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Anti-Cancer Potential of *Soodha Valladhi Urundai* - A Siddha Herbo-Mineral Formulation

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ABSTRACT

Cancer is a complex disease marked by uncontrollable cell growth, evading the body's regulatory mechanisms [1]. It can affect tissues throughout the body, each type presenting unique features despite a common underlying process. Oral Squamous Cell Carcinoma (OSCC), poses a significant public health challenge in India, where it ranks as the most prevalent cancer[3]. OSCC typically manifests as non-healing ulcers and other symptoms in the oral cavity, often diagnosed at advanced stages, contributing to a low survival rate[4].Existing cancer therapies including surgery, chemotherapy, and radiotherapy may lead to severe side effects like mucositis and infections, irreversible damage to oral tissues complicating recovery and impacting oral health and nutrition.[4,5,6,7]The Siddha system of medicine, originating from Southern India, offers potential solutions such as Soodha Valladhi Urundai (SVU) [8], a herbal-mineral formulation with anti-cancer properties. .The present study aimed to validate the *In vitro* Anticancer activity using MTT Assay and Acridine orange (ao) and Ethidium Bromide (EtBr) Double staining method in oral KB cell lines. The MTT Assay showed 66.3% and 41.6% cell viability at 50 µg/ml and 100 µg/ml respectively and the LC50 value was 94 µg/mL. In the double staining method, almost all of the cells were observed as uniformly orange-stained cell nuclei which indicate necrotic changes and a few of the cells were bright green with condensed or fragmented chromatin, which indicates early apoptotic changes[20]. The obtained results allowed us to conclude that *Soodha Valladhi Urundai* possesses a remarkably positive impact on cancerous growths.

Introduction:

Cancer is the uncontrolled proliferation of cells that have outgrown the body's innate control mechanisms [1]. Unlike other diseases, cancer is rich in its versatile features as it can be inflammatory, genetic, metabolic or all at once[2]. Cancer can develop in any part of the body tissues but the feature of each cancer is unique, although the basic process involved is the same. Oral cancer, characterized by uncontrolled cellular proliferation with the invasion of adjacent tissues in the oropharyngeal cavity, presents a significant public health crisis in India. Oral Squamous Cell Carcinoma (OSCC) is the most common type of cancer in India resulting in a public health issue [3]. OSCC commonly manifests as a non-healing ulcer involving the tongue along with varying degrees of xerostomia, mucositis and Ulcers, lymphadenopathy, restricted mouth opening and fistula were also observed[4]. It ranks as the sixth most prevalent cancer globally. However, India bears a disproportionate burden, accounting for an estimated one-third of all cases worldwide. India faces a staggering annual incidence of approximately 77,000 new oral cancer diagnoses and 52,000 associated deaths, representing a quarter of the global burden. Unlike Western nations where early detection is more common, around 70% of Indian cases are diagnosed at advanced stages. This significantly diminishes the five-year survival rate, which remains concerningly low at around 20% in India[5]. Several factors contribute to the heightened risk of oral cancer in India. Established risk factors include tobacco use (both smoking and smokeless forms), excessive alcohol consumption, inadequate oral hygiene, nutritional deficiencies, and chronic viral infections. However, the practice of chewing paan stands as a major local risk factor in India [5]. Furthermore, periodontal disease, often associated with paan use, further elevates the risk of oral malignancy in this population.

Generally, Radiotherapy, Chemotherapy before or after surgical removal of malignant growth are the most widely incorporated treatment avenue. These modes of treatment often present with adverse reactions such as mucositis, infection, salivary gland taste dysfunction, and pain which can further lead to secondary complications such as dehydration, dysgeusia, and malnutrition[4]. Radiation of the head and neck can irreversibly injure oral mucosa and its vasculature resulting in xerostomia, trismus, and necrosis of soft tissues and bones[4].In many cases, surgical removal of cancerous growth is not possible owing to its site, metastasis[6].A study conducted in China among aged individuals after treatment of oral cancer revealed the development of drug-resistant opportunistic microorganisms in the oral cavity. It is also

suggested that bacterial colonization in the oral cavity increases the risk of infections such as pneumonia and bacteraemia[7].

