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(RESEARCH ARTICLE)

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Study of cytotoxic effects of CO_2 extract of shigru (*Moringa oleifera* lam.) Root, in MCF-7 cell line of breast cancer

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Abstract

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors. With being the most common type of cancer in women, breast cancer accounts for 14% of cancers in Indian women. It is reported that with every four minutes, an Indian woman is diagnosed with breast cancer. Various breast cancer cell lines have been widely used for breast cancer modeling which encompasses a panel of diseases with distinct phenotypical associations. MCF:7, a widely studied epithelial cancer cell line derived from breast adeno-carcinoma has characteristics of differentiated mammary epithelium. Ayurveda is the oldest Indian indigenous medicine system of plant drugs is known from very early times for preventing or suppressing various tumors using various natural drugs. *Shigru* (*Moringa oleifera Lam.*) is one of the most important herbal drug having Multiple phytocomponents which possess the anti:cancer property. So by using this anti: tumor property of *Shigru* and to prove the efficacy of *Ayurvedic herbs* as an alternative and cost effective source of treatment of cancer, this study has been undertaken for an *In vitro* study on the breast cancer cell line MF-7 by using CO_2 extract of *Moringa oleifera* Lam. Root by using MTT assay.

Keywords: Breast cancer; Shigru; *Moringa oleifera* Lam; *In-vitro*; MCF-7 Cell-line

1. Introduction

Breast cancer is that commonest invasive cancer in women and the second leading cause of cancer death in women after lung cancer [1]. Breast cancer cell lines have been widely used for breast carcinoma modeling which encompasses a panel of diseases with distinct phenotypical association [2]. MCF:7, a widely studied epithelial cancer cell line derived from breast adenocarcinoma has characteristics of differentiated mammary epithelium[2]. In Ayurveda The word like Cancer or Karkaroga is not mention in literatures, but there are some diseases which resembles with Cancer, Acharya Sushruta mentioned diseases like Arbuda, Granth, Gulma, Vranashotha, Kustha etc[3] which shows similar signs and symptoms of tumors. The description of "Granthi" and "Arbuda" by Charaka and Sushruta can be considered as a tumor in the body.

Nowadays research is going to invent an Ayurvedic medicine to prevent or to cure cancer; Shigru (*Moringa oleifera* Lam.) is one of the most important drug having alkaloids, protein, quinine, saponins, anthraquinones, flavonoids, sitosterol, tannins, glycosides which possess the

anti:cancer property. According to Bhavprakasha Shigru (*Moringa oleifera* Lam.) is an Ayurvedic drug of family Moringaceae. It has Katu, Tikta Rasa with Katu Vipaka and Ushna virya which is useful in the treatment of Apachi, Gulma and Ganda [4]. By considering all the properties of *Moringa oleifera* Lam. Drug, here we took the CO_2 extract of root of Moringa tree and MCF-7 breast cancer cell line. This study are done by using the MTT assay for determine cell viability and cell toxicity.

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Aim and objectives

To Study of Cytotoxic and Apoptotic effect of CO_2 extract of Shigru (*Moringa oleifera* Lam.) Root, in MCF:7 breast cancer cell line

2. Material and methods

The present study was carried out under following headings

2.1. Section I

2.1.1. Collection of sample

The botanically identified sample of Shigru (*Moringa oleifera* Lam.) Roots was collected from local area and other parts of plant such leaves, pods, flowers, stems apply for further medicinal uses.

2.1.2. Authentication

The authenticity of this sample was confirmed by comparing their characters with various floras by the botanist from authorized institute.

Pharmacognostical, Physicochemical and Phytochemical study

It includes

- Raw material standardization
- In process standardization
- Finished product standardization

2.2. Pharmacognostical study

The parameters selected for Pharmacognostical study were as follows:

2.2.1. Part 1. Raw material standardization

Macroscopy of Shigru (*Moringa oleifera* Lam.) Roots[5]

Procedure

The external features of the test sample of Shigru (*Moringa oleifera* Lam.) Roots were examined by naked eyes, using magnifying lens.

Figure 1 Shigru (Root Powder)

Table 1 Organoleptic evaluation of *Shigru* (*Moringa oleifera Lam.*) Roots

Sr. No.	Characters	Observation
1.	Colour	Whitish grey
2.	Odour	Characteristic
3.	Taste	Pungent and spicy

2.3. In process drug standardization

Physico:chemical analysis: The air dried Roots of Shigru (*Moringa oleifera* Lam.) Roots powdered finely and subjected to various analyses, such as determination of foreign matter, moisture content, ash value, acid insoluble ash, water soluble ash etc. The extractive value in various solvents and ash value are important in identification and standardization of single drug.

