}} \times 100$$

#### IC<sub>50</sub> value

The IC<sub>50</sub> value is the half maximal inhibitory concentration of the sample. The IC<sub>50</sub> values were calculated using the equation for slope ( $y = mx + C$ ) obtained by plotting the average absorbance of the different concentrations of the test sample (6.25-100 µg/ml) in Microsoft Excel.

## RESULTS

*In vitro* cytotoxicity potential was evaluated for AMWE and AMWA on Hep G2 cell lines and IC<sub>50</sub> values were found out. The cell percentage viability decreased gradually when the doses have been increased in 24 hrs by using different concentration of 6.25, 12.5, 25, 50,

**Table 1: In vitro cytotoxic effect determination AMWE by MTT assay.**

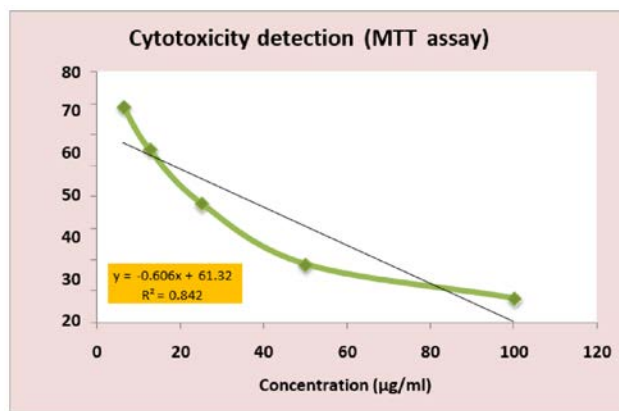
Sample concentration (µg/ml)	OD I	OD II	OD III	Average Absorbance at 570 nm	Percentage Viability
Control	0.872	0.865	0.876	0.871	100
6.25	0.792	0.786	0.773	0.784	89.97
12.5	0.712	0.724	0.706	0.714	81.97
25	0.644	0.632	0.623	0.633	72.68
50	0.555	0.547	0.541	0.548	62.88
100	0.443	0.431	0.417	0.430	49.41

**Table 2: In vitro cytotoxic effect determination AMWA by MTT assay.**

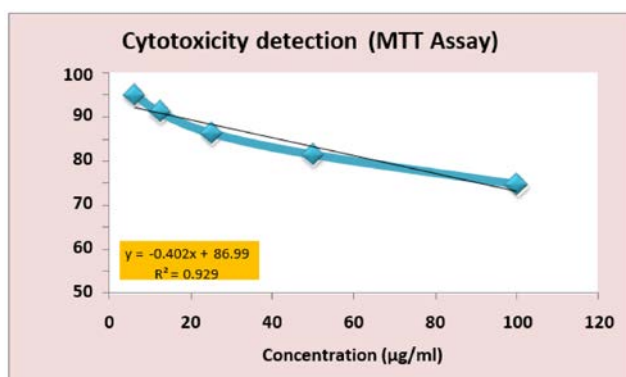
Sample concentration (µg/ml)	OD I	OD II	OD III	Average Absorbance at 570 nm	Percentage Viability
Control	0.872	0.865	0.876	0.871	100
6.25	0.766	0.752	0.744	0.754	86.57
12.5	0.622	0.601	0.611	0.611	70.19
25	0.522	0.507	0.516	0.515	59.13
50	0.504	0.481	0.476	0.487	55.91
100	0.388	0.365	0.356	0.369	42.44