Hence concerning comprehensive management of oral carcinoma, a drug which can control the growth of tumour cells, attenuate the nexus of symptoms, and help with pain management has to be employed. Siddha system of medicine, the homegrown system of medicine of Southern India has umpteen formulations that have strong anti-cancer potential. One of such formulations is *Soodha Valladhi Urundai (SVU)*. It is a classical herbo-mineral preparation consisting of *Serangottai (Semecardium anacarpus)*, Mercury and four other ingredients namely *Kurosani omam (Hyoscyamus niger), Thaneervittan Kizhangu (Asparagus racemosus), Nilapanai Kizhangu (Curculigo orchioides), Pooram (Hydrargyrum subchloride),* described in Agathiyar Valladhi 600[8]. It is indicated for Vaai *Soolai*(Painful oral conditions), *Kiranthi*(Adenitis), *Kuttam* (Chronic skin disorders), *Arayappu* (Venereal bubo/Inginal Bubo), *Soolai*(Painful conditions), *Powthiram*(Fistula), *Pilavai* (Carbuncle), *Puttru* (Cancer), *Vippuruthi* (Tumour), *Kandamaalai* (Cervical adenitis) and other *vatha* diseases. It is also indicated for the general improvement of health in the above-mentioned conditions. Therefore this study aims to evaluate the anti-cancer potential of *Soodha Valladhi Urundai* so that it can be carried over for human clinical trial and eventually into clinical practice for better management of all inflammatory conditions and malignancy.

MATERIALS AND METHODS:

Source of the Raw Drugs:

Kurosani omam *(Hyoscyamus niger)*, Thaneervittan Kizhangu *(Asparagus racemosus)*, Nilapanai Kizhangu (*Curculigo orchioides*), Soodham (Hydrargyrum), Pooram (Hydrargyrum subchloride) were procured from a reputed country drug store in Parrys Corner, Chennai.

Serangottai *(Semecarpus anacardium)* was procured from a country drug store in Nagercoil.

The herbal ingredients were identified and authenticated by the Botanist (NISIMB6042023), The metal drugs were identified by the Head of the Department, Department of Gunapadam. (Gun/Aut/11/23).

All the ingredients of the trial drug Soodha Valladhi Urundai mentioned here were purified as per the Siddha literature. Kurosani omam, Thanneervittan kizhangu, and Nilapanai kizhangu were pounded and made to a fine powder. Rasam was triturated along with Pooram to form a fine powder. Purified Serangottai was pounded one after the other along with the above-mentioned powders until it attained a waxy paste consistency. The paste was divided into 22 parts and made into round tablets. The tablets were dried in the shade and stored in a glass container.[7]

Cell line and culture:

KB (Human oral cancer) cell line was procured from the National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM. (Sigma-Aldrich, USA).

The cell line was grown in a 25-centimeter tissue culture flask with DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum), sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO 2 incubator (NBS Eppendorf, Germany).

The viability of cells was assessed by direct observation of cells by an Inverted Phase Contrast microscope and followed by the MTT assay method.[9]

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells was trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10 3 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO 2 incubator.

Preparation of compound stock:

1mg of sample was weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility.

The growth medium was removed after 24 hours, and the freshly prepared compound in DMEM were five times serially diluted by two-fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM) and each concentration of 100µl was added in triplicates to the

respective wells and incubated at 37ºC in a humidified 5% CO 2 incubator. Non-treated Control cells were also maintained.

Anticancer Assay by Direct Microscopic Observation:

After 24 hours of treatment, the entire plate was observed in an Inverted Phase Contrast Tissue Culture Microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Anticancer Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.[10]

After 24 hours of the incubation period, the sample content in wells was removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO 2 incubator for 4 hours.

After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 540 nm.[10]

The percentage of growth inhibition was calculated using the formula:

% of viability= Mean OD Samples x 100 / Mean OD of the control group

Determination of apoptosis by acridine orange (ao) and ethidium Bromide (EtBr) double staining.

DNA-binding dyes Acridine Orange (AO) and Ethidium Bromide (EtBr) (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells. AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into a double-stranded nucleic acid (DNA). EtBr is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA[11].

Procedure:

KB cell line was cultured as per standard procedures described earlier and treated with LC50 Value: 94.004862µg/mL from a stock of 1mg/ml was added and incubated for 24 hours.