Table 2 Physicochemical parameters

Sr no	Tests	API Standard value	Powder of Moringa Root
1	Loss on drying[9]	-	4.92
2	Total Ash[10]	Not more than 18 per cent	5.22
3	Acid Insoluble Ash[10]	Not more than 10 per cent	0.67
4	Water Soluble Ash[10]	-	0.83
5	Alcohol Soluble extractive[11]	Not less than 3 per cent	4.06
6	Water Soluble Extractive[11]	Not less than 11 percent	12.24
7	pH[12]	-	5.30

2.4. Finished product standardization

2.4.1. Preparation of CO_2 extrac t[8] of Roots of Shigru

Figure 2 Powder of Shigru Root

Figure 3 Co_2 extraction unit

Figure 4 CO_2 extract of Shigru root

Table 3 Finished product standardization

Sr. No.	Tests	CO_2 extract of Root of *Moringa oleifera*
1.	pH[12]	5.30
2.	Specific gravity[13]	0.8708
2.	Total solid[14]	57.74%

Table 4 Heavy Metal Tests

	Result	Permissible limits
Lead	<1 ppm	Not more than 3 ppm
Arsenic[16]	<5 ppb	Not more than 1.0 ppm
Mercury[17]	<2 ppb	Not more than 0.1ppm
Cadmium[15]	<0.1 ppm	Not more than 0.3 ppm

Table 5 Microbial contamination

	Limits	Result
Total Plate Count	Not More Than 10'5cfu/gm	30 cfu/gm
Total Yeast and Mold	Not More Than 10'4 cfu/gm	<10 cfu/gm
Count		
E.coli	Should be absent in 1 gm	Absent
Salmonella	Should be absent in 25 gm	Absent
Enterobacteria	Not More Than 104 cfu/gm	Absent

Table 6 Phytochemical analysis of CO_2 extract of Roots of *Moringa oleifera Lam*

Phytochemical test	Result
Carbohydrate	
Fehling	Absent
Benedict's	
Alkaloids	
Wagner's	Present
Mayer's	
Glycoside	
Legal test	Present
Tannin	
Gelatin	Present
Fecl3	

Flavonoids	
alkaline reagent	Present
Protein	
Biurette test	Present
Saponin	
Froath test	Absent

2.4.2. HPTLC

HPTLC is used for identification of constituents, identification and determination of impurities, and quantitative determination of active substances.

Table 7 HPTLC

Application Mode	**CAMAG Linomat:5 Applicator**
Filtering System	Whatman filter paper No.1
Stationary Phase	MERCK:TLC/HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start	10mm
Development End	80mm from plate base
Sample Application	Shigru (CO_2 extract of *Moringa oleifera* Lam.) 3.0µL
DiStance Between	20mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber	30 minutes
Mobile Phase	Toluene: Ethyl acetate: Acetic acid (7: 2: 1 v/v)
Visualization	@254nm, @366nm (after derivatization) and @540 nm (after derivatization)
Spray Reagent	Anisaldehyde: sulphuric acid reagent
Derivatization Mode	CAMAG :Dip tank for about 1 minute
Drying Mode, Temp.	TLC Plate Heater Preheated at 100±± 5 degree Celsius for 3 minutes

2.4.3. Observation

HPTLC plate of CO_2 extract of Roots of *Moringa oleifera* scanned at different wavelength such as 254 nm wavelength, 366 nm wavelength and 540 nm wavelength which showed the presence of various phytochemical constituents at different ranging of Rf values.

2.5. Section II

In vitro study (assessment of anti:cancer activity of *Moringa oleifera Lam.*)

Table 8 Cell line of breast cancer used for the study

Sr. No.	**Cell lines**	**Tissue of organ**
1.	MCF:7	BREAST CANCER

2.5.1. Method of In vitro anti cancer study

The cytotoxicity are checked by using MTT assay. The general purpose of MTT assay is to measure viable cells in relatively high throughput (96:well plates) without the need for elaborate cell counting.

2.5.2. Cancer cell line culture

Day 1

- 104 cells/well was added in a 96 well tissue culture treated plate (Cell count was taken on a Neubauer's chamber).
- The plate was incubated at 37°C in a 5% CO_2 incubator for 24 hours.

Day 2

- After 24 hrs incubation, the plate was observed under inverted microscope to check the morphology of the cells and confluency of the wells in the 96 well plate.
- Sterile test sample was suspended in DMEM containing 10% FBS at a known concentration and dilutions for the same were made accordingly.
- 100 µl of each of the test samples were added in triplicates along with the positive control (DMSO) and normal control (cells with medium and no test sample).
- Post sample addition, the plate was incubated at 37°C in a 5% CO_2 incubator for 24 hours.

