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Original Article

Evaluation of *in vitro* Anti-cancer Activity of *Linum usitatissimum* and *Mentha spicata* Combination Extract against MCF7 Breast Cancer Cell-line

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ABSTRACT

Background/Aim: Breast cancer is one of the most common cancers among women in India. Though the current treatment is beneficial, it is complicated with lots of risks and disadvantages. So, the search for alternatives especially phytochemicals is increasing recently. *Linum usitatissimum* (Flaxseed) and *Mentha spicata* (Spearmint) are plant-based products, which are known for their various health benefits, exhibited anticancer activities independently *in vitro*. With the Proven importance of drug synergism, it is hypothesized that a combination of products would exhibit synergistic activity. **Objectives:** The present study aimed at evaluating the anticancer activity of a combination extract of *Linum usitatissimum* and *Mentha spicata* at MCF7 breast cancer cell line using MTT Assay. **Materials and Methods:** A combination methanolic extract of *Linum usitatissimum* and *Mentha spicata* was added to the MCF7 cell line at different concentrations ranging from 20 to 100 µg/mL. The IC_{50} value was calculated based on the percentage of inhibition. **Results:** Cell viability decreased to 24.6% at the maximum measured concentration of 100 µg/mL. The IC_{50} value was found to be at 70 µg/mL. **Conclusion:** Our study results showed that there is a significant anticancer property in combination extract of *Linum usitatissimum* and *Mentha spicata* in a concentration-dependent manner *in vitro*.

Keywords: Anti-cancer activity, Flaxseed, Spearmint, Breast cancer, MTT assay.

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INTRODUCTION

Cancer is globally the foremost and most common cause of mortality and morbidity.[1] Carcinoma of the breast is the most typical type of cancer among Indian women. As per WHO, 23 lakhs women are diagnosed with carcinoma of breast and 685000 deaths per year by 2020 worldwide.[2] Breast cancer treatment is highly effective and has a better survival rate when identified and treated early. Breast cancer treatment depends on the tumor stage, and subtypes like hormone receptor status, nodal status, and HER2 status, and consists of surgery, radiation, and anti-cancer medications which include endocrine treatment, chemotherapy, and targeted biologic therapy[3] Hormonal therapies can be given to progesterone receptor-positive Breast Carcinoma, and estrogen receptor-positive Breast Carcinoma.[4] Chemotherapeutic agents can be given to hormone-negative breast cancer.[5] But both these hormonal and chemotherapeutic agents have certain disadvantages like drug resistance, adverse effects, and cost, which may ultimately affect the effectiveness and prognosis of the treatment. So, counteracting these disadvantages, research towards novel anticancer agents with fewer adverse events and better treatment effects is widely done nowadays.[6] Medicinal plants have always been an important source for discovering new treatments for human diseases. As per WHO many countries use natural sources, mainly plants for chemotherapeutic purposes and almost 60% of anti-cancer agents have been derived from natural sources globally.[7] Some of the plant-derived compounds are in use as anticancer medications are vinca alkaloids, Podophyllotoxin derivatives, Taxol analogs.[8]

Among the various phytochemicals researched, Flaxseed (*Linum usitatissimum*) has been one of the most studied components in breast cancer.[9] *Linum usitatissimum* is a plant-based fibre crop with a high nutritive value and possesses antioxidant, and anti-tumorigenic properties.[10] The antitumorigenic activity of flaxseed is mainly because of the Lignan, fibers, Omega-3 Fatty acids, and alpha-linolenic acid constituents present in flaxseed. Lignans are Phyto estrogens, its structure is similar to that of the main form of estrogen which binds to the cell receptor that inhibits the growth of the cancer cell. Thereby exhibiting an antiestrogenic effect.[9] So, it can probably be used in Hormone receptor-positive breast cancer. Flaxseed also has a few side

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effects like gastrointestinal disturbance. Because of its treatment selective nature and few side effects, there is a need for synergistic components for better efficacy with fewer side effects.

