Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 September; 6 (1): 2078-2084

Anticancer Activity Of Nanosuspension Of Extract Of Malaxis Acuminata On Breast Adenocarcinoma Cell Line (MCF-7 Cell Line) And Hepatocellular Carcinoma Cell Line (Hep G2 Cell Line) – An In Vitro Study

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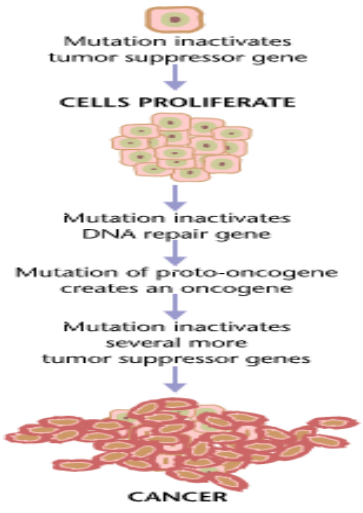
Abstract :
The cytotoxic activity of Malaxis Acuminata Nanosuspension (MANS) was evaluated using the MTT assay on two cancer cell lines: MCF-7 (breast adenocarcinoma) and Hep G2 (hepatocellular carcinoma). The study measured cell viability at various concentrations of Malaxis Acuminata Nanosuspension and compared it to the standard chemotherapeutic agent Doxorubicin**.** For MCF-7 cells, the IC50 value of Malaxis Acuminata Nanosuspension was 63.97 µg/mL, indicating a moderate cytotoxic effect, while Doxorubicin had an IC50 value of 1.81 µg/mL, demonstrating a higher efficacy. Similarly, for Hep G2 cells, Malaxis Acuminata Nanosuspension exhibited an IC50 value of 71.6 µg/mL, compared to Doxorubicin's IC50 value of 2.23 µg/mL. The results suggest that MANS shows considerable cytotoxicity in both breast and liver cancer cell lines, though Doxorubicin was more potent. Malaxis Acuminata Nanosuspension holds promise as a potential therapeutic agent for cancer treatment, particularly as a natural-based alternative.

Introduction :

A tumor is a mass of tissue that forms due to the uncontrolled, excessive, and abnormal growth of cells. Tumors are classified as either "benign," meaning they grow slowly and stay localized without causing major harm, or "malignant," meaning they spread quickly, invade other parts of the body, and can ultimately be fatal. The general term for all malignant tumors is "cancer." Cancer can develop in various areas of the body, with hundreds of distinct types identified. [1]
Cancer can result from abnormal proliferation of any of the different kinds of cells in the body, so there are more than a hundred distinct types of cancer, which can vary substantially in their behavior and response to treatment. The most important issue in cancer pathology is the distinction between benign and malignant tumors.Malignant conversion involves further changes in the cell, such as:

- Unregulated growth of cells, resulting in the loss of control over cell division.
- The capacity for local invasion and metastasis, where cancer cells can invade surrounding tissues and disseminate to remote locations within the body.
- Resistance to apoptosis, allowing cancer cells to evade programmed cell death.
- An angiogenic tendency, fostering the creation of fresh blood vessels to supply the growing tumor with nutrients and oxygen. [2,3]



Now a days natural products are considered to be safer than the synthetic product that are regarded as unsafe to human being. Although huge number of synthetic drugs are being added to the pharmacopoeia, but still no system of medicine within the world has been ready to settle all the health issue, such as Cancer. Plant-derived compounds have played an vital role in the establishment clinically useful anti-cancer agents. Many promising new agents are under development based on selective activity against tumor-related molecular targets, In India, there is an ocean of knowledge about medicinal plants and rich medicinal flora, but still only a few pearls are searched as therapeutic agents. [4]

According to World Health Organization, 80 % look after the people living in rural areas rely upon medicinal herbs as primary healthcare system. The synthetic anticancer remedies are beyond the reach of human due of cost factor. Herbal medicines have an important role within the prevention and treatment of cancer and medicinal herbs are commonly available and relatively economical. Scientists everywhere in the planet are concentrating on the herbal medicines to spice up immune cells of the body against cancer. By understanding the complex synergistic interaction of varied constituents of anticancer herbs, the herbal formulations is designed to attack the cancerous cells without harming normal cells of the body. [5]

Plants are considered as a extremely good source of the numerous Ayurvedic drug formulations with rejuvenating and health-promoting properties, besides these it also help to strengthen the system. Attention possess to be focused on natural products as potential sources of anti-tummor drugs with high efficiency and low toxicity. The issues like drug resistance, cancer recurrence and drug side effects stick to this therapies. Therefore, the event of latest chemotherapeutic agents has become an urgent need for patients to profit and better survival [6].

