Scientific Analysis of Classical Siddha formulation Sanda Rasa Parpam for Breast Cancer- A Review

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ABSTRACT
Cancer is a deadly disease that threatens mankind and the number of people dying from cancer has increased recent times. Most women are affected by breast cancer in general, also a person's cancer can mean a huge economic, emotional and very distressing situation not only for the individual but also for the entire family. Typical modern treatments currently used for Cancer have some adverse effects. Therefore, there is a need to search for alternative drugs from natural derivatives that are more potent but have less side effects. Siddha medicine, traditional system of healing that originated in south India and is considered to be one of India's oldest systems of medicine. Siddha medicine deals extensively with cancer and its treatment. In the ancient Siddha literature, the name putru is interpreted as Arpudham, vanmeegam and vippuruthui. This review paper aim to investigate the pharmacological analysis of Sanda Rasa Parpam (SRP), a classical Siddha formulation mentioned for Cancer.

1. Introduction

Cancer is a major life threatening disease. It is also said to be a lifestyle disorder which is knowingly or unknowingly incorporated in our day to day activities. Cancer is the second leading cause of mortality. In India, Tumor is the third cause of mortality. There are more than 100 types of cancers. Researchers defined cancer as a disease characterized by uncontrolled or unregulated proliferation of cells with the potential to invade or spread to other parts through blood and lymphatic vessels [1] Breast cancer is one of the most common cancers that affects women. Breast cancer typically affects women age 50 and older. Traditional siddha medicine explained about various types of cancer in the name of Putru, vippuruthi, pilavai, odu-pilavai, thurmangisam, katti kandamalai and few other traditional tamil names. Based on the onset of cancer in various parts of the body, Siddhars has coined its name like yoni putru (vaginal cancer), kazhundhu putru (Cervical cancer) nagir putru (Breast cancer), linga putru (penile cancer) [2]

Types of Breast Cancer [3]

According to the site,

- Non- invasive Breast cancer
- Invasive breast cancer

Frequently occurring breast cancer

- Lobular carcinoma in situ
- Ductal carcinoma in situ
- Infiltrating ductal carcinoma

Less commonly occurring Breast Cancer

- Medullary carcinoma
- Mutinous carcinoma
- Tubular carcinoma
- Inflammatory breast carcinoma
- Paget's diseases of the nipple
- Phylloides tumor

2. Prevalence

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths.[4] The most common cancers are breast, lung, colon and rectum and prostate cancers.

- Breast (2.26 million cases);
- lung (2.21 million cases);
- colon and rectum (1.93 million cases);
- prostate (1.41 million cases);
- skin (non-melanoma) (1.20 million cases); and
- Stomach (1.09 million cases).

The most common causes of cancer death in 2020 were:

- lung (1.80 million deaths);
- colon and rectum (916 000 deaths);
- liver (830 000 deaths);
- stomach (769 000 deaths); and
- Breast (685 000 deaths).

Each year, approximately 400 000 children develop cancer. The most common cancers vary between countries. Cervical cancer is the most common in 23 countries.

In 2020, there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life.[5]

In India, the incidence of cancer cases is likely to increase from 1.46 million in 2022 to 1.57 million in 2025. The national average for the year 2022 of crude rate of incidence per 100,000 is 100.4; for males, 95.6 and females, 105.4. Lung and breast cancers in males and females, respectively, remain to be the leading sites of cancer [6]

With being the most common type of cancer in women, breast cancer accounts for 14% of cancers in Indian women. It is reported that with every four minutes, an Indian woman is diagnosed with breast cancer. Breast cancer is on the rise, both in rural and urban India. A 2018 report of Breast Cancer statistics recorded 1,62,468 new registered cases and 87,090 reported deaths.

Cancer survival becomes more difficult in higher stages of its growth, and more than 50% of Indian women suffer from stage 3 and 4 of breast cancer. Post cancer survival for women with breast cancer was reported 60% for Indian women, as compared to 80% in the U.S.[7]

Ingredients of Sanda Rasa Parpam:

- Purified *Lingam* (Cinnabar)

For distillation of dravagam:

