Title: Pharmacopeial standardization and anticancer evaluation of canceroso-1 a spagyric-based electrohomeopathic medicine prepared by King Herbal.

Hypothesis:

Canceroso-1, a spagyric-based electrohomeopathic medicine, has garnered significant attention due to its potential therapeutic applications in cancer treatment. This report aims to explore the pharmacopeial standardization and anticancer evaluation of this unique medicinal formulation. We hypothesize that the drug C1, a mixture of plant Spagyric extracts, and its components exhibit potent antiproliferative activity against ER-positive (MCF-7) and triple-negative (MDAMB-231) breast cancer cells.

Objectives

1. Studies on the antiproliferative effect of plant extracts C1 and its components in the ER-positive (MCF-7) and triple-negative (MDAMB-231) breast cancer cells in vitro.
2. Studies on the cancer hallmarks with the most potent extracts in vitro.

Summary of Studies (Pharmacopeial Standardization): Pharmacopeial standardization is critical to ensuring medicinal products' quality, safety, and efficacy. It involves establishing and adhering to specific standards and guidelines outlined in pharmacopeias, such as the Indian Pharmacopoeia (IP) or the United States Pharmacopoeia (USP).

For Canceroso-1, the following pharmacopeial parameters should be considered:

- We develop the Pharmacopeial standardization, method for validation, and quantification of secondary metabolites of Canceraso-1. The proposed method can be used as a standard method for further use.
- Identification tests (e.g., TLC, HPLC/LCMS) of each plant extract used in Canceraso-1.
- It helps identify quality planting material for various pharmaceutical preparations.
- Creation of a repository of distinct plants that were used in the formulation of Electrohomoeopathy.

TLC fingerprinting of Canceroso-1 (In different solvent systems which is illustrated below).

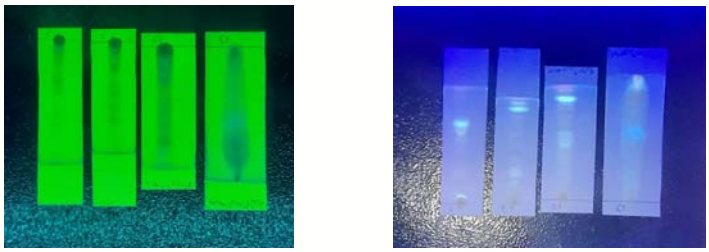
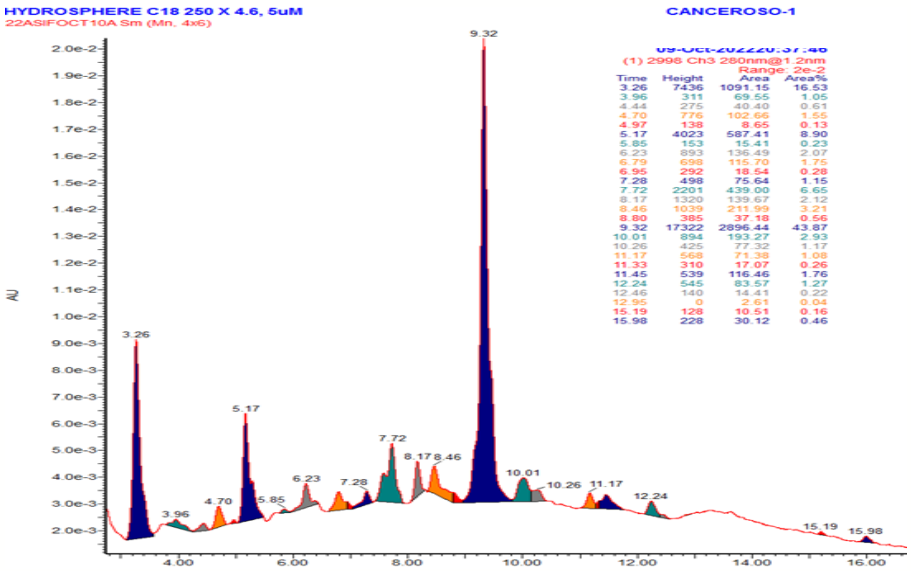
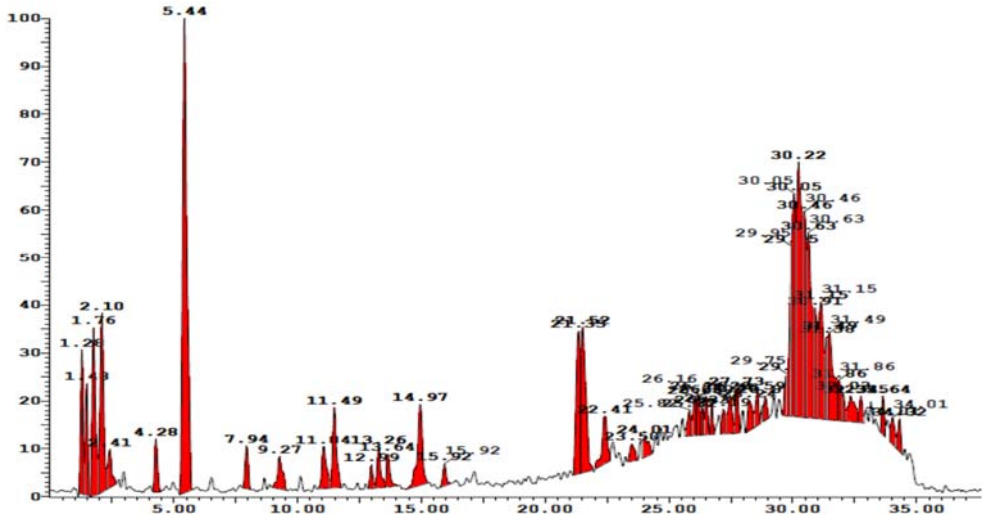