Spearmint (*Mentha spicata*) is a herb that is mainly used for the treatment of gastrointestinal symptoms like nausea, vomiting, etc.[11] *Mentha spicata* possesses antioxidant, anti-microbial, anti-inflammatory, and antitumorigenic properties.[12] L-Carvone is the major constituent of *Mentha spicata*, proven to have anticancer activity by inducing apoptosis, cell cycle arrest, hyper-reactive oxygen species generation and thereby inhibiting the growth of the tumor cell.[13] In addition to it, spearmint may also reduce the chemotherapy-induced Gastrointestinal adverse effects.[14]

Flaxseed (*Linum usitatissimum*) and Spearmint (*Mentha spicata*) works on different mechanism in the management of breast cancer, and also it is thought that the Gastrointestinal side effects of flaxseed can be counteracted by spearmint. Therefore, combining Flaxseed and Spearmint might have a beneficial effect on both efficacy and safety. Since there is no study evaluating the anticancer activity of their combination, the present purpose of our research aims to assess the *in vitro* anticancer capability of the combination of Flaxseed extract and Spearmint leaf extract in MCF7 Breast cancer cell line through MTT assay.

MATERIALS AND METHODS

Preparation of extract

Flaxseed and Spearmint leaf were taken, dried, and grinded into fine powder respectively. 50 g of flaxseed powder and 50 g of Spearmint leaf powder were taken and mixed, and placed in a clean conical flask. 250 mL of methanol was added to the mixed powder in the conical flask and then the extraction was carried out in the Soxhlet apparatus for 72 hr. The solvent was evaporated and extracts were stored at 4°C for further use.

Cell Culture Maintenance

The human breast cancer cell line, Michigan Cancer Foundation-7 was obtained from NCCS (National Centre for Cell Sciences), Pune, India. In DMEM (Dulbecco's modified eagle media) supplemented with 100 g/mL streptomycin, 100 U/mL penicillin, and 10% (v/v) heat-inactivated Foetal Bovine Serum, cells were kept in the logarithmic phase of growth (FBS). They were upheld at 37°C with 5% CO_2 in 95% air humidified incubator.

Cytotoxic effect by *in vitro* cell viability assay

By using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test), the cytotoxic effect of the material was determined against the MCF-7 cell line (Mossman, 1983). The cells were diluted in 96-well microplates (1 x 10^6 cells/well) and cultured at 37°C for 48 hr in a 5% CO_2 incubator, they were allowed to reach 70-80% confluence. The medium was then changed, and the cells were exposed to varied sample concentrations for 24 hr. After 24 hr, the Morphological (structural) changes of untreated (control) and the sample-treated cells were examined and photographed using a digital inverted microscope (20X magnification). The cells were then rinsed with 20 µL of (MTT) solution (5 mg/mL in PBS) and each well received an addition of phosphate-buffer saline (PBS, pH-7.4). The plates were then left at 37°C for 2 hr in complete darkness. The absorbance was determined spectrophotometrically at 570 nm after the formazan crystals were dissolved in 100 µL DMSO. Cell viability Percentage was calculated using the formula,

$$\text{Cell viability (\%)} = (\text{Absorbance of sample}/\text{Absorbance of control}) \times 100$$

A graph was created by plotting the sample concentration on the X-axis and the cell viability % on the Y-axis.[15]

RESULTS

MTT assay is a safe and reliable *in vitro* method for assessing the cytotoxicity against many cancer cell lines. The MTT assay was used to determine if the sample extract was cytotoxic to the MCF7 cell line, and the experiment was carried out in triplicates. For both the control (untreated) and the test (treated with extract), the percentage of inhibition used to quantify cell viability was calculated. Cell viability ranged from 20 µg/mL to 100 µg/mL concentrations. The cell viability of the sample at the different concentration on the MCF7 cell line were shown in Table 1. IC_{50} value (half maximal inhibitory concentration) was identified to be 70 µg/mL. Concentration-dependent inhibitory effects in cell viability was noted (As shown in Figures 1-6).

Table 1: Anti-cancer activity of *Linum usitatissimum* and *Mentha spicata* combination extract on MCF7 breast cancer cell line.