Using nanosuspension technology, the medication is preserved in its necessary crystalline form, but with smaller particles, resulting in an improved dissolution rate and, consequently, improved bioavailability. [7]

Malaxis Acuminata is a worldwide soil loving plant belongs to the family Orchedaceae, commonly named as Jeevak. This species grow in colonies and one colony may contain 5-25 individuals. *Malaxis Acuminata* forms colonies in shady places, moist ground and in the area that are wet & mossy.

Malaxis Acuminata is an important medicinal plant having immense ethnomedicinal potential. The dried pseudobulbs known as 'jeevak' are important ingredients of 'Chyavanprash' which is a polyherbal immune booster known to restore vigour, vitality and youthfulness. [8,9]

Qualitative analysis of plant metabolites (primary and secondary both) of *Malaxis accuminata shows the presence of* Phytochemicals such as Alkaloid, glycosides Carbohydrate,Resin, Saponin, Starch, Steroids, Tannin. [10]

Methods and Materials :

Formulation of nanosuspension from plants extract:

The formulation of the nanosuspension of the plant extract will involve the use of the Nano precipitation technique, followed by lyophilization with mannitol as a cryoprotectant. The formulation of the nanosuspensions was conducted through the nano-precipitation method with slight modifications. A solution was prepared by dissolving 2.5g of plant extract in 15 ml of acetone and ethanol (3:1) through sonication for 60 seconds.

The prepared solution was then slowly injected (1 ml/min) using a syringe connected to a thin Teflon tube into 25 ml water containing 1.5% w/v PVA, under continuous magnetic stirring at 1000 rpm. The resulting emulsion was further diluted in 50 ml PVA solution (0.2% w/v in water) to minimize coalescence. The mixture was stirred continuously (500 rpm) for 6 hours at room temperature to facilitate solvent evaporation and the formation of nanoparticles. The resulting nanosuspension was then be cooled to -18°C and subjected to lyophilized to obtain dry powder[11].

PHARMACOLOGICAL INVESTIGATION :

The various cell culture was use for the anticancer activity as follows: -

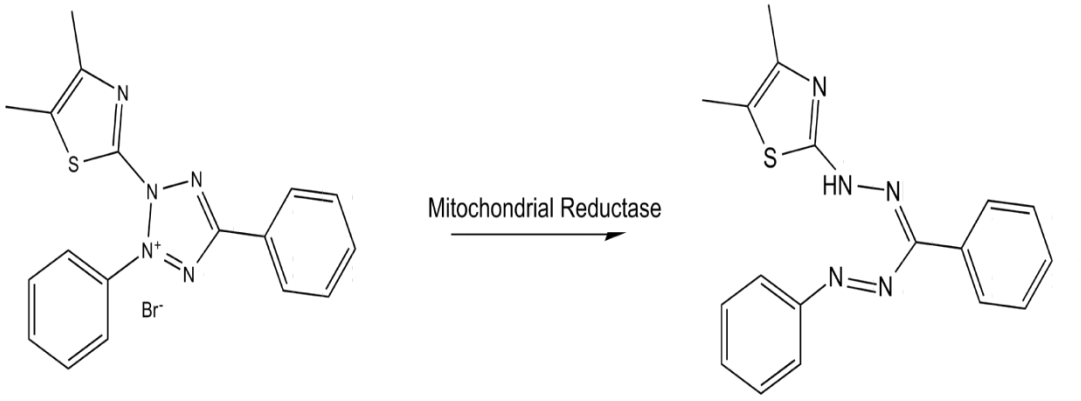
1. Hep G2 cell line
2. MCF 7 cell line

Determination of anticancer activity by In vitro method :

Cytotoxic activity carried by MTT assay

Principle of assay:

This is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinatedehydrogenase. The MTT enters the cells and passes into the mitochondria where it isreduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (eg. DMSO, Isopropanol), and the released, solubilized formazan reagent is measured spectrophotometrically. Since reductions of MTT can onlyoccur in metabolically active cells the level of activity is a measure of the viability of thecells.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(MTT)