Fig. 1: showing the spots of a sample using solvent systems: (1) $CHCl_3$:MeOH (95:5) (2) $CHCl_3$:MeOH (9:1) (3) $CHCl_3$:MeOH (8:2) (4) $CHCl_3$:MeOH: H_20 (5:4:1) respectively.

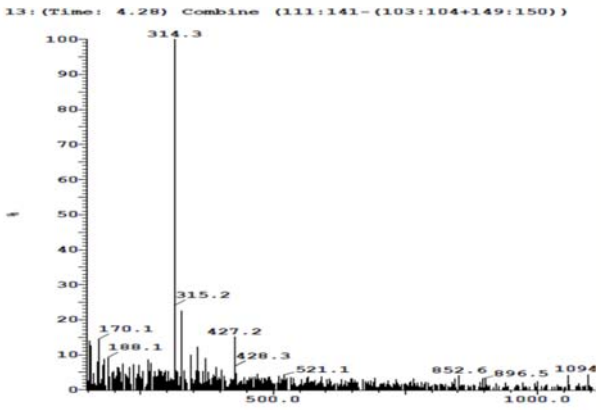
HPLC Chromatogram of **canceroso-1**



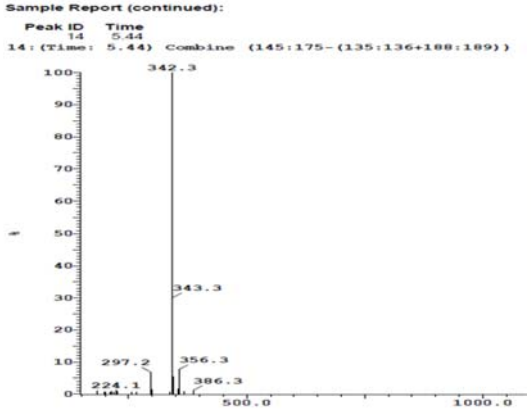
LC-MS Chromatogram of **canceroso-1**

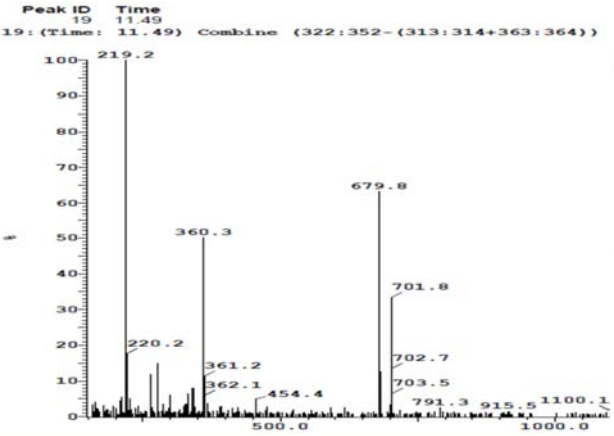


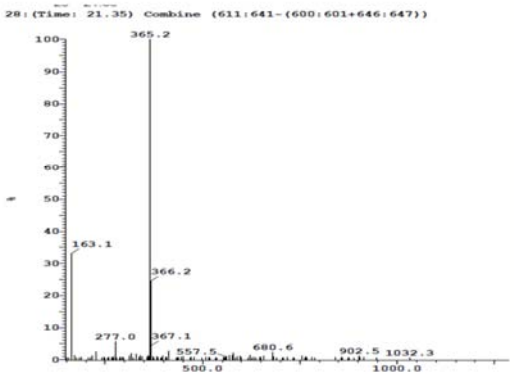
LCMS-Molecular Ions peak of canceroso-1



Sample Report (continued):







In the Biological assay, we screened the Canceraso-1 and its distinct fraction.

In the current set of results, we have used the MTT assay for studies on the antiproliferative activity of the extracts.

In this assay, we measure the cell viability 24h after the treatment to understand the plant extracts' anti-proliferative activity.

- ❖ We checked the 12 distinct fractions in ER-positive MCF-7 (4 extracts) and Triple Negative MDAMB-231 (8 extracts) cell lines.
- ❖ TheC1 served as a control in both cell lines studied.

We have also checked the extracts in the noncancerous MCF10A cell line. All the extracts showed anti-proliferative activity in the normal epithelial cells, almost at 10-fold concentrations indicating that these extracts are safe with good specificity against breast cancer cells.

Background:

Breast cancer is the most prevalent cancer among women worldwide. Breast cancer is a complex heterogeneous disease and based on histological features, it can be classified into hormone-receptor-positive, human epidermal growth factor receptor-2 over expressing (HER2+) and triple-negative breast cancer (TNBC). Mda-MB-231 is triple negative breast cancer cells. Amongst the breast cancer subtypes, the triple negative breast cancer (TNBC) is the most aggressive, metastatic subtype lacking estrogen, progesterone, and HER-2/neu receptors. The TNBC have complex histopathology, enhanced proliferation and lung, brain metastases. Mutational inheritance in two vulnerable genes, BRCA1/2 leads to the onset and progression of breast cancer in majority of the cases. It is utmost need to develop new therapeutic strategies to overcome the resistance of chemotherapeutics drug.

Therapeutic drugs for breast cancer under clinical development:

S.N.	Classes of drug	Drugs
1.	Anthracyclines	Doxorubicin, mitoxantrone, daunorubicin
2.	Antimetabolites	5-Fluorouracil, Gemcitabine,
3.	Alkylating agents	Cisplatin, Cyclophosphamide, nitrosourea,
4.	Taxanes	Paclitaxel, docetaxel
5.	Vinca alkaloids	Vincristine, Vinblastine
6.	Tyrosine kinase inhibitor	bevacizumab, imatinib, sunitinib, sorafenib
7.	Hormones	Tamoxifen, letrozole
8.	others	Bleomycin, etoposide, irinotecan

Hypothesis:

We hypothesise that the mixture of plant extracts C1 and its components have potent antiproliferative activity against triple negative (MDAMB-231) breast cancer cells.