Non-treated controls were also maintained and incubated for 24 hours. After incubation, the cells were washed with cold PBS and then stained with a mixture of AO (100 µg/ml) and EtBr (100 µg/ml) at room temperature for 10 minutes. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in a blue filter of a fluorescent microscope (Olympus CKX41 with Optika Pro5 camera).

The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange-stained cell nuclei).

Results:

Cytotoxicity Assay by MTT Assay on KB cell line:

Table No. 1: Cytotoxic effect of *Soodha Valladhi Urundai*

Sample Concentration (µg/ml)	**OD I**	**OD II**	**OD III**	**Average Absorbance @ 540nm**	**Percentage Viability**
CONTROL	0.5668	0.5608	0.5647	0.5641	100
Sample – Soodha Valladhi Urundai					
6.25	0.5484	0.5435	0.5438	0.5452	96.6559
12.5	0.4955	0.4954	0.4929	0.4946	87.6813
25	0.44	0.4429	0.4437	0.4422	78.3926
50	0.378	0.3726	0.3709	0.3738	66.2706
100	0.2347	0.2346	0.2354	0.2349	41.6423

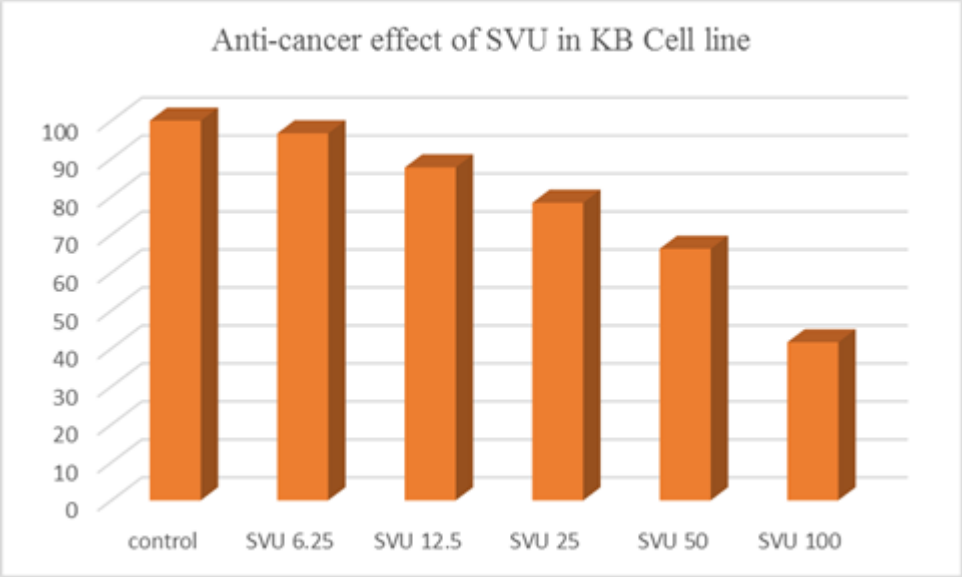
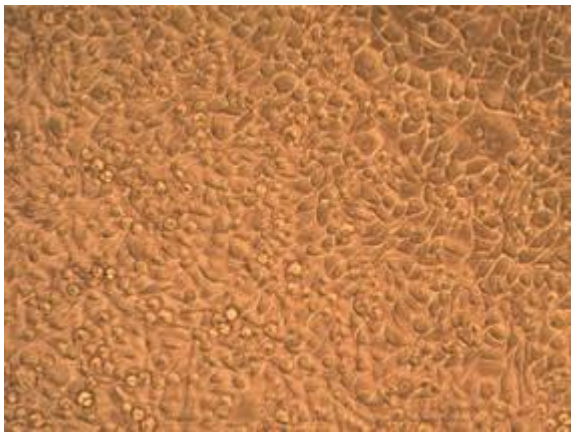