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
(Formazan)

Protocol-
Cytotoxicity
1. The cells were seeded in a 96-well flat-bottom microplate and maintained at 37ºC in 95% humidity and 5% CO_2 overnight.
2. Different concentration (75, 50, 25, 12.5, 6.25, and 3.125 %) of samples were treated.
3. The cells were incubated for another 48 hours.
4. The wells were washed twice with PBS and 20 µL of the MTT staining solution was added to each well and the plate was incubated at 37°C.
5. After 4h, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using microplate reader [12-14].

Formula:
Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100

Results and Discussion :

Determination of anticancer activity of nanosuspension of *Malaxis Acuminata* plants extracts by In-Vitro method :
The nanosuspension of *Malaxis Acuminata* was evaluated for its cytotoxic effects using the MTT assay across two distinct human cancer cell lines: MCF-7 (breast adenocarcinoma) and HepG2 (hepatocellular carcinoma). The results are summarized in table 1 & 2 and depicted in figure from 1 -8, provide insight into the nanosuspension's effectiveness in inhibiting cell proliferation, as indicated by the IC50 values for each cell line.

Cytotoxic activity carried by MTT assay Materials
MCF 7

Table.No.1. Cell Viability of MCF 7 for *Malaxis Acuminata* Nanosuspension (MANS)

CELL VIABILITY OF MCF 7						
CONCENTRATION (%)	**MANS SAMPLE**			**Doxorubicin**		
75	41.21	40.65	41.13	10.74	11.35	10.95
50	64.29	64.37	64.70	13.58	14.26	14.32
25	73.36	73.12	74.25	15.88	15.95	16.08
12.5	84.53	84.70	84.78	21.15	21.55	21.42
6.2	94.33	94.01	94.17	22.64	23.04	23.45
3.125	97.57	97.25	97.49	27.23	27.36	27.64
Negative Control	100			100		
IC_{50} value (µg/ml)	63.97			1.81		
STANDARD DEVIATION	0.29			0.03		

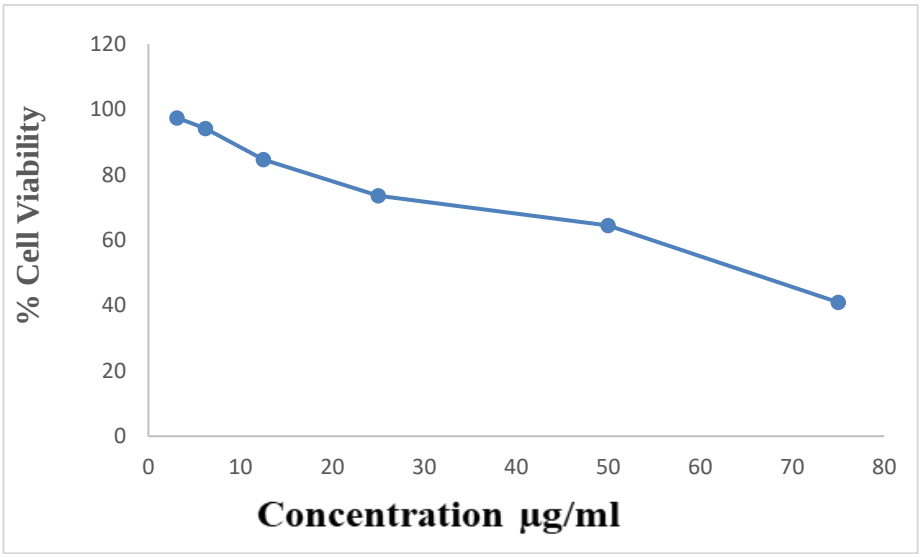


Fig.No.1 : Percentage of cell viability at various concentration of *Malaxis acuminata nanosuspension* on MCF 7