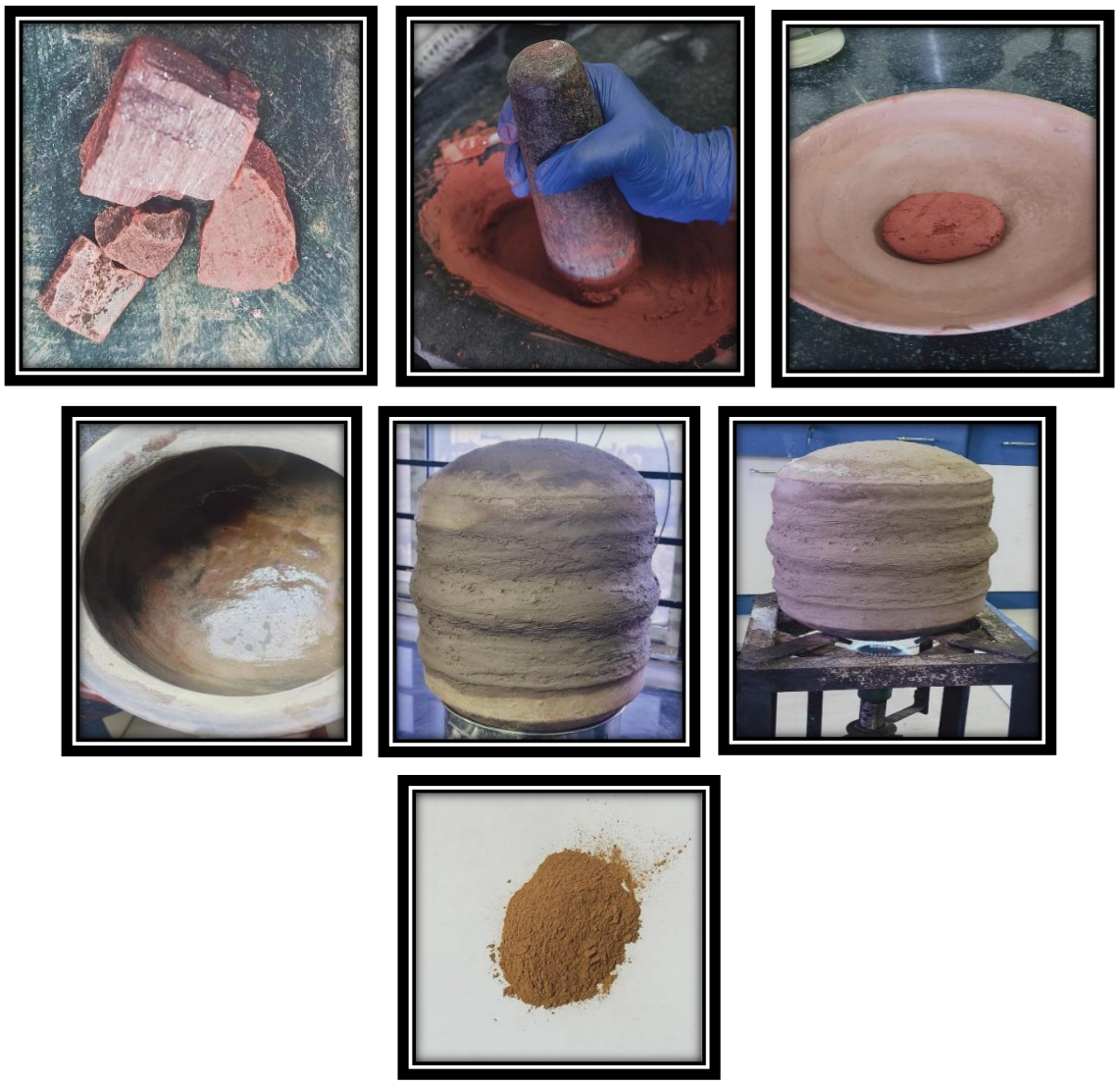
- Purified *Vediyuppu*
- Purified *Savakkaram*

Purified *Vediyuppu* – 100 *palams* and Purified *Savarkkaram* – 50 *palams* to be taken and rubbed finely putting them in a kalvam. In a distillation apparatus, the Mixture is to be placed and dravakam to be distilled.

Process of Distillation

The method of preparation of the drug is mentioned below [8]:

Purified Lingam (Cinnabar) – 4 palams has to be taken and triturated rubbing with the Dravakam for three hours, made into a small round cake and dried. The cake has to be placed in an agal (earthern plate), another plate smeared with Murukkampoo charu in the inner layer and dried has to be placed over and closed using seven layers of cloth cover smeared with wet clay and dried. This closed Agals are to be placed on an oven and heated for three hours. Opened after cooling down, the sediment and sublimation to be collected. Both are to be rubbed again using Dravakam for three hours and the whole process has to be repeated for a total of seven times. The final white sublimation (Sanda Rasa Parpam) has to be collected and stored in a suitable container.