**Table 3: *In vitro* cytotoxic effect determination Doxorubicin by MTT assay.**

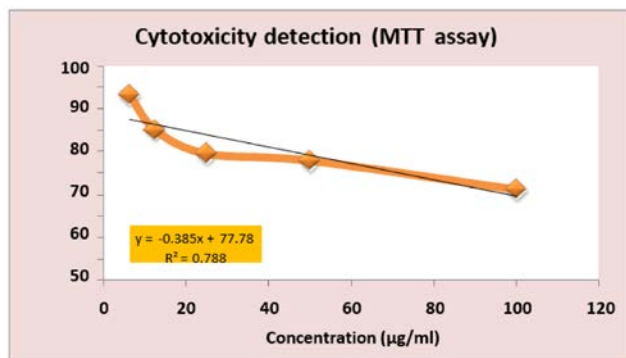
Sample concentration (µg/ml)	OD I	OD II	OD III	Average Absorbance at 570 nm	Percentage Viability
Control	0.872	0.865	0.876	0.871	100
6.25	0.611	0.6	0.591	0.600	68.96
12.5	0.481	0.492	0.474	0.482	55.37
25	0.344	0.322	0.331	0.332	38.15
50	0.174	0.166	0.151	0.163	18.79
100	0.082	0.066	0.059	0.069	7.92



**Figure 3: *In vitro* cytotoxicity detection by MTT assay on Hep G2 cell lines (Doxorubicin).**



**Figure 1: *In vitro* cytotoxicity detection by MTT assay on Hep G2 cell lines (AMWE).**



**Figure 2: *In vitro* cytotoxicity detection by MTT assay on Hep G2 cell lines (AMWA).**

100µg/ml and is presented as Table 1 and 2 respectively. A decreased absorbance in the cells treated with drugs suggests cytotoxicity.<sup>[6]</sup> The cell percentage viability with doxorubicin is shown in Table 3.

Concentration of the extract that produce 50% cell growth inhibition (IC<sub>50</sub>) were calculated from curves constructed by plotting cell survival (%) versus drug concentration. (Figure 1, 2, 3).

The IC<sub>50</sub> value was observed at 92.01 µg/ml concentration for AMWE and 72.16 µg/ml for AMWA. The results showed that both the crude extracts has significant toxicity to Hep G2 cell lines when compared with the standard drug doxorubicin.

Dose dependant reduction in cell viability was observed in Hep G2 cells administered with different concentrations of the standard anticancer drug, doxorubicin. The IC<sub>50</sub> value was observed with 18.68 µg/ml concentration of this sample.

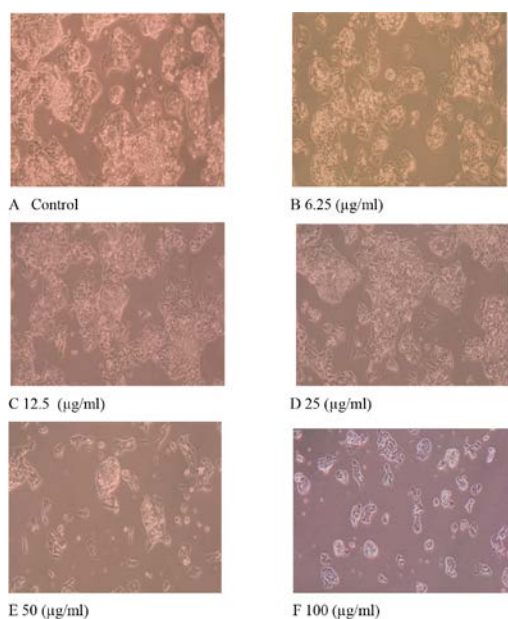
Anticancer assay by direct microscopic observation

The entire plate was observed after 24 hrs of treatment in an inverted phase contrast tissue culture microscope and microscopic observation was recorded as images. Any detectable changes in the morphology of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity. (Figure 4, 5, 6).

