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Evaluation of *in-vitro* anticancer activity assessment on breast cancer cell line MCF-7

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ABSTRACT
The aim of research work to investigate the in-vitro anticancer activity on breast cancer cell line MCF-7 using poly-herbal hydroalcohalic extract of Ocimum sanctum, Curcuma longa, Emblica officinalis, Teriminalia bellerica, Teriminalia chebula, Piper longum, Piper nigrum and Zingiber officinale. The poly-herbal hydro-alcoholic extract prepared were using coarsely powder of Ocimum sanctum 50mg, Curcuma longa 50mg, Emblica officinalis 50mg, Teriminalia bellerica 50mg, Teriminalia chebula 50mg, Piper longum 50mg, Piper nigrum 25mg, and Zingiber officinale 25mg. The poly-herbal hydroalcohalic extract was used for development of poly-herbal capsule for the treatment and management of cancer disease. The developed poly-herbal combination showed significant anticancer activity against MCF-7 breast cancer cell line. The poly-herbal hydroalcohalic extract was found to be IC50 35.44 µg/ml effective against MCF-7 breast cancer cell line. Thus, the poly-herbal hydroalcohalic extract showed the therapeutic potential against cancer cells which can be exploited in further investigation against carcinogenesis. These potential bioactive compounds having combination might be effective for the treatment and management of breast cancer.
Keywords: *Breast cancer, poly-herbal formulation, MCF-7 breast cancer cell line.*

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INTRODUCTION

Medicinal drugs are most important for all humans. These herbal drugs are used for treatment and management of diseases by peoples from ancient times. As per the world health organization (WHO), breast cancer disease is a increasing health issue worldwide and is the second most cause of death. Average 1 in 10 women is diagnosed with breast cancer at some stage of life. World Health Organization (WHO), approved that "natural plants are having more bioactive molecules that have been reported their therapeutic benefits, or which are mother sources of chemo-pharmaceutical semi-synthesis [1-2].

According to various researchers reports estimated that even modern time approximately 65-75% of the World's populations depend on medicinal plants for treatment and management of diseases [3-4].

Breast cancers diseases have been reported worldwide in woman. Breast cancer creates significant morbidity and mortality amongst women [5]. Lack of effective therapeutic drugs for control and treatment of breast cancers, and the huge financial burden placed on individuals and nations mean urgent action must be taken in the fight and treatment and management of breast cancer disease. The sides' effects produce by conventional chemotherapy for long time treatment. These side effects can be removed by using herbal bioactive medicines at economic level. In the recent years, peoples interest highly increases towards natural products for safe cancer prevention, current approaches have been focused on the use of food and ethno medicinal plants as sources of natural products that could used for effectively control cancer diseases [6-8].

In poly-herbal formulation is a excellent concept and suitable and right for the treatment and management of chronic diseases such as cancer diseases. Bioactive compounds have been used by Ayurveda doctors to treat and manage the condition of breast cancer. The beneficial compounds flavonoids, saponins, tannins, gums, and mucilage are all secondary metabolites of natural plants [9-10]. Natural remedies are incredibly efficient, safe, and economical for treating numerous diseases [11]. The anticancer activity of a poly-herbal combination extract (PHCE) containing leaf extracts of *Ocimum sanctum, Curcuma longa, Emblica officinalis, Teriminalia bellerica, Teriminalia chebula, Piper longum, Piper nigrum*, and *Zingiber officinale* was selected [12] Selected poly-herbal combinations have never been employed before by any researcher, and there are currently no research publications available. These are

all medicinal herbs potential anticancer bioactive molecules. As a result, these PHCE were chosen for the treatment and management of breast cancer disease. Many researchers have been reported and found good results but need to more research work for development of potential natural products. Our objective of present research work was the development of poly-herbal combination for the treatment and management of breast cancer disease [13-17]. Name of plants and their potential bioactive molecules pharmacological properties are shown in below Table 1.