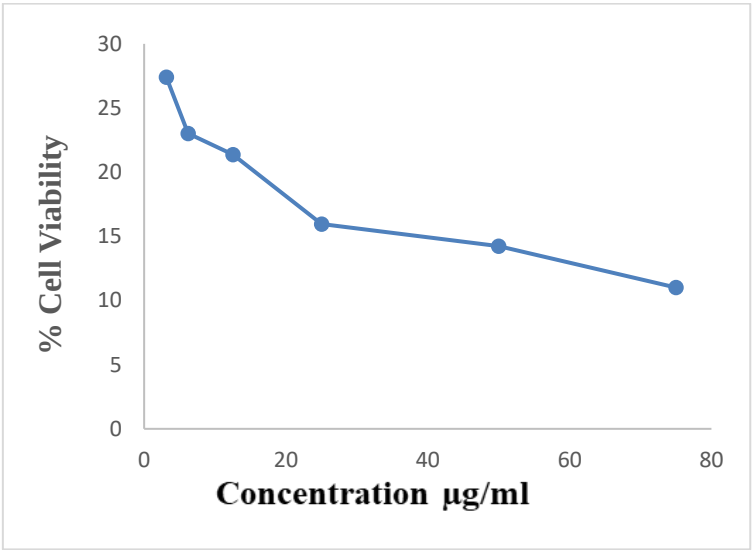
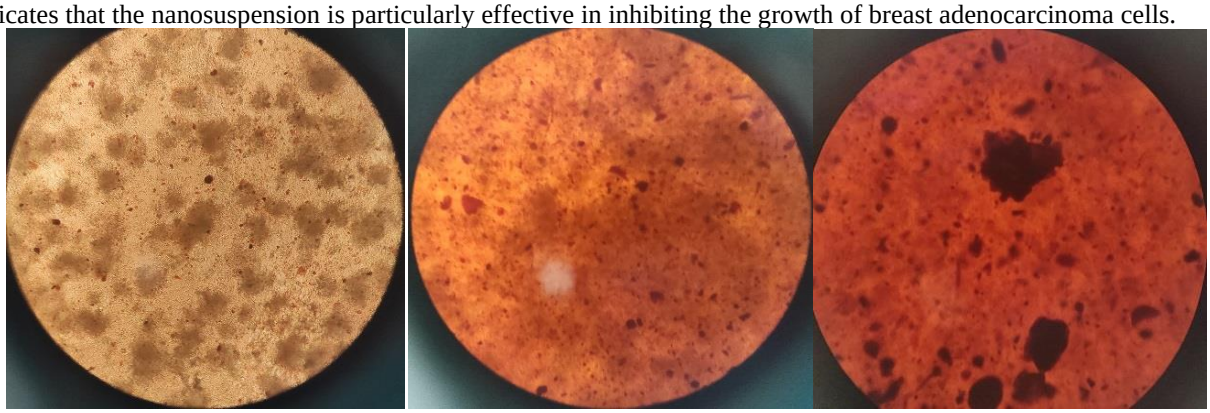


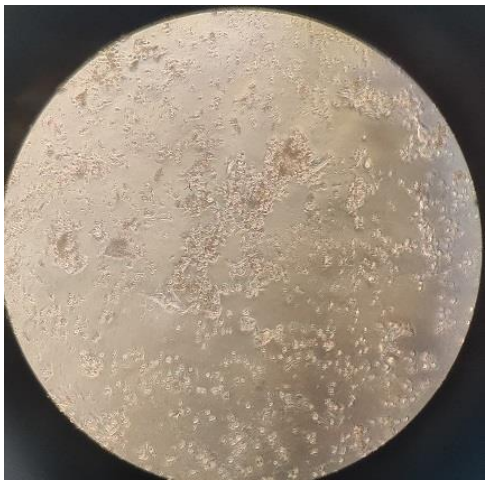
Fig.No.2 :Percentage of cell viability at various concentration of Doxorubicinon MCF 7

The concentration of Malaxis Acuminata Nanosuspension was evaluated in triplicates by serial dilution. The results showed that nanosuspension of ethanolic extract of Malaxis Acuminata significantly inhibited the MCF 7 cell line.
For the MCF-7 cell line, the IC50 value was determined to be 63.97 µg/mL. This value suggests the concentration at which the nanosuspension of *Malaxis Acuminata* inhibits the viability of MCF-7 cells by 50%. The lower IC50 value indicates that the nanosuspension is particularly effective in inhibiting the growth of breast adenocarcinoma cells.