Concentrations (µg/mL)	Absorbance			Average	Cell Viability (%)
	I	II	III		
Control	0.93	0.919	0.916	0.9216667	100
20	0.846	0.832	0.84	0.8393333	91.06690778
40	0.737	0.728	0.712	0.7256667	78.73417722
60	0.533	0.541	0.55	0.5413333	58.73417722
80	0.39	0.379	0.383	0.384	41.6636528
100	0.217	0.23	0.235	0.2273333	24.66546112

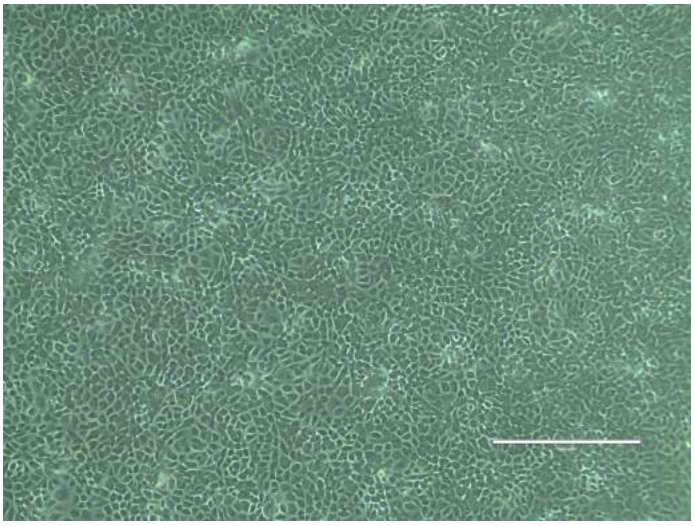

Figure 1: Picture showing cell viability of Control.

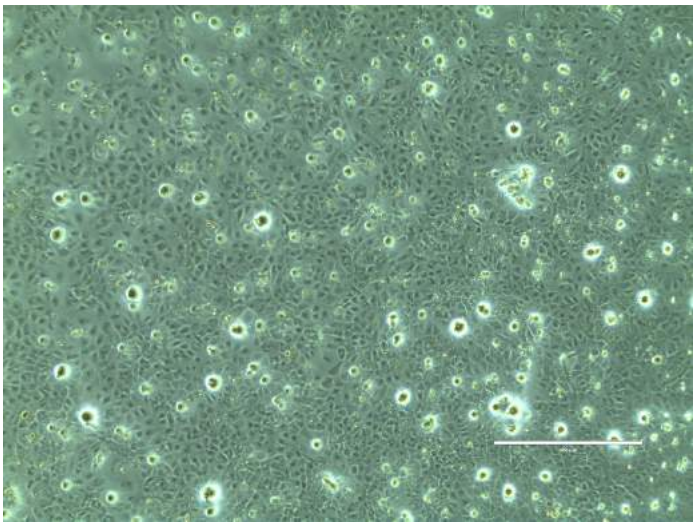

Figure 2: Picture showing viability of cell at sample concentration 20 µg/mL.

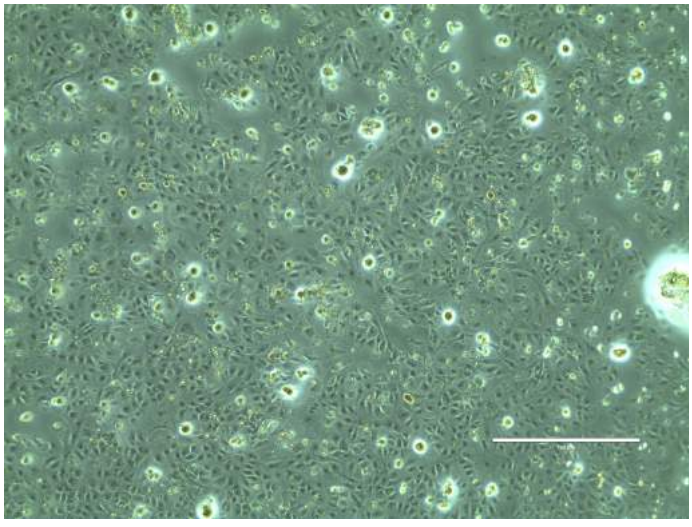

Figure 3: Picture showing viability of cell at sample concentration 40 µg/mL.