Table 1 Name of plants and their potential bioactive molecules pharmacological properties

Name of plants	Phytochemicals	Pharmacological properties
Ocimum sanctum [18].	Carvacrol, methyl carvicol, urosolic acid, limatrol, Caryophyllene, sitosterol; xylose and polysaccharides	Anticancer, anti-diabetic, antifungal, antimicrobial, cardioprotective etc.
Curcuma longa [19-20].	Curcuminoids, turmerone (40%) and curlone demethoxycurcumin, bisdemethoxycurcumin and many more.	Treatment for anti-inflammatory, antiallergic, antimicrobial, anticancer, hepatoprotective, neuroprotective etc.
Emblica officinalis [21].	Ellagic acid, tannins, gallic acid, minerals, vitamins, amino acids, fixed oils, and flavonoids such as rutin and quercetin are all polyphenols.	Digestive problems, cancer, osteoporosis, hypertension, neurological problems etc.
Mixture of Haritaki, Bibhitaki and Amalaki [22-23].	Flavonoids, alkaloids, phenols	Hepatoprotective, ulcerative colitis, cardiovascular illness, low liver function, large intestine inflammation
Trikatu (Mixture of black pepper, ginger and long pepper [24-25].	Piperine, gingerols, shogaols, and paradols, oleoresins, and alkaloids are some of the compounds found in ginger.	Fever, gastrointestinal and GI issues, urinary difficulties, hepatoprotective, neuralgia, and boils, to name a few.

MATERIALS AND METHODS

Collection of plants and analytical reagents

Selected plants materials were collected from local market like as *Ocimum sanctum, Curcuma longa, Emblica officinalis, Teriminalia bellerica, Teriminalia chebula, Piper longum, Piper nigrum* and *Zingiber officinale.* The plants materials are verified by the Central Ayurvedic Research Institute, Jhansi, Uttar Pradesh with accession number CARI/H/13212021, CARI/H/13222021, CARI/H/13232021, CARI/H/13242021, CARI/H/13252021, CARI/H/13262021, CARI/H/13272021, CARI/H/13282021, CARI/H/132292021, by Pharmacognosist, Dr. Sandeep Kumar Singh. All the required chemicals and reagents are analytical grade were used. The cancer cell lines were obtained from the National Center for Cell Sciences Pune, India.

Table 1: Herbal Drugs Used in Poly-herbal powder Formulation

Name of the plant	Common Name	Parts Used
Ocimum sanctum	Tulsi	Leaves
Curcuma longa	Turmeric	Rhizomes
Emblica officinalis	Amla	Fruits
Teriminalia bellerica	Baheda	Fruits
Teriminalia chebula	Harde	Fruits
Piper longum	Long pepper	Fruit
Piper nigrum	Kali mirch	Fruit
Zingiber officinale	Ginger powder	Rhizome

Formula for Poly-herbal formulation

The poly-herbal hydro-alcoholic extract prepared were using coarsely powder of *Ocimum sanctum* 50mg, *Curcuma longa* 50mg, *Emblica officinalis* 50mg, *Teriminalia bellerica* 50mg, *Teriminalia chebula* 50mg, *Piper longum* 50mg, *Piper nigrum* 25mg, and *Zingiber officinale* 25mg [18-20].

Extraction

The prepared poly-herbal combination of coarsely powder was used and extracted with using distilled water 30%: ethanol 70% ratio with maceration method. The powder poly-herbal combination was

dipped into the solvent ration (30:70) for 15 days and after completion of extraction, filtered and concentrated with the help of rotator evaporator method. The hydroalcohalic extract stored in airtight container and stored for further used [21-28].

Maintenance of cell lines:

The MCF-7 breast cancer cell line was obtained from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^{o}C temperature in the CO_2 incubator and sub-cultured for every 2days.

Background of the study:

3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm [29-31].

***In-vitro* anticancer activity assessment (MTT assay)**

The prepared poly-herbal hydroalcohalic extracts were tested for anticancer activity assessment using MTT- assay MCF-7 breast cancer cell line. In-vitro anticancer activity assessment under seed 200µl cell suspension is in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet). Incubate the plate for 24hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume. Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.) Remove the MTT reagent and then add 100µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength [Sangeeta et al., 2012, Kaur et al., 2018]. The IC50 value was determined by using linear regression equation i.e.

$Y = Mx + C.$

Here, Y = 50, M and C values were derived from the viability graph.