a. MANS Sample at 6.25 % b. MANS Sample at 50 % c. MANS Sample at 75 %

Fig. No. 3. a,b,c. Cell Vaibility of MCF 7 for Malaxis Acuninata Nanosuspension



Negavite Control of MCF 7

Fig. No. 4.Cell Vaibility of MCF 7 for Negative Control Material Hep G2

Table.No. 2. Cell Viability of Hep G2 for *Malaxis Acuminata* Nanosuspension(MANS)

CELL VIABILITY OF Hep G2						
CONCENTRATION (µg/ml)	**MANS SAMPLE**			**Doxorubicin**		
75	44.93	45.17	45.01	6.10	6.15	6.11
50	70.38	70.46	70.31	10.16	10.26	10.19
25	72.11	71.80	72.03	12.65	12.95	12.79
12.5	78.87	79.10	78.95	15.71	15.61	15.65
6.2	87.12	86.65	86.80	18.91	18.65	18.78
3.125	95.13	95.37	95.29	21.65	21.31	21.21
Negative Control	100			100		
IC_{50} value (µg/ml)	71.6			2.23		
STANDARD DEVIATION	0.12			0.02		

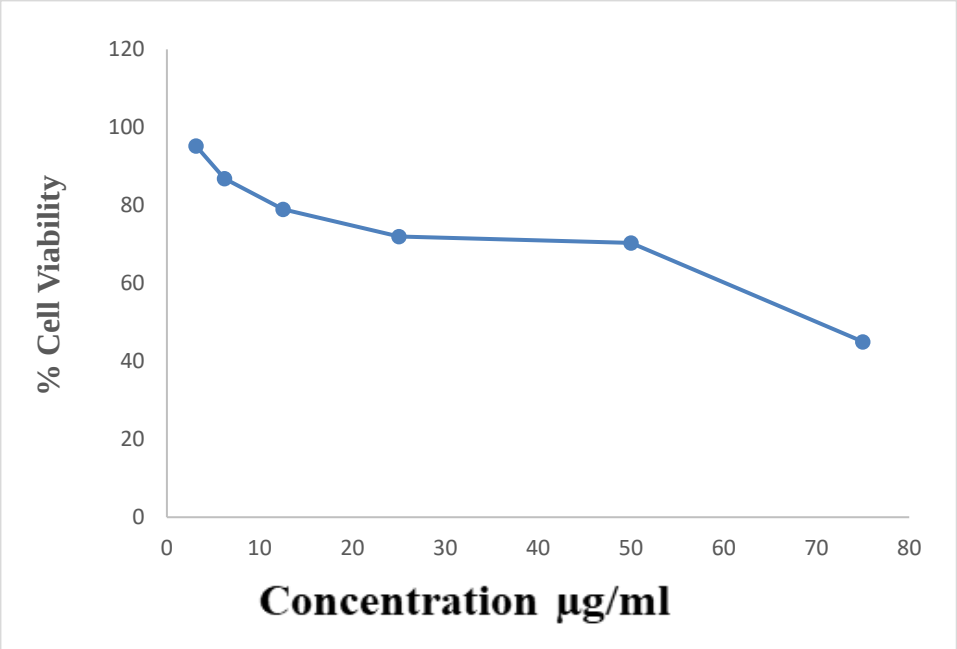


Fig. No. 5 : Percentage of cell viability at various concentration of *Malaxis acuminata nanosuspension* on Hep G2