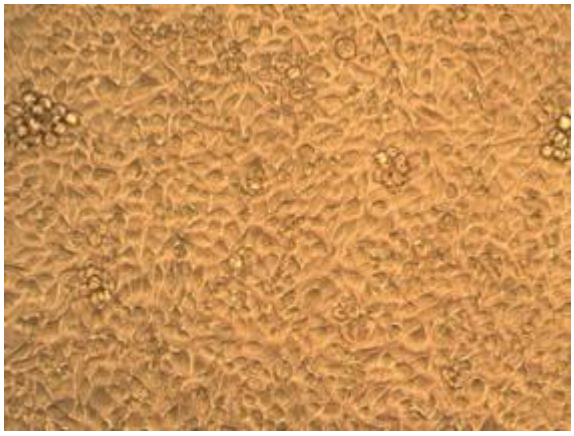
Figure no I : Graphical representation of Cytotoxic effect of *Soodha Valladhi Urundai* on KB cell line by MTT assay

Along the Y-axis Percentage viability, Along the X-axis varied concentrations of Soodha Valladhi Urundai. All experiments were done in triplicates and results were represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. ***p < 0.001 compared to control groups, **p < 0.01 compared to control groups

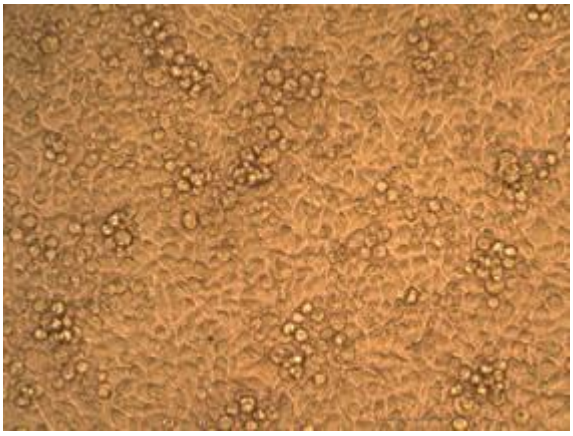
Control



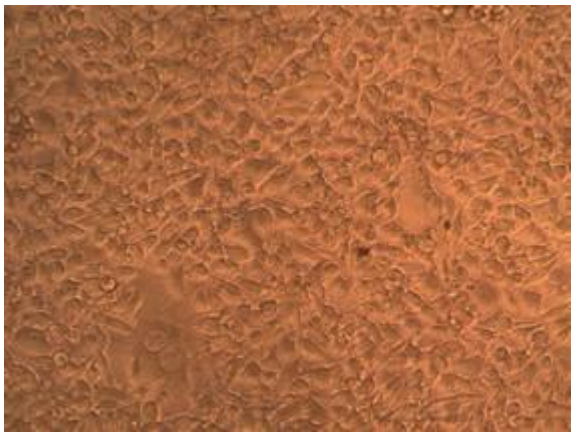
SVU Treated with 6.25 µg/mL



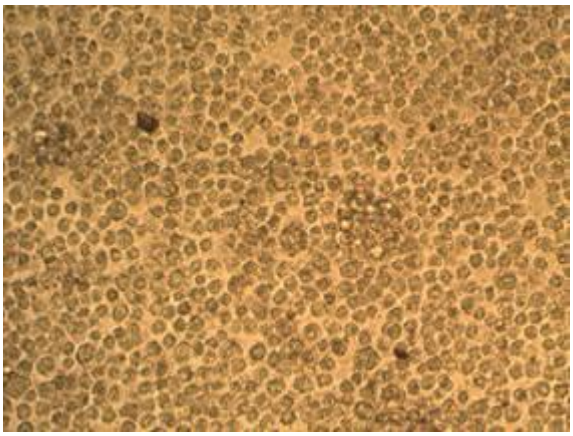
SVU Treated with 12.5 µg/mL



SVU Treated with 25 µg/mL



SVU Treated with 50 µg/mL



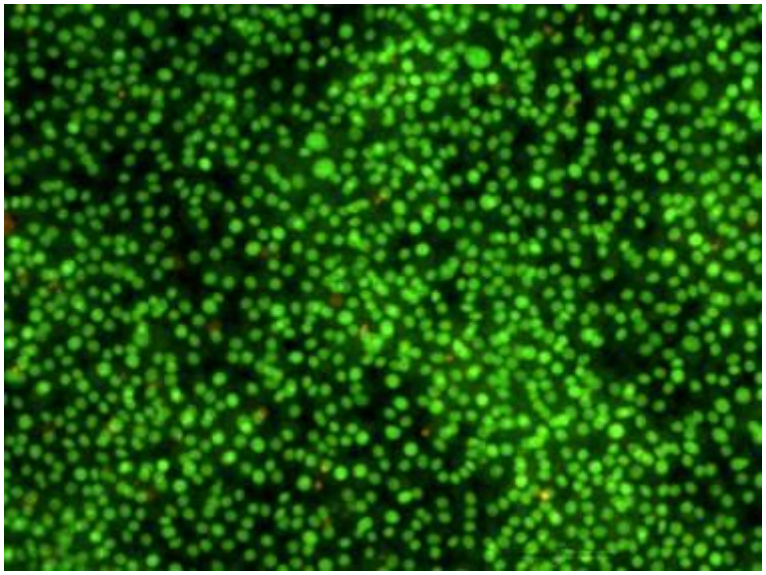
SVU Treated with 100 µg/mL

Figure II: Cancer cells treated with various concentrations of SVU

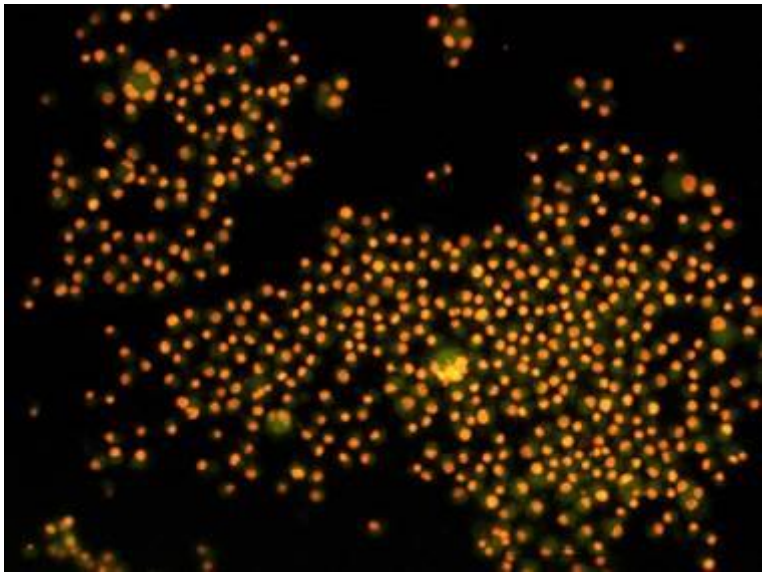
LC_{50} VALUE

LC_{50} Value (*Soodha Valladhi Urundai*) **-94.004862µg/mL** (Calculated using ED_{50} PLUS V1.0)

Figure III: Determination of Apoptosis by Acridine Orange(AO) and Ethidium Bromide(EtBr) Double Staining



Control



SVU treated at 94 µg/mL

Discussion:

All the constituents of the formulation were previously investigated to possess promising anti-cancer activity. The main components namely Serangottai (*Semearpus anacardium*) and Mercury are the herbal and mineral archetypes respectively in cancer management in the Siddha system of medicine. *Semecarpus anacardium* (SA) has been extensively studied for its inhibitory effect on various cancer cell lines including the human breast cancer cell line (T47D)[12], the human epidermoid larynx carcinoma cell line (Hep 2)[13], breast cancer cell lines, MCF-7 (estrogen receptor-positive) and MDA 231 (estrogen receptor-negative)[14].

The Alkaloidal extract of Hyoscyamus niger, was shown to possess cytotoxic activity, reduced the spontaneous frequency of chromosomal aberrations, micronuclei assay, and increased the mitotic index in mice bone marrow cells. The extract also exhibited cytotoxic activity against the cancer cell line Hep-2 in all periods. It was found to possess a moderate cytotoxic effect on Rhabdomyosarcoma (RD cell line) and murine mammary adenocarcinoma cell line (AMN-3) and no effect on normal cell lines[15].