Day 3

- After 24 hours incubation, the plate was observed under the inverted microscope and photographs were taken of the recorded observations.
- Test sample was removed and 90 µl fresh DMEM containing 10% FBS was added.
- Then 10µl of MTT reagent was added in each well.
- The plate was wrapped in aluminium foil and incubated at 37 °C in a 5% CO_2 incubator for 4 hours.
- Post 4 hours incubation, the entire medium was removed by flicking the plate and 100 µl of solubilisation buffer was added in each well and incubated at 37°C in a 5% CO_2 incubator for about 20 minutes. 6. Post incubation, absorbance was measured at 570nm and 630 nm on 96 well Plate reader.

Table 9 Observation of Drug Concentration with O.D

Sr. No	Drug concentrations µg/ml	Average O.D values
1.	C	0.268
2.	Positive control	0.114
3.	100µg/ml	0.175
4.	200µg/ml	0.162
5.	300µg/ml	0.142
6.	400µg/ml	0.125
7.	500µg/ml	0.100

In vitro (Fig no.5) screening of CO_2 extract of Root of *Moringa oleifera Lam.* Optical density at different concentration on MCF:7 cell line

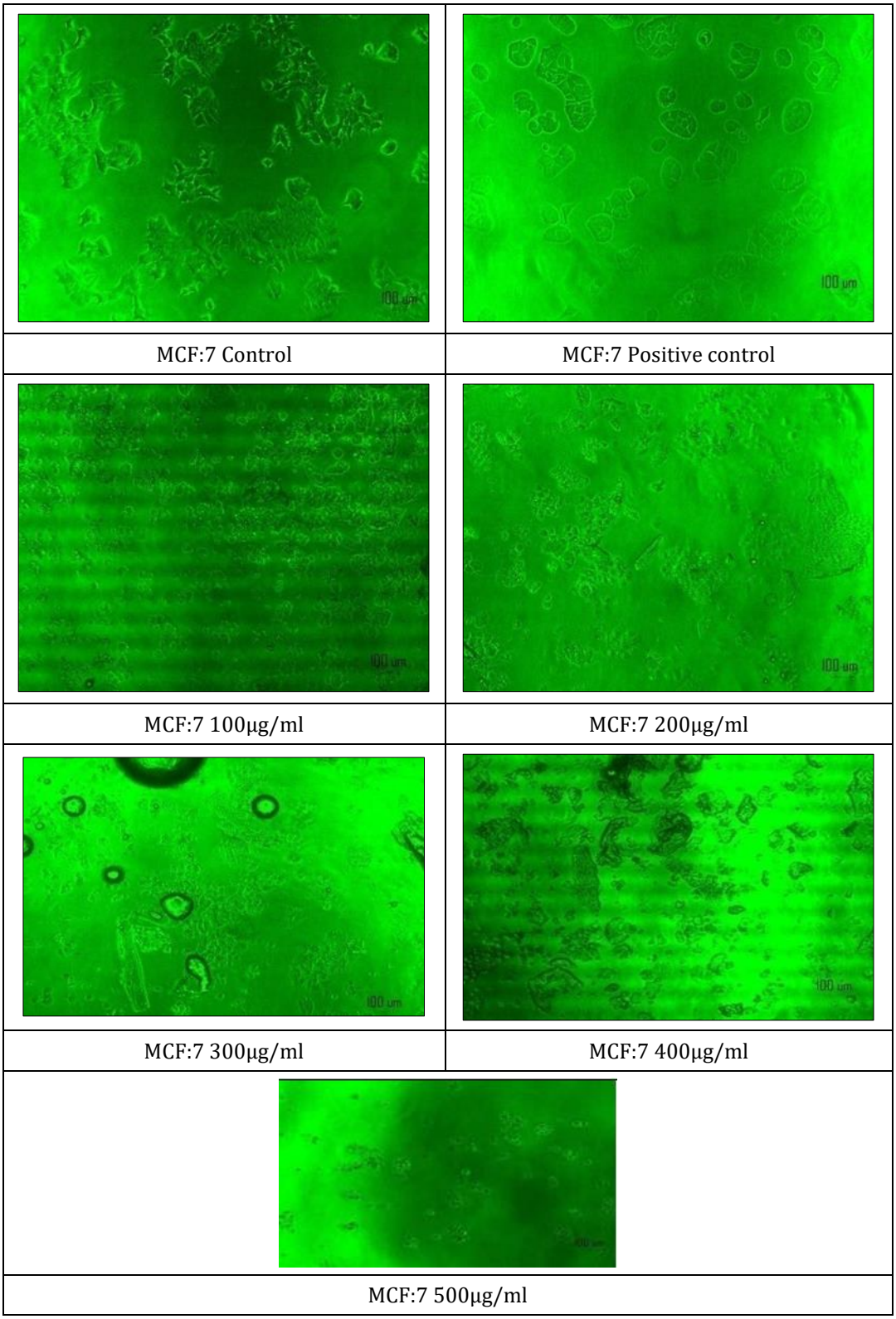

Figure 1 Optical density at different concentration on MCF:7

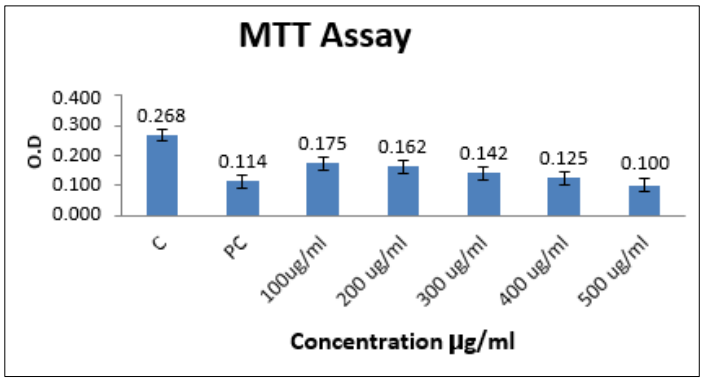

Figure 2 Graphical description of Optical density at different concentration on MCF:7

Observation: The concentration increases cytotoxic activity increases and as the concentration decreases cytotoxic activity decreases. It suggests that cytotoxic activity is dependent on the concentration of the drug. So, the cytotoxicity is dose-dependent. The highest toxicity was seen at the concentration of 500 µg/ml as compared to the positive control. Whereas the remaining concentrations i.e. 100 µg/ml, 200 µg/ml, 300µg/ml, and 400 µg/ml have shown less toxicity as compared to the positive control

- Cytotoxicity of the sample was carried out on MCF:7 cell lines by using MTT assay.
  As compared to C (control group), optical density (OD) of PC (positive control) was significantly ($P<0.001$) less indicating significant cell death. According to the result it can be concluded that the increase in concentration of CO_2 extract of Shigru Root shows significant increase (<0.0001) in cytotoxicity in cell line as compare to control group.

3. Discussion

The test drug proved standard by comparing all API standard values by doing various standard test such as Pharmacognostical, Physicochemical, phytochemical, Heavy metal test, Microbial contamination.