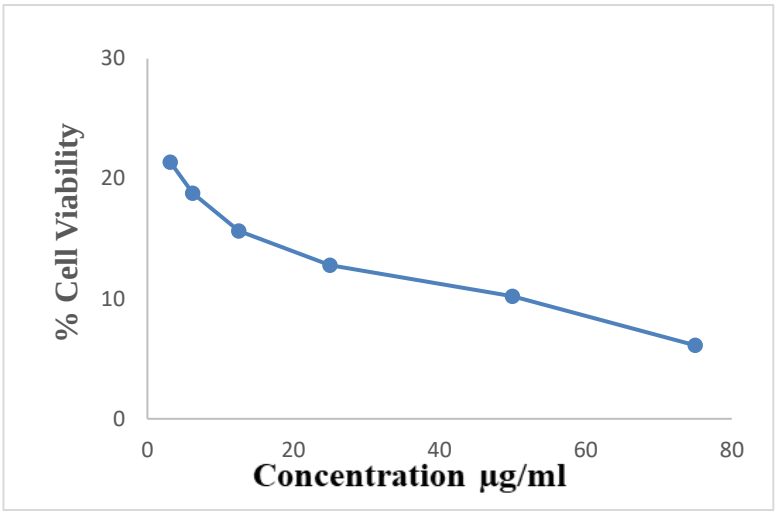
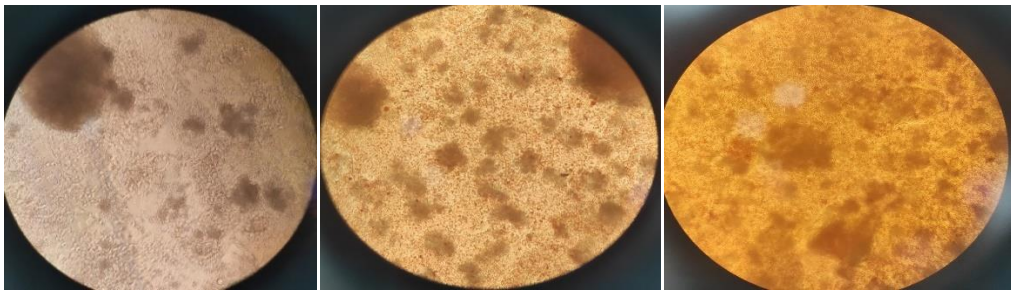


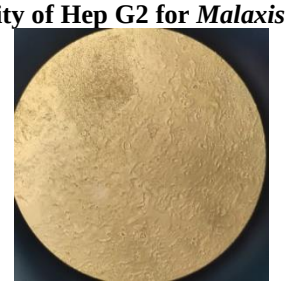
Fig.No. 6 :Percentage of cell viability at various concentration of Doxorubicinon HepG2

The concentration of Malaxis Acuminata Nanosuspension was evaluated in triplicates by serial dilution. The results showed that nanosuspension of ethanolic extract of Malaxis Acuminata significantly inhibited the Hep G2 cell line. In the case of the HepG2 cell line, the IC50 value was found to be 71.6 µg/mL. The moderate cytotoxicity observation suggests that the nanosuspension of *Malaxis acuminata* has potential of effective therapeutic agent for hepatocellular carcinoma.



a. MANS Sample at 3.125 % b. MANS Sample at 12.5 % c. MANS Sample at 50 %

Fig. No. 7. a,b,c. Cell Vaibility of Hep G2 for *Malaxis Acuninata* Nanosuspension



Negavite Control of Hep G2

Fig. No. 8.Cell Vaibility of Hep G2 for Negative Control

Conclusion :

In the present study cytotoxic effect was analyzed by MTT bio assay. The MTT assay results showed growth inhibition in MCF 7 and HepG2 cell lines when subjected with nanosuspension of ethanolic extract of Malaxis Acuminata. The concentration of Malaxis Acuminata Nanosuspension was evaluated in triplicates by serial dilution. The results showed that nanosuspension of ethanolic extract of Malaxis Acuminata significantly inhibited the MCF 7 and Hep G2 cell lines and was the most potent nanosuspension with IC50 value at 63.97 µg/ml for MCF 7 cell lines and for HepG2 cell line it was 71.6 µg/ml. The MTT assay results for Malaxis Acuminata Nanosuspension (MANS) demonstrated significant cytotoxic activity against both MCF-7 (breast adenocarcinoma) and Hep G2 (hepatocellular carcinoma) cell lines.

For the MCF-7 cell line, the IC50 value of 63.97 µg/mL indicates that MANS effectively inhibits 50% of the cell viability at this concentration. This suggests that MANS has strong anti-cancer potential against breast adenocarcinoma, though Doxorubicin, with a lower IC50 of 1.81 µg/mL, remains more potent. For the Hep G2 cell line, the IC50 value

of 71.6 µg/mL for MANS suggests moderate cytotoxicity against hepatocellular carcinoma cells, highlighting the potential for MANS as a therapeutic agent. Again, Doxorubicin showed greater potency, with an IC50 value of 2.23 µg/mL. The obtained data showed variability of result of the investigated nanosuspension in the same experimental condition.

Overall, Malaxis Acuminata Nanosuspension exhibited promising cytotoxic effects on both cell lines, supporting its potential use as a natural-based therapeutic agent in cancer treatment.

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