The anticancer activity of shatavarins (containing shatavarin IV) isolated from the roots of *Asparagus racemosus* (Wild) has been evaluated using *in vitro* and *in vivo* experimental models. Oral administration of Shatavarins (AR-2B) to Ehrlich ascites carcinoma (EAC) tumour-bearing mice at doses of 250 and 500 mg/kg body weight for 10 days, showed a significant reduction in tumour volume, viable tumour cell count, and increased non-viable-cell count when compared to the untreated mice of the EAC control group. The haematological parameters were restored to normalcy suggesting strong anti-cancer activity[16]. The *in vitro* cytotoxicity of synthetic nanoparticles using Curculigo orchioides rhizome extracts (CoREs) was studied which showed that the inhibitory concentration of the nanoparticles was 18.86 and42.43 μg mL−142.43 μg mL−1for human breast cancer cell line (MDA-MB-231) and Vero cells line, respectively, after 48 h of incubation suggesting that the synthesized metallic silver nanoparticles could possess potential anticancer activity[17]. A Mercury (II) complex based on quinoxaline–amino antipyrine was evaluated for its anticancer activities. The synthesized compounds were tested against MCF-7 and HT-29 cancer cells using a cell viability assay that measures the ability of cells to grow and divide. The results showed that the Hg(II) complex had higher activity than the related free ligand in killing cancer cells after 48 hours[18]. The anti-cancer effect of Rasa Karpoora Kuligai containing Pooram, one of the ingredients in Soodha Valladhi urundai on HeLa cell lines showed significant inhibition of the proliferation of human cervical cancer HeLa cells[19].

In order to determine the cytotoxic effect of the novel Siddha formulation *Soodha Valladhi Urundai* against Oral KB cells. The experiment was screened at different concentrations to determine the LC50 using an MTT assay. A chart was plotted using the % cell viability on the Y-axis and the concentration of the test sample on the X-axis. The percentage of growth inhibition was found to be increasing with increasing concentrations of the test drug. The LC50 of the test sample in the Oral KB cell line was found to be 94 μg/mL. This confirms that the trial drug *Soodha Valladhi Urundai* has a promising anti-cancerous effect. SVU at

different doses (6.5-100 µg/ml of 5% MEM) was administered for 24 hrs. Results show that the number of cells decreases as the dose increases and at approximately 94µg/ml dose of extract, 50% of the cells (KB cells) were less as compared to normal control. The percentage of cell viability was determined by calculating the O.D. of the treated against the control. Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 540nm. Comparison values are made based on 50% inhibition of growth (IC50) in treated cells with specific agents. Morphological changes such as changes to the cell membrane, loss of membrane asymmetry and cell shrinkage, which are the early stages of apoptosis are analyzed by the Double staining method with Acridine Orange(AO) and Ethidium Bromide(EtBr).

Apoptosis or programmed cell death of the abnormally proliferating cells and restoring the usual metabolism of the cells is the main target in the treatment of cancer. Cancer cells often develop mechanisms to evade apoptosis, which contributes to their uncontrolled growth and survival[20]. The LC dose (94 µg/ml) treated cancer cells show features of apoptosis whereas treated with the same amount of dose, normally treated cells appeared without any significant changes. As shown in Fig no. II Almost all of the cells were observed as uniformly orange-stained cell nuclei which indicates necrotic changes and a few of the cells were bright green with condensed or fragmented chromatin, which indicates early apoptotic changes[20].

Hence from the previously established anti-cancer potential of the constituents of SUV and the findings of the present study, it can be suggested that *Soodha Valladhi Urundai* (SVU) has remarkable medicinal value in treating oral cancer.

Conclusion:

Earlier studies indicated that each component of the test drug *Soodha Valladhi Urundai* is a potential source in curtailing cancer growth. The current study is an initial effort to unveil the anti-cancerous potential of a supreme herbo mineral Siddha formulation. The results show that the anticancer activity as measured by the MTT method with an LC50 value of 94 µg/mL and early apoptotic changes with minimal effects on normal cells. Hence Soodha Valladhi Urundai has been shown to possess promising anti-cancerous effects that shall be studied further for its safety profile and eventually make it a part of cancer therapy.

