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RESEARCH ARTICLE
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PHARMACOLOGICAL ASSESSMENT OF ANTI-MAMMARY CARCINOMA ACTIVITY OF *ANETHUM GRAVEOLENS* SEEDS AND *SPINACIA OLERACEA* LEAVES.

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ABSTRACT

Background: Breast cancer is the most commonly diagnosed malignancy in women and a leading cause of cancer-related mortality worldwide. Despite advancements in diagnostic and therapeutic strategies, breast cancer remains a significant clinical challenge due to its heterogeneity and the adverse effects of conventional treatments. The use of traditional herbal medicine, known for its minimal side effects and efficacy, offers a promising alternative.

Material & Methods: Aerial parts of ***Anethum graveolens*** seeds and ***Spinacia oleracea*** leaves were extracted using the Soxhlet extraction method. This study investigates the anti-mammary carcinoma potential of *Anethum graveolens* (dill) seeds and *Spinacia oleracea* (spinach) leaves, known for their bioactive compounds with anti-cancer properties.

Result & Discussion: At the different doses (10 to 100 µg/ml) of polyherbal extracts carried out for anticancer activity against MCF-7 cell line., the polyherbal extracts showed good activity against breast cancer cell line when compared with the standard drug 5 FU. These findings highlight the anti-cancer potential of polyherbal extract of *Anethum graveolens* seeds and *Spinacia oleracea* leaves as natural therapeutic agents for the management of mammary carcinoma.

Keywords: Mammary carcinoma, *Anethum graveolens*, *Spinacia oleracea*, Anti-cancer, Polyherbal.

INTRODUCTION

Breast cancer is the most frequent malignancy in women and is a heterogeneous disease on the molecular level. It is a malignant tumor that forms in the breast tissue, commonly affecting women but also occurring in men. It arises when cells in the breast mutate and multiply uncontrollably, potentially spreading to other parts of the body. Over the past 10-15 years, treatment concepts have evolved to take this heterogeneity into account, with emphasis being placed on more biologically-directed therapies and treatment descalation to reduce the adverse effects of treatment. Despite the inherent molecular heterogeneity, which is a driving principle of modern-day treatments, some

features such as the impact of loco-regional tumors burden or metastatic patterns are shared and influence therapy. Early breast cancer that is, cancer that is contained in the breast or that has only spread to the axillary lymph nodes is considered curable. Improvements in multimodal therapy have led to increasing chances for cure in ~70-80% of patients.

National Cancer Institute has evaluated about 35,000 plant species for Anti-cancer properties. Around 3,000 plant species have been found to have Anti-cancer action that can be replicated. *Anethum graveolens* and *Spinacia oleracea* is a medicinal herb mentioned in Ayurveda and it contains a variety of phytochemicals constituents that have been linked to a variety of pharmacological effects like Anti-diabetic, Antimicrobial, Anti-inflammatory, Immunomodulatory, Antispasmodic, & Diuretic. These two plant contains Flavonoids (Quarcetin), Phenolic compounds, carotenoids, saponins, beta carotene which are known for their therapeutic benefits and possess potential Anti-cancer effect.

MATERIALS AND METHODS

Fig no.1 *Anethum graveolens* seeds and *Spinacia oleracea* leaves

Collection of plant material:

Anethum graveolens belonging to Apiaceae and *Spinacia oleracea* belonging to Amaranthaceae family, was collected from local market of Nashik region. (Maharashtra).

Drying and pulverizing of plant material

The seeds of *Anethum graveolens* and leaves of *Spinacia oleracea* plant were dried in shade for 1 weeks and triturated to a fine powder. The powder was further passed through a 2 mm sieve to obtain fine particles.

Extraction of plant material

The triturated powder of *Anethum graveleons* seeds and *Spinacia oleracea* leaves was weighed seperately approximately 20-25g sample in filter paper. Close the filter paper and keep in siphon tube. Fit water IN and OUT in condenser must remember cool water should pass through below to top of condenser to avoid air bubbles. Then after fill solvent Ethanol (70- 80^0C) in siphon tube it would first siphon then fill half as well on siphon tube (Soxhlet extraction). Start heating on water bath the temperature of water bath should be 70-75^0C. Run the system till 7 hours, for clearance. Ethanol will clear at siphon tube, it means extraction is completed. Remove the sample from siphon tube, recover Ethanol, Keep sample in drying oven for 3 hours. Both the extract obtained are taken in equal quantity (3 gm) and mixed together to prepare a polyherbal extract.