Methodology:

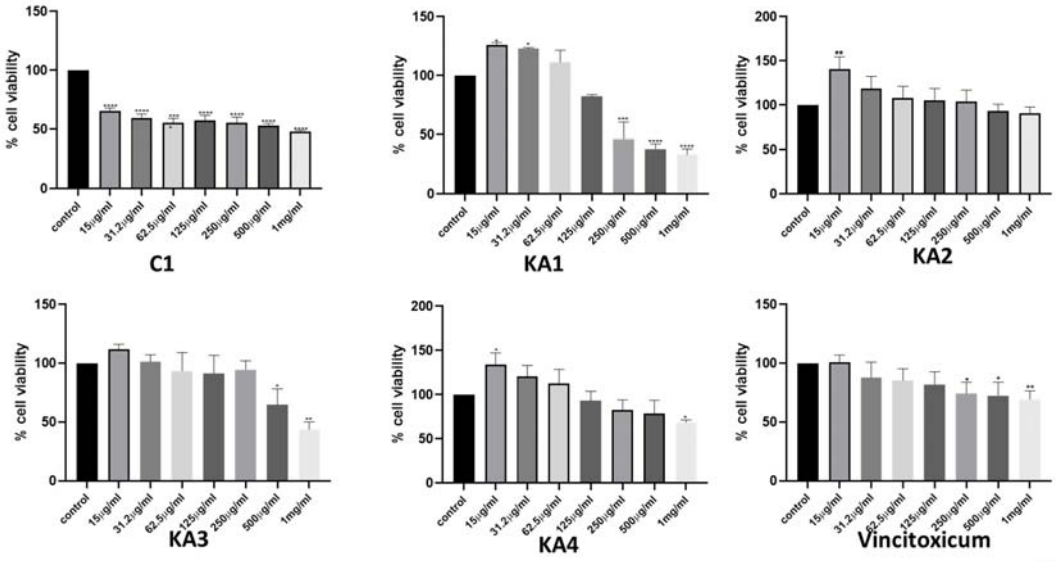
Cell culture: The human breast cancer cell line MDA-MB-231 purchased from ATCC and cultured and maintained in a humidified atmosphere containing 5% CO2. cells were maintained in RPMI culture media containing 10%FBS (HiMedia) along with 1% penicillin–streptomycin.

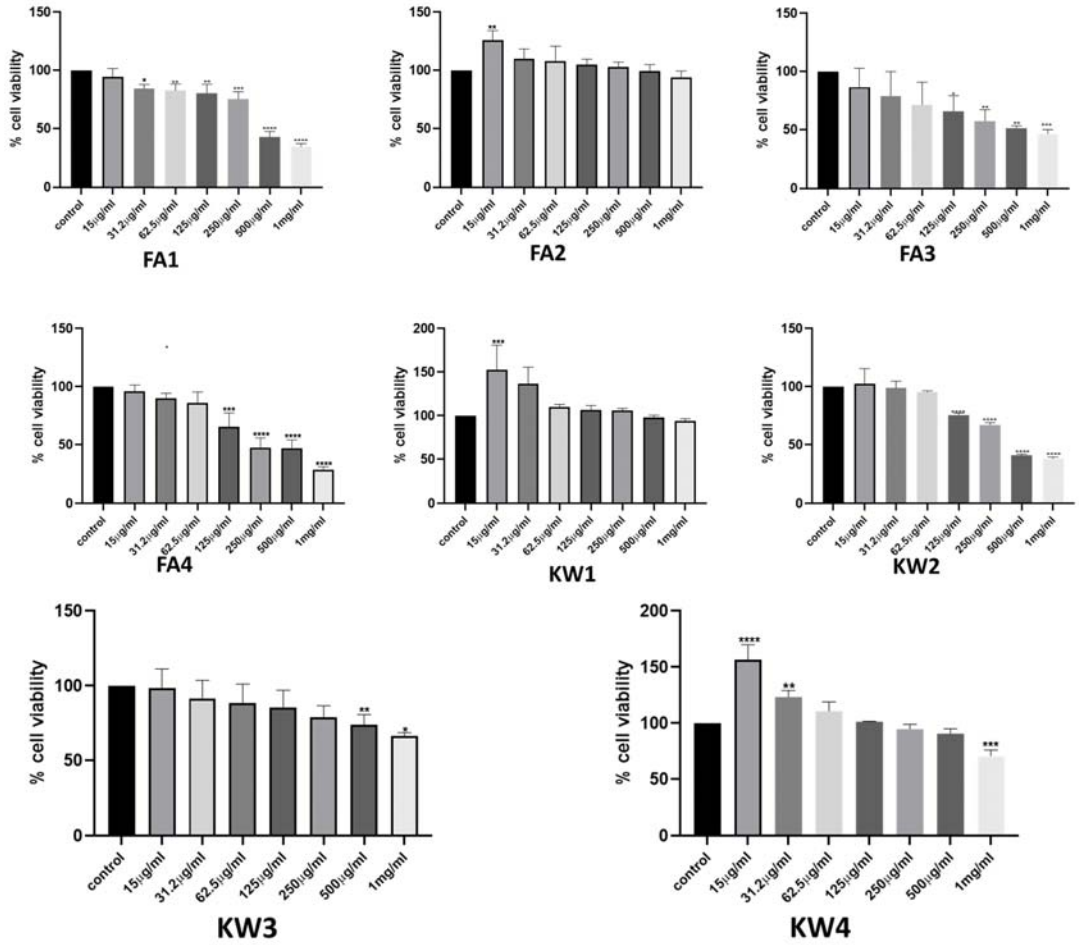
Cell viability assay:

To check the cytotoxicity of compounds, MTT assay was performed according to previously standardized protocol. $5x10^3$ cells/per well were seeded in 96 well plate with RPMI containing 10% FBS, 5% CO_2 and treated with different concentration of test compound. After completion of treatment 10µl of 5mg/ml MTT was added in each well of 96well plates and incubated at 37°C for 2 hours. After 2-hour incubation, media containing MTT solution was replaced with 100µl DMSO to dissolve formazan crystals. Absorbance was measured at 570 nm using ELISA plate reader.

RESULT:

We have assessed the therapeutic potential of plant extracts in breast cancer. In this study, our aim to study the anti-cancer activity of plant extracts in breast cancer cell. The results of cell viability assay of plant extracts are shown below:





Summary of studies:

Table showing IC_{50} of Plant extracts in MDA-MB-231 and MCF10A cells:

S.N.	Compounds name	IC_{50} in MDA-MB-231	IC_{50} in MCF-10A cells
1.	KA-1	**328** µg/ml	**2.2** mg /ml
2.	KA-2	>1 mg/ml	9 mg /ml
3.	KA-3	764 µg/ml	
4.	KA-4	>1 mg/ml	10 mg/ml
5.	FA-1	499 µg/ml	6mg/ml
6.	FA-2	>1 mg/ml	10 mg/ml
7.	FA-3	587.6µg/ml	

8.	FA-4	**325.3** µg/ml	
9.	KW1	>1 mg/ml	10 mg/ml
10.	KW2	415.5µg/ml	
11.	KW3	>1 mg/ml	
12.	KW4	>1 mg/ml	
13.	Vincitoxicum	>1 mg/ml	10 mg/ml
14.	C1	874 µg/ml	2.5 mg /ml

Potential Mechanisms of Action:

Given the unique nature of spagyric preparations, the anticancer mechanisms of Canceroso-1 may involve:

Synergistic Interactions: Combinations of plant extracts and minerals may exhibit enhanced therapeutic effects.

Modulation of Cellular Pathways: Canceroso-1 could target key signaling pathways involved in cancer cell proliferation, survival, and invasion.

Immunomodulation: The formulation might stimulate the immune system to recognize and attack cancer cells.

Conclusion:

After screening 12 distinct fractions of different Plant extracts, six fractions were found to be active in MDA-MB-231 cells and non-toxic in normal breast cancer (MCF-10A) cells. Pharmacopeial standardization and anticancer evaluation are essential steps in validating the therapeutic potential of Canceroso-1. By adhering to rigorous quality control measures and conducting comprehensive biological assessments, researchers can establish a strong scientific foundation for the clinical application of this spagyric-based electrohomeopathic medicine.

Note: This report provides a general framework for the evaluation of Canceroso-1. Specific experimental designs and methodologies may vary depending on the research objectives and available resources.

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