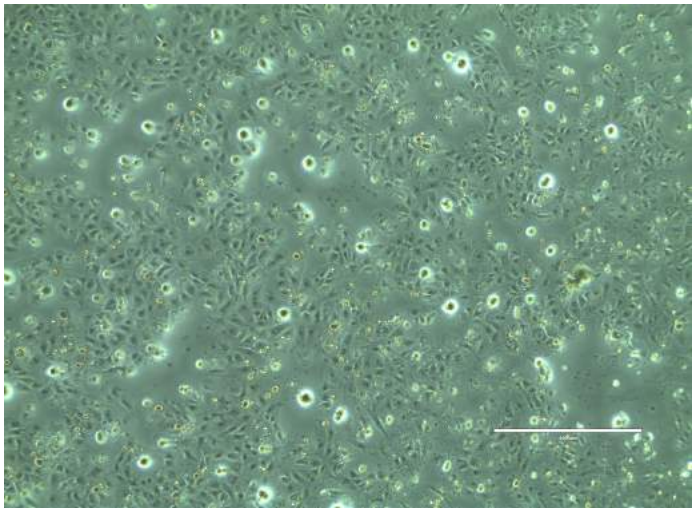

Figure 4: Picture showing viability of cell at sample concentration 60 µg/mL.

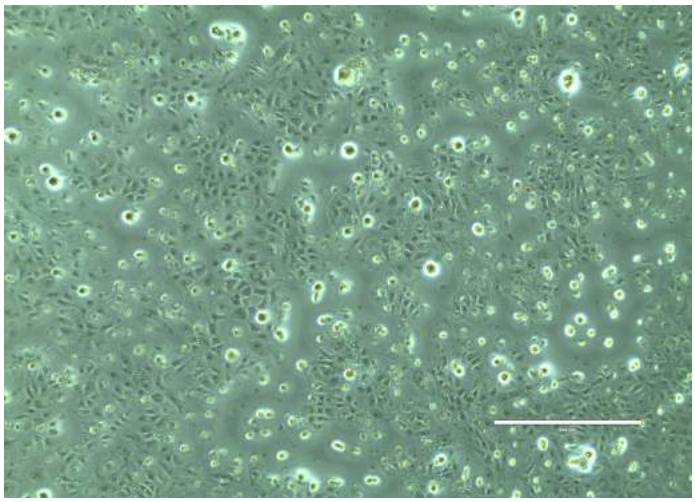

Figure 5: Picture showing viability of cell at sample concentration 80 µg/mL.

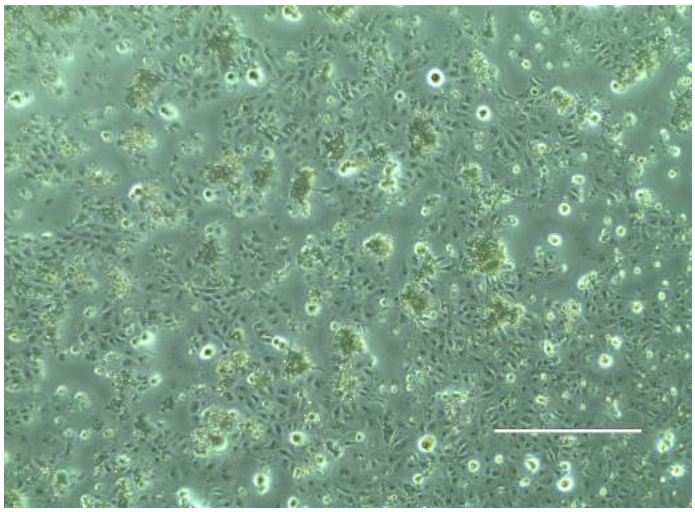

Figure 6: Picture showing viability of cell at sample concentration 100 µg/mL.

DISCUSSION

Carcinoma of the Breast has been one of the foremost causes of death due to cancer in women worldwide. Early recognition and treatment are proven to be more successful in terms of the survival rate. Medical Treatment depends on the hormone receptor status expressed on the cancer cell. Hormone receptor-positive respond to endocrine therapy whereas Hormone receptor Negative are needed to be treated with Chemotherapy. However, over the past decades, the use of these conventional drugs has not significantly improved the survival rate. So, the search for novel agents for the treatment of cancer as a complementary or as an add-on to current therapy is needed and is increasing nowadays. Phytochemicals Present in medicinal plant-based products have expanded more attention in the treatment of cancer as they are most cost-effective, natural, less side effects, and easily available.