## DISCUSSION

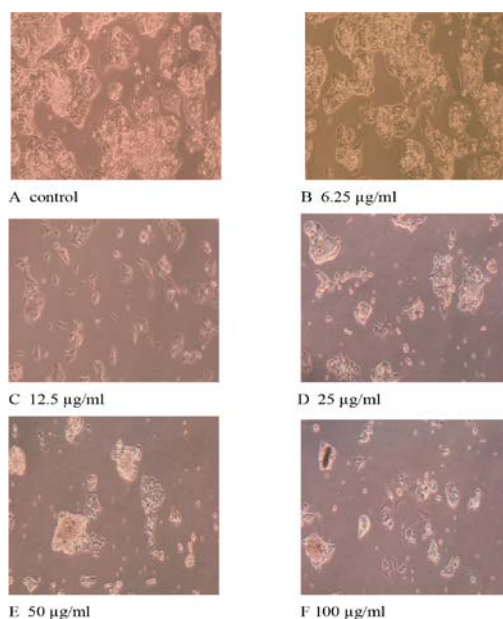
Plants contain many phytoconstituents and are used as a rich source of therapeutic agents which can be used as a basis for synthetic drugs.<sup>[17]</sup> About 55% of chemotherapeutic agents are based upon natural products. These natural products act as main agents in the research and development of newer anticancer agents. The interest in alternative therapies increasing day by day due to the higher number of cancer cases worldwide. To develop new therapeutic agents for cancer therapy, many plant extracts and active principles have been studied on *in vitro* and *in vivo* cancer models, and the correlation of both studies became one of the key steps for the success of anticancer research.<sup>[18]</sup>

In this study ethanol and acetone extract of *Abelmoschus moschatus* was evaluated for their cytotoxic potential on Hep G2 cell lines. Doxorubicin was used as the standard



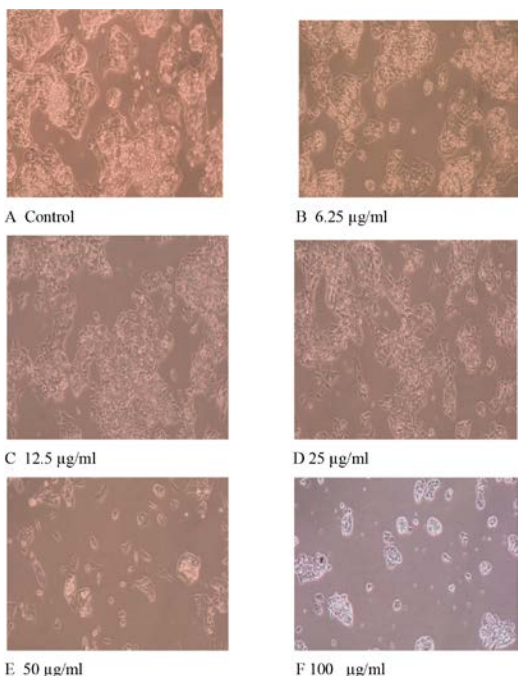
**Figure 4: *In vitro* cytotoxicity of Ethanol extract of *Abelmoschus moschatus* against Hep G2 cell lines.**

- A - Control
- B - Hep G2 cell line treated with 6.25 µg/ml of ethanolic extract of *Abelmoschus moschatus*
- C - Hep G2 cell line treated with 12.5 µg/ml of ethanolic extract of *Abelmoschus moschatus*
- D - Hep G2 cell line treated with 25 µg/ml of ethanolic extract of *Abelmoschus moschatus*
- E - Hep G2 cell line treated with 50 µg/ml of ethanolic extract of *Abelmoschus moschatus*
- F - Hep G2 cell line treated with 100 µg/ml of ethanolic extract of *Abelmoschus moschatus*



**Figure 6: *In vitro* cytotoxicity of Doxorubicin against Hep G2 cell line.**

- A - Control
- B - Hep G2 cell line treated with 6.25 µg/ml of Doxorubicin
- C - Hep G2 cell line treated with 12.5 µg/ml of Doxorubicin
- D - Hep G2 cell line treated with 25 µg/ml of Doxorubicin
- E - Hep G2 cell line treated with 50 µg/ml of Doxorubicin
- F - Hep G2 cell line treated with 100 µg/ml of Doxorubicin