% Cell viability is calculated using the below formula:

% Cell viability= [Abs of treated cells/Abs of Untreated cells] *100

RESULTS

The developed poly-herbal powder formulation of anticancer activity assessment test was performed by using in-vitro method. The Observations in Statistical data of MTT cytotoxicity Study suggesting us that against MCF-7 breast cancer cell line, Test Compounds namely S1 showing significant cytotoxic potential properties with the IC50 concentrations at 35.44uG/ml used in the study. The compound, S1 showing effective cytotoxicity on MCF-7 breast cancer cell line and may be considered as potent anti-breast cancer agent due to their low IC50 values on MCF-7 breast cancer cell line. Overall, S1 compound showing effective anti-cancer potency on Human breast cancer cells. Further studies like Cell Cycle Study by PI staining, Apoptosis study by Annexin V/PI staining, Apoptotic Protein expressions like Caspase 3,7,9, Bcl2,p53 and ROS study to evaluate the mechanism of action of test compounds viz., S1 behind the anticancer potential in invitro conditions. The results of in-vitro cancer cell line study shown in Table 2 and Fig.1 respectively.

***In-vitro* anticancer activity assessment**

Table 2: Table showing the IC_{50} concentrations of the Test compounds, S1 against MCF-7 breast cancer cell lines after the incubation period of 24hrs.

S. NO	Sample code	IC_{50}(µG/ml)
		MCF-7 Cell
1	F1	35.44

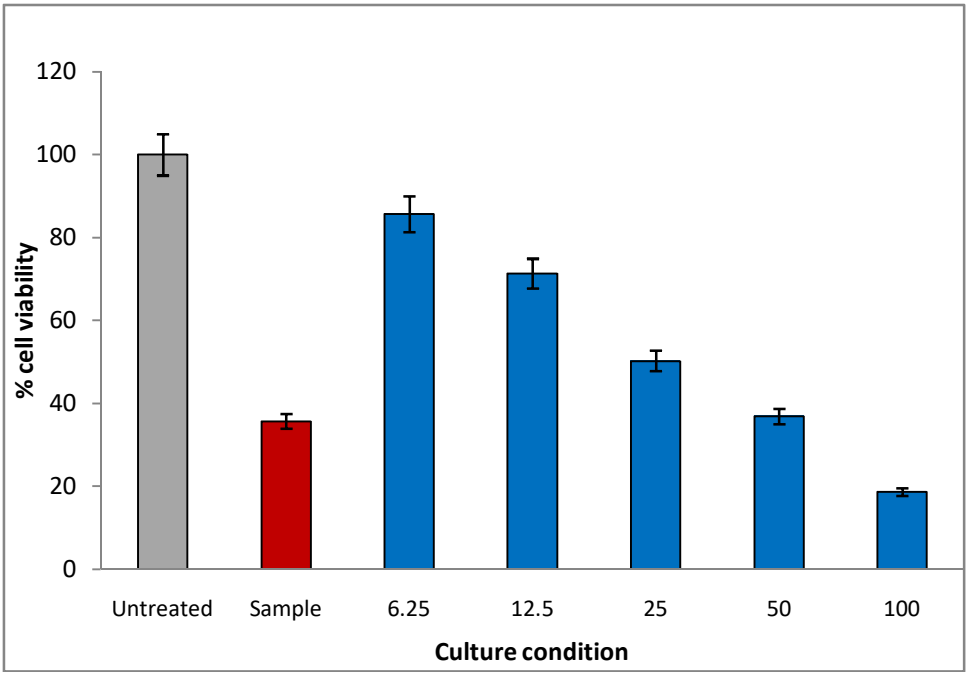

Fig. 1. Bar graph showing the % cell viability of Test compounds against MCF-7 breast cancer cell line by MTT study.

DISCUSSION

Various types of herbal medicines have been used as curative agents in different parts of the world. Drugs derived from traditional herbs may have possible therapeutic significance in the treatment of cancer diseases. In the present research work poly-herbal powder mixtures of selected plants were screened for their anti-cancer activity. Poly-herbal mixture of plant shows significant anti-cancer activity on MCF-7 breast cancer cell line. Further studies using more specific methods are required to explore the constituents responsible for the activity and the mechanism of this activity which might prove important and improved therapies for the treatment and management of breast cancer diseases [32-38].

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