Physiochemical evaluation and Phytochemical study

The crude drug is tested for the quality and purity parameters which include total ash value, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, alcohol and water soluble extractive value and foaming index. The obtained extract is further studied for phytochemical evaluation which include carbohydrates, proteins, fats and oils, phenolic compounds, flavonoids, cardiac glycosides, alkaloids, terpenoids, saponins, tannins, coumarin, anthraquinone & thin layer chromatography was also performed.

PHARMACOLOGICAL STUDY

Determination of IC_{50} concentration by using *In-vitro* MTT assay

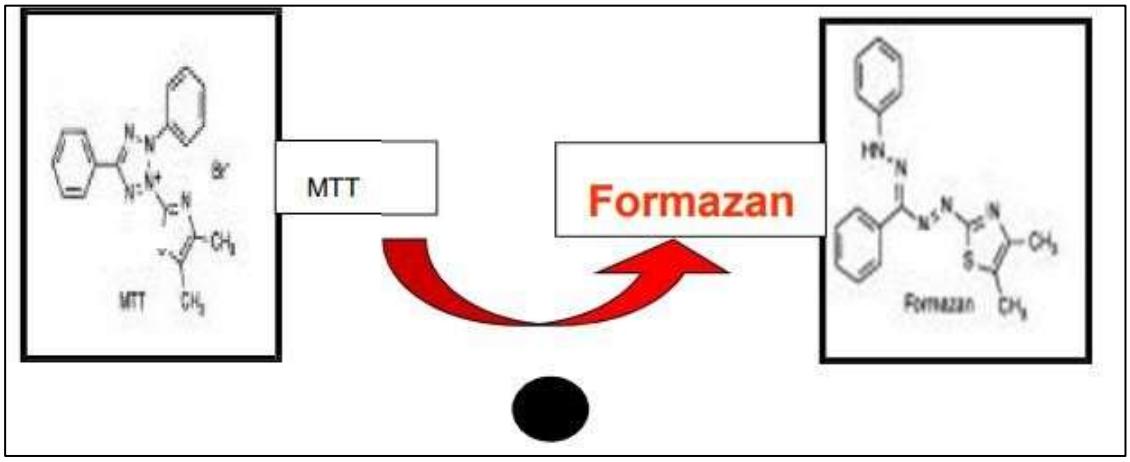
Cell line: MCF-7 (Human Breast Cancer Cell line)

Media:

- DMEM with high glucose (Cat No-11965-092),
- FBS (Gibco, In-vitrogen) Cat No -10270106
- Antibiotic-Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062

Principle of assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, purple colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.



Experimental procedure

MCF-7 Human breast cancer cell line was procured from National center for cell sciences (NCCS), Pune maintained in DMEM medium supplemented with 10 % fetal bovine serum. Cells were incubated at a concentration of 1 × 104cells/ml in culture medium for 24 h at 37°C and 5% CO Cells were seeded at a concentration (70µl) 104cells/well in 100µl culture medium and 100µl Sample (10 -100 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientific BB150) After incubation, the medium was completely removed and Added 20 µl of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4 hrs at 37oC in CO2incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only. After removing the medium completely. Added 200µl of DMSO (kept for 10 min) and incubate at 370C (wrapped with

aluminium foil). Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa microplatereader (Benesphera E21) at a wavelength of 570 nm.

Statistical analysis

The study was performed by using MTT assay on MCF-7 cell line. The MTT assay of polyherbal extract with standard drug 5- Fluorouracil was carried out. The MTT assay revealed a concentration-dependent decrease in cell viability in MCF-7 cells treated with polyherbal extract. Specifically, treatment with higher concentrations of both extracts resulted in a significant reduction in cell viability compared to untreated control cells. The IC50 values, representing the concentration of extract required to inhibit cell viability by 50%, were calculated for polyherbal extract.