**Figure 5: *In vitro* cytotoxicity of Acetone extract of *Abelmoschus moschatus* against Hep G2 cell lines.**

- A - Control
- B - Hep G2 cell line treated with 6.25 µg/ml of acetone extract of *Abelmoschus moschatus*
- C - Hep G2 cell line treated with 12.5 µg/ml of acetone extract of *Abelmoschus moschatus*
- D - Hep G2 cell line treated with 25 µg/ml of acetone extract of *Abelmoschus moschatus*
- E - Hep G2 cell line treated with 50 µg/ml of acetone extract of *Abelmoschus moschatus*
- F - Hep G2 cell line treated with 100 µg/ml of acetone extract of *Abelmoschus moschatus*

for the study. Cell viability was assessed by MTT assay, which is a colourimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT, to its insoluble formazan, giving a purple colour.<sup>[14]</sup> Dose dependant reduction in cell viability was observed in HepG2 cells administered with different concentrations of the sample, AMWE and AMWA. The IC<sub>50</sub> value was observed at 92.01 µg/ml concentration for AMWE and 72.16 µg/ml for AMWA. The results showed that both the crude extracts have significant toxicity to Hep G2 cell lines when compared with the standard drug doxorubicin. The activity of the extracts is low in comparison to the standard drug. This may be due to the crude nature of the extracts and can be further enhanced by purification.

Mir Zahoor Gul, conducted a study on the anti-proliferative effects of *Abelmoschus moschatus* seed and leaf extracts using two human cancer cell lines COLO 205 and Y 79. In this study proliferation was inhibited in a concentration dependent manner after the exposure to seed and leaf extracts.<sup>[19]</sup> The study concluded that the hydroalcoholic extracts of seed and leaf of *A. moschatus* might be useful as an ant proliferative agent due to the presence of potent bioactive principles.

The present study highlights the antiproliferative effects of acetone and ethanolic extract of *Abelmoschus moschatus* (subspecies *tuberosa*) on Hep G2 cell lines. The antiproliferative effect may be due to the phytoconstituents present in *Abelmoschus moschatus*. Phytoconstituents like megastigmatrienone and phytol present in *Abelmoschus moschatus* possess antioxidant and antitumor properties.<sup>[20]</sup> Loliolide is a monoterpene lactone and possess significant antioxidant and cell protective effects against the cellular damage produced by H<sub>2</sub>O<sub>2</sub>.<sup>[21]</sup> Literature survey reveals that gamma sitosterol has a role in the activation of components involved in extrinsic apoptotic pathway in human lung and breast adenocarcinoma cells.<sup>[22]</sup>

The active components present in such extracts may inhibit the process of carcinogenesis in a synergistic manner. The identification and characterization of components with potential anti-cancer activity may be a stepping stone for the synthesis of lead compounds having anticancer activity. These extracts can be tested on various *in vivo* models to establish its cytotoxic potential.

## CONCLUSION

The MTT cleavage assay was found to be a quick (24 hrs) and easy to perform system for the evaluation of the biological activity of new drugs and plant extracts. The objective of this work was to study the cytotoxic activity of ethanolic and acetone extracts of *Abelmoschus moschatus* on Hep G2 cell lines. The data revealed the acetone extract has high cytotoxicity effect compared to ethanol extract. Hence it was anticipated as that *Abelmoschus moschatus* extracts can be a useful pharmaceutical material to protect or treat cancer cells. Further studies will be conducted using these plant extracts to identify the molecular mechanism of cytotoxic effect. Gene expression studies are under investigation to know the possible mechanism of cytotoxic effect of *Abelmoschus moschatus* extracts against Hep G2 cell lines.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**Hep G2 Cell lines:** Human hepatoma cell lines; **MTT assay:** Methylthiazoltetrazolium assay; **IC<sub>50</sub>:** Half maximal inhibitory concentration.

## SUMMARY

*Abelmoschus moschatus* is an aromatic medicinal plant belonging to Malvaceae family. Different parts of the plant has uses in ayurvedic medicine. The aim of the present study was to evaluate the cytotoxic potential of *Abelmoschus moschatus* on Hep G2 cell lines. Both acetone and ethanolic extracts were studied for the evaluation of cytotoxic activity. The preliminary data from MTT assay revealed that both the extracts of *abelmoschus moschatus* possess significant cytotoxic activity against Hep G2 cell lines. This calls for further studies for the proper assessment of their chemotherapeutic properties as well as the development of promising anticancer drugs.

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