Dosage: 488 mg.

Adjuvant: Palm jaggery

Indication: Vadha diseases, Pakka soolai, Syphilis, Cancer, Leprosy, Pricking Pain, Eczema, Myocardial infarction.

Siddha Aspects

Lingam

Synonyms: Natural cinnabar, Vermilion [9]

Chemical name: Red sulphide of mercury.

Other names: Inkuligam, Raasam, Kadai vanni, Karpam, Kalikkam, Kaanjanam, Kaaranam, Sandagam, Samarasam, Saaniyam, Chendooram, Maniragam, Milechem, Vaniand Vanni.

Gunam (General Properties): It is hard, when it is put into fire it becomes smoke; not soluble in water, has no smell and taste and has hot potency.

Action: Tonic

பேதிசுரஞ் சந்நி பெருவிரண நீரோடுத
காதகடி காசங் கரப்பான் புண்- ணோத
வுருவிலிங்க சங்கதமா யூறுகட்டி யும்பொங்
குருவிலிங்க சங்கமத்தைக் கொள்

It is effective in the treatment of diarrhea, pyrexia, delirium, urticaria, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain and vatha-diseases and it is also cures hidden diseases of the body.

Method of Purification:

- Algangium bark (Alangium savlifolium) – 1400gm is powdered and added with vinegar 5.2 liter and placed in dews in the night.
- The next day it is rubbed and kindled well. 35gm. Of cinnabar is tied well in a cloth and put into the above liquid.
- The pot is covered with another pot concealed with cloth over which mud get gummed, after which it is dried and exposed in dew for one day.
- It is heated with low intensity fire (flame) until the liquid is dehydrated for 24 hours. Then the cinnabar is taken out and cleaned well.
- This procedure is repeated using the vinegar soaked individually with the whole plant of Vitis lanata (puli karunai) and Indian sarasaparilla root.

Modern Aspect of Cinnabar [10]

Cinnabar (Red Mercury (II) Sulfide (HgS), vermilion) is the ordinary ore of Hg. It is normally found in a substantial, granular form and is bright scarlet to brick-red in color. It is a chemical compound composed of the chemical elements Mercury and Sulphur (Mercury 86.22 % Sulphur 13.78 %).

- Formula - Mercury (II) sulfide
- Symbol - HgS Properties: Molecular formula – HgS
- Number - 32 Color - brownish red and lead-gray
- Specific gravity - 8.176
- Solubility - Soluble in water,
- Molecular Weight - 232.66 gm
- Melting point - 580 °C decomp.
- Other anions - Mercuryoxide, Mercury selenide, Mercury telluride
- Other cation’s - Zinc sulphide, Cadmium sulphide
- Fermion Index - 0.26
- Boson Index - 0.74
- Radioactivity - 0GRapi i.e. not radioactive (Gamma Ray American Petroleum Institute Units)

Uses of cinnabar [11]

- It is used as a single remedy or as an ingredient in various herbo -mineral preparations in Ayurveda and siddha. It also has cell rejuvenating, anti-aging, and digestion strengthening properties.
- One of the major health benefits of Cinnabar in Ayurveda is its ability to treat various skin ailments. Skin problems are mainly caused due to the imbalance of pitta dosha. It is highly beneficial for skin and skin-related problems.
- Cinnabar is a powerful ingredient used to improve digestion. It brings immunity to the digestive system and strengthens the whole naturally. It also fights nausea. Cinnabar is cooling and soothing to the human body.
- Consuming very small doses of *Linga chenduram* helps bring down high body temperature during fever. It also works against microbial infection.
- Cinnabar has immunity-boosting properties that help build a stronger immune system. Consuming Cinnabar with long pepper powder flushes out the toxins out of your body and thus makes your immunity system strong and healthy.
- According to Indian medicine, Cinnabar is one of the powerful medicines to treat liver and spleen diseases. Cinnabar's hepato-protective properties protect the liver from damages and improves liver strength. It is used to treat arthritis and jaundice.
- Although Cinnabar is a metallic ingredient, Indian system of medicine has found a useful way to purify it and use it for the benefit of mankind. So, these are the powerful health benefits of cinnabar

Vediyuppu (Potassium Nitrate) [9]

Other names:

Pottiluppu, Inangan, Padairasan, Bhoomi koormai, Navachara mithru

General characteristics of Vediuppu:

மல்லாரு மட்டகுன்ம மாதருத ரக்கட்டி

கல்லா மதைப்புநீர்க் கட்டருக- லெல்லமே

கம்பிகம்பி யென்றுங் கருவுண்டா மங்கிநின்ற

கம்பிகம்பி யென்றுரைக்குங் கால்

It cures diseases like 8 types of Gunmam (ulcer), Sobai (dropsy), Karpashayakatti (uterine tumour), neersurukku (dysuria), and moothira kiricharam (burning micturition).