RESULTS

Physiochemical evaluation and Phytochemical study

This phytochemical study confirmed the presence of Flavonoids, Phenolic compounds present in polyherbal extract possess the anti-mammary carcinoma activity.

Pharmacological study

Effect of compound against MCF-7 cell line

Table no. 1 Group wise percentage cytotoxicity on MCF-7 cell line (Standard)

Sr. no	Concentration (µg/ml)	Absorbance (OD)				Cell inhibition (%)	IC_{50} (µg/ml)
	5-FU	1	2	3	Average		
1	10	0.394	0.465	0.393	0.417	32.10	53.32
2	20	0.48	0.376	0.34	0.398	35.14	
3	40	0.333	0.325	0.396	0.351	42.84	
4	80	0.292	0.241	0.208	0.247	59.81	
5	100	0.189	0.186	0.124	0.166	72.93	

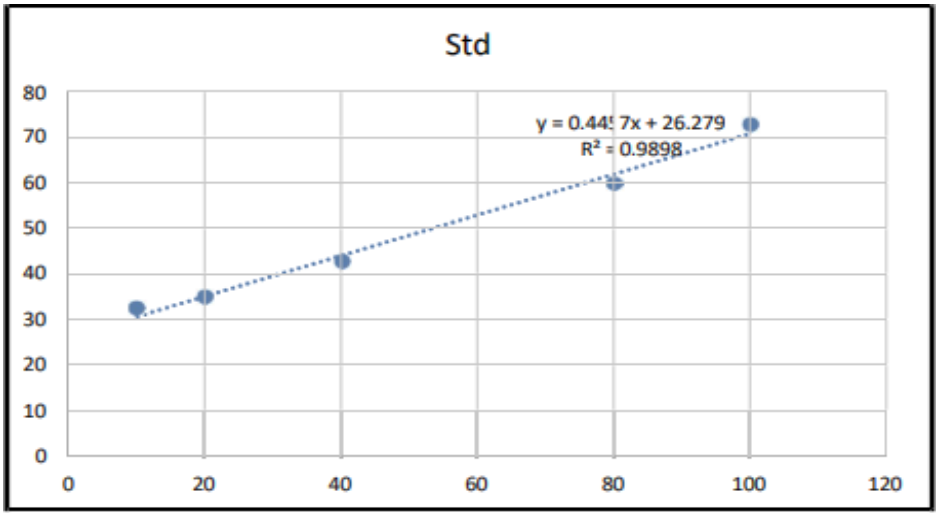


Fig no. 2 Group wise percentage cytotoxicity on MCF-7 cell line (Standard)

Table no. 2 Group wise percentage cytotoxicity on MCF-7 cell line (Test)

Sr. no	Concentration (µg/ml)	Absorbance (OD)				Cell viability (%)	Cell inhibition (%)	IC_{50} (µg/ml)
		1	2	3	Average			
1	10	0.856	0.824	0.794	0.824	70.08	29.91	85.86
2	20	0.712	0.728	0.712	0.717	60.96	39.03	
3	40	0.699	0.678	0.681	0.686	58.30	41.69	
4	80	0.642	0.661	0.565	0.622	52.91	47.08	
5	100	0.536	0.546	0.515	0.532	45.24	54.75	