As per result obtained from MCF 7 breast cancer cell line, the concentration of the study was increased upto 500µg/ml. DMSO was used as control group. Bar diagram is plotted on the basis of average value obtained from each experiment which denotes the effect of drug and control drug on selected cancer cell line. The results can been discussed of anticancer study on breast cancer cell line MCF 7 were as follows:

Statistical analysis of various concentration from 100 µg/ml to 500µg/ml shows significant anti- cancerous action i.e. cytotoxic activity on MCF 7 breast cancer cell lines. Study drug was taken in 5 concentrations i.e. 100 µg/ml, 200µg/ml and 300µg/ml, 400µg/ml, 500µg/ml. Each was compared to the Standard drug (DMSO). The highest toxicity was seen at the concentration of 500µg/ml as compared to the positive control. Whereas the remaining concentrations i.e. 400µg/ml, 300µg/ml, 200µg/ml, 100µg/ml have shown less toxicity as compared to the positive control. As per the above obtained results, it can be stated that as the concentration increases cytotoxic activity increases and as the concentration decreases cytotoxic activity decreases. This suggests that cytotoxic activity is dependent to the concentration of our drug. So, we can conclude that cytotoxicity is dose-dependent. And the drug has shown positive results at 500ug/ml concentration.

4. Conclusion

- The study was carried out by using all standard protocol the test drug *Moringa oleifera* Lam was checked by doing all standard parameter tests such as Pharmacognostical, Physicochemical, phytochemical, Heavy metal test, Microbial contamination and the drug was proved by all API standard values.
- Cytotoxicity of the sample was carried out on MCF:7 cell lines by using MTT assay. According to the results it can be concluded that the increase in concentration of CO_2 extract of Shigru (*Moringa oleifera* Lam.) Root shows increase in cytotoxicity in MCF-7 cell line.

Further scope and Limitation of the study

CO_2 extract of the root of *Moringa oleifera* lam. has shown promising anticancer potential. The following are the future scope of the current research to develop these extracts as a drug.

- Standardization of CO_2 extract of the Root of *Moringa oleifera* Lam. should be done with the help of more precise and advanced parameters of testing.
- Further study required to comparative study on different cell line for the analysis of efficacy and toxicity of CO_2 extract of *Moringa oleifera*.
- The study should be conducted using different extraction method like soxhlet extraction, decoction, percolation etc.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that there is no conflict of interest.

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