Action:

Refrigerant, Demulcent, Astringent, Diuretic

Savakkaram (Fuller' S Earth)

Impure Sodium Carbonate

Other names:

Pooneeru[12] poovazhalai, uzha man

General properties

பார்த்திட்ட பூநீற்றின் பருவங்ளு
பங்குனியுஞ் சித்திரைவை காசிகுள்ளே
பூர்திட்ட ரவிசுருக்கிற் பொங்கிநீறும்
பூப்போன்மே னிற்குமதை வாரிக்கொள்ளு[2]

Uses:

- Pooneeru and limestone are added in equal ratio and obtained clear water solution. The solution is used to purify the tortoise shell. Egg shell pearl oyster, asbestos, fossil of crab, conch shell. The above materials are individually kept with the above said solution and boiled to get purified form. Arsenic compound may be purified with this solution.
- Pooneeru is mixed with the hot water. For curing, arthritis in the ankle joint and the foot is kept in the above solution for sometimes.

Preclinical Studies of Anticancer, Anti-Tumor and Cytotoxicity Of Lingam Containing Siddha Formulations

Linga chenduram

The growth inhibitory nature of *Linga chenduram* against the HeLa cell lines with different concentrations was studied. When the medicine increased in its concentration, there was an increase in cell growth inhibition. The 50% 0f inhibitory concentration (IC50) of drug value was obtained at 147.573389µg/ml (Calculated using ED50 PLUS V1.0 Software). With this concentration it inhibits cell growth effectively [13]

Veera mezhugu

In vitro cytotoxicity study employing MTT assay was performed as per standard textual procedures [12]. Ehrlich ascites carcinoma (EAC) cells were cultured in RPMI-1640 medium supplemented with 2nM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin, maintained at 37ºC in an atmosphere of 95% O2 and 5% CO2. In each assay, 0.1X106 EAC cells were harvested in culture medium and plated in 96-well flat bottom culture plates and incubated at 37ºC for 24 h in humidified 5% CO2. After 24 hours, 10 µL aliquots of serial dilutions of plant extract (1000-1.95 µg/ml) in DMSO were added to EAC cells and incubated

for 48 h. Cell viability was assessed through the MTT assay. The cytotoxicity of *Veera Mezhugu* was evaluated using in vitro MTT assay on EAC cell lines. Various concentrations of test drug (VM) ranging from 7.8 to 1000 µg/ml were reacted with EAC and the cytotoxicity was assessed. In the present study, 74.05 % cytotoxicity was observed at 1000µg/ml concentration of the test drug *Veera Mezhugu* [14].

Panchamuga chenduram-

In vitro studies showed that after 24 hrs and 48 hrs of incubation, the IC 50 values of PMC were found of 65.03 ± 0.05 µg/ml, 70.51±0.01 µg/ml in compared with the standard drug taxol 72±2.4, 75±3.2 µg/ml. Thus the Siddha formulation PMC shows a potent anticancer effect in MCF7 cell line determined by MTT assay [15]

Pancha paasana chenduram

Human breast cancer MCF-7 cells were procured from National Centre for Cell Science, Pune, India. Growth inhibition of MCF-7 cells by Pancha paasana chenduram (PPC) was determined by MTT assay. Cytotoxicity assay was used to determine the IC_{50} concentration of drugs or chemicals. MCF-7 cells were maintained as a monolayer culture in DMEM, supplemented with 10% FBS, in a humidified atmosphere at 37°C and 5% CO_2. Cells were seeded at a density of 8000/well in 96-well micro plate and treated with PPC (10–320 µg/mL) for 20 h. After drug exposure, 5 µL of MTT (5 mg/mL) dye was added to wells and wrapped with aluminum foil and incubated for 4 h. After incubation, medium was removed cautiously and 200 µL of DMSO was added to each well to solubilize the formazan crystals; it was then placed still in the dark for 15 min. MTT reduction was quantified by measuring the absorbance at 570 and 630 nm in enzyme-linked immunosorbent assay (ELISA) Plate reader (Bio-Rad Laboratories, Inc., Berkeley, California, United States). Each experiment was repeated at least three times.[16]

Asta bairava chooranam

Ashta Bairava Chenduram at different doses (6.25-100 µg in100 µl of 5% MEM) was administered for 24 hrs. It was found that the number of cells decreases as the dose increases and at approximately 50 µg/ml dose of extract, 50% of the cells (KB cells) were less as compared to normal control as shown in figure(15). The percentage of cells viability was determined by calculating the O.D of treated against the control. Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 540 nm. Comparison values are made on a basis of 50% inhibition of growth (IC50) in treated cells with specific agents [17].