Our study showed the potential anti-neoplastic activity of a combination extract of flaxseed (*Linum usitatissimum*) and spearmint (*Mentha spicata*) against MCF 7 cell line via MTT assay. IC_{50} was calculated and it is found to be 70 µg/mL (Table 1). Initially at 20 µg/mL of sample concentration, the viability of the Breast cancer cell was decreased to 91%. The percentage of cell viability progressively declined at each treated concentration and reaches until 24.6% at 100 µg/mL. So, from this study, we could say that the extract showed a potential anti-cancer activity in a concentration-dependent manner.

There are various *in vitro* studies available which proved the anticancer potential of flaxseed. In the study conducted by Alison L *et al*, MCF 7 cancer cell line are been treated with flaxseed oil which proved the inhibitory effect in dose-dependent manner.[16] In the study conducted by Shahira *et al* Flaxseed showed significant anticancer activity against MCF7 cancer cell line.[17] Numerous studies including an animal study on mice also proved that flaxseed alone or in combination with tamoxifen has an inhibitory effect on breast cancer cells.[18]

The Other component of our combination extract, Spearmint (*Mentha spicata*) is the herb that is most commonly used in Gastrointestinal disturbances and it also has many biological properties. There are various studies available that proves the *in vitro* anticancer activity of *Mentha spicata* individually. *Mentha spicata* has *in vitro* anticancer potential against the MCF7 cell line, according to a study by Sanaa *et al.* that looked at its anti-proliferative activities.[12] *Mentha spicata* was tested for its *in vitro* anticancer properties using methanolic extracts at a concentration of 100 µg/mL in the study done by Vikas Sharma *et al.* It was shown that *Mentha spicata* had an antiproliferative potential of 70-97% versus Breast cancer cell line MCF 7.[19] The research conducted by Akhilesh Kumar *et al.* showed that methanolic extracts of *Mentha spicata* has an anti-oxidant property and thereby it can protect organisms from oxidative DNA damage associated with cancer.[20]

Flaxseed (*Linum usitatissimum*) is an annual herb, which are rich in omega-3 fatty acid, alpha linolineic acid, lignans, fibres. All of these components in flaxseed mainly lignans play a significant role in inhibiting the development of breast carcinoma cells.[21] It's proved that *Mentha spicata* essential oil is safe and effective against chemotherapy-induced nausea and vomiting.[14] L-Carvone is the major component present in *Mentha spicata*. Research have exposed that L-carvone brings apoptosis and inhibits the development and migration of breast carcinoma cell lines.[13] These proven mechanisms may be the probable reason for the anticancer property of our combination of Flaxseed (*Linum usitatissimum*) and Spearmint (*Mentha spicata*) extract.

LIMITATIONS

We proved anti-cancer activity of combination of *Linum usitatissimum* and *Mentha spicata* on MCF 7 Breast cancer cell line *in vitro*. Further *in vivo* and clinical studies are needed to support this evidence.

CONCLUSION

Our study investigated the combination of *Linum usitatissimum* extract and *Mentha spicata* extract on *in vitro* MCF7 Breast cancer cell line. Results showed that combination extract has Significant anti-cancer activity in a concentration-dependent manner. However, Further animal and clinical studies are needed to prove its clinical implication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MCF7: Michigan Cancer Foundation 7; **MTT:** (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **µg/mL:** Microgram/milliliter; **IC_{50}:** Half maximal Inhibitory concentration; **WHO:** World Health Organisation; **HER2:** Human Epidermal Growth Factor Receptor2; **g:** Gram; **hr:** Hour; **NCCS:** National Center for Cell Sciences; **DMEM:** Dulbecco's Modified Eagle Medium; **CO_2:** Carbon dioxide; **PBS:** Phosphate Buffer Saline; **nm:** Nanometer.

SUMMARY

Breast cancer is one of the commonest malignancies among women in India. Since the current management of it involves lots of adverse effects, the search for plant-based alternatives

have gained attention. *Linum usitatissimum* (Flaxseed) and *Mentha spicata* (Spearmint) synergistically exhibited significant anticancer activities at MCF7 breast cancer cell line using MTT Assay done *in vitro*.

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