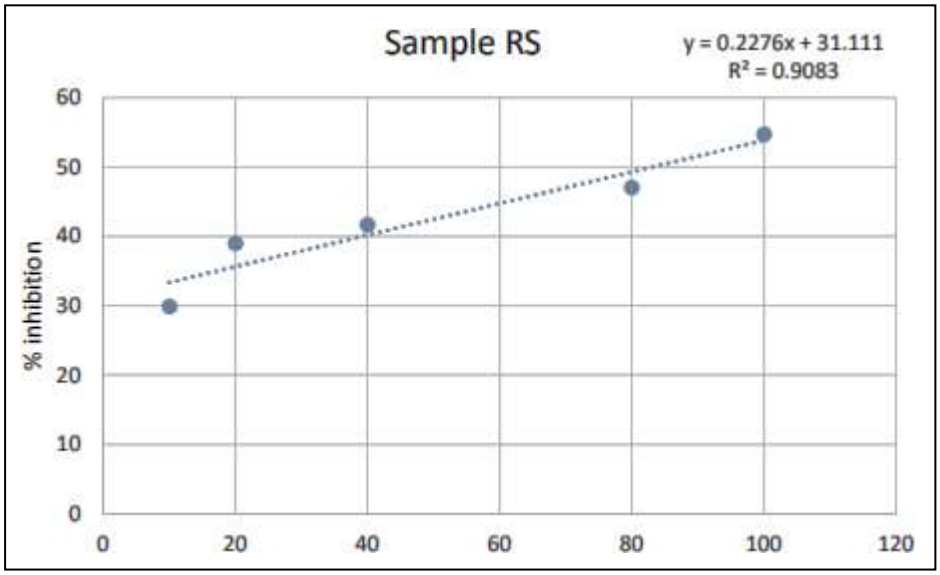


Fig no. 3 Group wise percentage cytotoxicity on MCF-7 cell line (Test)

The percent cell viability counts of polyherbal extract on MCF-7 cell line was calculated by using the formula (100 X Control-Sample/Control). A significant difference is observed in cytotoxicity on cancer cell lines when the polyherbal extract is compared with standard (5-Flurouracil). Similarly, more pronounced effect was observed in estrogen receptor-positive cells. At the concentration of 100 µg/mL, polyherbal extract has significantly reduced the growth of cancerous cell line and found effective against MCF-7 Human breast cancer cell line. So, it has been concluded that the *Anethum graveleons* seeds and *Spinacia oleracea* leaves polyherbal extract acts as potential Anti-cancer agent against MCF-7 (Human breast cancer) cell line (Figure 2 & 3).

DISCUSSION

Our results show that *Anethum graveolens* seeds and *Spinacia oleracea* leaves extracts have potential anti-mammary carcinoma actions on MCF-7 breast cancer cells. The concentration-dependent decline in cell viability that was found indicates that the bioactive chemicals in spinach leaf and dill seed extracts are both able to stop MCF-7 cell growth. Numerous processes, including

as the activation of apoptosis, cell cycle arrest, and the inhibition of cell signaling pathways implicated in the genesis of cancer, may be responsible for this suppression of cell viability.
According to earlier research, extracts from spinach leaves and dill seeds both contain phytochemicals with anti-cancer qualities. For example, flavonoids, polyphenols, and essential oils found in dill seed have been demonstrated to have anti-proliferative and apoptotic properties in a variety of cancer cell lines. In a similar vein, spinach leaves are abundant in antioxidants including flavonoids, carotenoids, and vitamins that have anti-cancer properties by scavenging free radicals and preventing the formation of tumor cells.

CONCLUSION

At the different doses (10 to 100 µg/ml) of polyherbal extract carried out for anticancer activity against MCF-7 cell line., the compounds polyherbal extract showed good activity against breast cancer cell line when compared to standard drug 5 FU.

FUTURE PROSPECTIVE

Targeted Therapies: Advancements in molecular biology and genomics can lead to the development of targeted therapies that focus on specific molecular pathways or genetic mutations driving breast cancer. This approach can enhance treatment efficacy while minimizing side effects.

Immunotherapy: Harnessing the immune system to target and destroy cancer cells has revolutionized cancer treatment. Researching immunotherapeutic approaches specific to breast cancer can lead to innovative treatments, such as checkpoint inhibitors or CAR-T cell therapies.

Natural Products and Drug Discovery: Continued research on plants and natural compounds can uncover novel bioactive molecules with potent anticancer properties. High-throughput screening methods and advanced analytical techniques can accelerate the discovery and development of these natural anticancer agents.

Personalized Medicine: With advancements in technology like next-generation sequencing, personalized treatment strategies based on a patient's unique genetic makeup and tumor profile are becoming a reality. Future research can further refine these approaches for more effective and personalized breast cancer care.

Combination Therapies: Combinations of traditional chemotherapy, targeted therapies, and immunotherapies can provide synergistic effects, improving treatment outcomes and reducing resistance. Future studies can focus on optimizing these combination therapies to maximize efficacy.

Health Technology and Data Analytics: Utilizing artificial intelligence (AI) and machine learning algorithms can help analyze large datasets, predict treatment outcomes, and identify new drug targets. Integrating health technology into breast cancer research can accelerate discoveries and improve patient care.

CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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