Kandha Rasa villai

To determine the number of cells undergoing apoptosis upon Kandha Rasa villai treatment, HeLa, SiHa and C33A were plated at a seeding density of 5x105 cells/well in 6-well plates and allowed to grow overnight at 370 C inCO2 incubator. Next day, the cells were treated with various concentrations of Kandha rasa villai (0–80 mg/ml) and incubated for 24 h. Cells were stained with Annexin V-FITC according to manufacturer‟s instructions. A total of 10,000 events were acquired and dual parameter dot plot of FL2-H(X-axis; PI fluorescence, linear scale) versus FL1-H (Y-axis; Annexin V-FITC-fluorescence, linear scale) was recorded. The data was analyzed using the FACS Calibur Cell Quest software.[18]

Namachivaya chendururam

The HeLa cell line was cultured in 25 cm2 tissue culture flask with DMEM supplemented with 10% FBS L-glutamine, sodium bicarbonate 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% CO2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT (4,5-dimethylthiazol-2-yl) assay method.[19]

Putru pathangam

Cell viability assay

The effect of the test sample was analyzed using Hek 293 (embryonic kidney-derived) and MCF-7 (breast cancer) cell lines, which are procured from National Center for Cell Science (NCCS), Pune, India. The culture was maintained in DMEM Medium supplemented with 10% FBS. The sample stock solution was prepared by dissolving 1mg/ml. The MTT assay was performed with the seven different concentrations from 10 µg/ml to 70 µg/ml along with control. The cell viability was done in triplicates and analyzed the effective concentration by spectrophotometer at 545nm [20]

Cellular apoptosis was determined using the AnnexinV-FITC Apoptosis Detection Kit I (Clontech Laboratories Inc, USA) according to the manufacturer's protocol. OSCC and HOS cell lines were cultured at 6 × 10cells/ml and seeded in 60 mm dish. The cells were treated with free medium containing various concentrations of ABC for 6, 12 and 24 hour. Cells were harvested by trypsinization, then washed twice with cold PBS and centrifuged at 1000 rpm. About 1 × 105 -1×106 cells were then re suspended in 400 µl1×binding buffer, centrifuged again at 1000 rpm for 5 minutes and then supernatant was removed. Cells were re-suspended in 200 µl 1× binding buffer and transferred to a sterile flow cytometry glass tube. Five µl Annexin V-FITC and 10 µl propidium iodide were added and then incubated in the dark at room temperature. Cells were analyzed by flow cytometer at 488 nm. The distribution of cells was analyzed using Cell Quest software (Becton-Dickinson) in the flow cytometer within 1 hour of staining. Data from 10,000 cells was collected for each data file. Apoptotic cells were identified as Annexin V-FITC-positive and P-negative cells[21]

3. Disscusion

In siddha system, commonly used anti-cancer drugs are listed in Siddha formulatory of India are, Rasagandhi mezhugu, Chitramoola kuligai, Sivanar amirtham, Nandhi mezhugu, Poorna Chandrodayam, Mahavallathi Ilagam, Kandhaga Rasayanam. Of these some drugs specially treating for specific cancers, chitramoola kuligai is specifically using for uterine cancer. Some of the lingam formulations with anti-cancer properties like pancha muga chenduram, pancha pasana chenduram, putru pathankam etc., have been demonstrated in MCF-7 cell lines for breast cancer. So lingam has the ability to act on the breast cancer cells and prevent proliferating of the cells.

4. Conclusion

This review shown that the Lingam (Cinnabar) the main ingredient of Sanda rasa parpam has anti-cancer, anti-tumor, analgesic and antioxidant activities to varying degrees. The anti cancer effects and pharmacological activities of Sanda Rasa Parpam (SRP) mentioned in siddha literature and its scientific interpretations have proved its importance in cancer treatment, so it needs collaborative research and clinical trials to overcome the present day challenge of breast cancer.

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