REFERENCES:

1. Krieghoff-Henning E, Folkerts J, Penzkofer A, Weg-Remers S. Cancer – an overview. Med Monatsschr Pharm. 2017 Feb;40(2):48–54.
2. Gyamfi J, Kim J, Choi J. Cancer as a Metabolic Disorder. Int J Mol Sci. 2022 Jan 21;23(3):1155.
3. PIRES FR, RAMOS AB, de OLIVEIRA JBC, TAVARES AS, da LUZ PSR, dos SANTOS TCRB. Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single Oral Pathology service during an 8-year period. J Appl Oral Sci. 2013;21(5):460–7.
4. Minhas S, Kashif M, Altaf W, Afzal N, Nagi AH. Concomitant-chemoradiotherapy-associated oral lesions in patients with oral squamous-cell carcinoma. Cancer Biol Med. 2017 May;14(2):176–82.
5. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sens Int. 2020;1:100046.
6. Tohme S, Simmons RL, Tsung A. Surgery for Cancer: A Trigger for Metastases. Cancer Res. 2017 Apr 1;77(7):1548–52.
7. PAN J, ZHAO J, JIANG N. Oral cavity infection: an adverse effect after the treatment of oral cancer in aged individuals. J Appl Oral Sci. 2014;22(4):261–7.
8. Agathiyar. Agathiyar Vaithiya Valladhi 600. 1st ed. Central Concil for Research in Ayrveda and Siddha; 1980. 3–6 p.
9. (PDF) Rosmarinic Acid-Rich Fraction from Mentha arvensis Synchronizes Bcl/Bax Expression and Induces Go/G1 Arrest in Hepatocarcinoma Cells [Internet]. [cited 2024 Jul 13]. Available from: https://www.researchgate.net/publication/334473155_Rosmarinic_Acid-Rich_Fraction_from_Mentha_arvensis_Synchronizes_BclBax_Expression_and_Induces_GoG1_Arrest_in_Hepatocarcinoma_Cells
10. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec;65(1–2):55–63.
11. Talarico LB, Zibetti RGM, Faria PCS, Scolaro LA, Duarte MER, Noseda MD, et al. Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds Gymnogongrus griffithsiae and Cryptonemia crenulata. Int J Biol Macromol. 2004 Apr;34(1–2):63–71.
12. Mathivadhani P, Shanthi P, Sachdanandam P. Apoptotic effect of Semecarpus anacardium nut extract on T47D breast cancer cell line. Cell Biol Int. 2007 Oct;31(10):1198–206.
13. (PDF) In Vitro Cytotoxicity Activity of Semecarpus anacardium Extract Against Hep 2 Cell Line and Vero Cell Line [Internet]. [cited 2024 Jul 13]. Available from: https://www.researchgate.net/publication/242364569_In_Vitro_Cytotoxicity_Activity_of_Semecarpus_anacardium_Extract_Against_Hep_2_Cell_Line_and_Vero_Cell_Line
14. Sowmyalakshmi S, Nur-E-Alam M, Akbarsha MA, Thirugnanam S, Rohr J, Chendil D. Investigation on Semecarpus Lehyam--a Siddha medicine for breast cancer. Planta. 2005 Apr;220(6):910–8.
15. Al-Snafi A. Therapeutic importance of Hyoscyamus species grown in Iraq (Hyoscyamus albus, Hyoscyamus niger and Hyoscyamus reticulates)-A review. 2018 Jun 1;8.
16. Mitra SK, Prakash NS, Sundaram R. Shatavarins (containing Shatavarin IV) with anticancer activity from the roots of Asparagus racemosus. Indian J Pharmacol. 2012;44(6):732–6.
17. Kayalvizhi T, Ravikumar S, Venkatachalam P. Green Synthesis of Metallic Silver Nanoparticles Using Curculigo orchioides Rhizome Extracts and Evaluation of Its Antibacterial, Larvicidal, and Anticancer Activity. J Environ Eng. 2016 Sep;142(9):C4016002.
18. Alyaninezhad Z, Bekhradnia A, Gorji RZ, Arshadi S, ahmadi S, Gorji MZ. Mercury (II) complex based on quinoxaline–aminoantipyrine: Synthesis, crystal structure, computational studies and anticancer activities evaluation. J Mol Struct. 2023 Mar 5;1275:134607.
19. Karthikraja S. Pre-Clinical Study of Herbo Mineral Formulation Rasa Karpoora Kuligai for its Anti-Cancer, Anti-Oxidant and Anti-Inflammatory Activities. The Tamilnadu Dr.MGR Medical University;
20. Jan R, Chaudhry G e S. Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. Adv Pharm Bull. 2019 Jun;9(2):205–18.