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Anti-cancer potential of siddha herbo mineral formulation parangi rasayanam against hela cell lines: A *In vitro study*

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Abstract
Siddha system of medicine is a system blended with Tamil culture. In Siddha system, there are many higher order herbo mineral/ herbo metallic formulations which are exclusively indicated for the management of Non – communicable diseases like Diabetes, Hypertension, Cancer, etc.., Among this, Cancer is considered to be one of the leading cause of morbidity and mortality worldwide with a mortality rate of 9.6 million in 2018. Of which, Cervical cancer is found to be one of the common types of cancer in women representing 6.6% of all female cancers. Though many modern therapies like surgery, radiation therapy, chemotherapy and targeted therapy, palliative care, stem cell therapy, adjuvant therapy are available they contain certain risks and side effects. Hence, the use of herbal medicines in cancer treatment has received increasing attention because of their easy availability, low cost and minimal side effects. Their therapeutic efficacy is attributed due to their various phyto-metabolic contents with multiple biological activities. *Parangi Rasayanam* (PRM) is one such herbo mineral siddha formulation which is indicated for Penkuri putru (Cervical cancer); *Aankuri putru* (Penile cancer); *Megam* (Veneral diseases); *Parangipun* (Veneral sore); *Kandamalai* (Nodular swelling of neck) and *Kiranthi* (Nodules). Hence, the study is aimed to evaluate the anti-cancer effect of *Parangi Rasayanam* in HeLa Cell Lines using MTT Assay. The result of the study reveals that it has 49.53 % of cell viability and 50.47% of cell death at a concentration of 200 microgram/ml with IC_{50} value as 183.7. This anti-proliferation may be due to the presence of Kaempferol (Flavonoid) in Smilax china which inhibits cell multiplication through induction of G2/M phase growth arrest. Hence, this study concludes that *Parangi Rasayanam* possess anti-cancer potential and further efficacious pharmacological and clinical studies have to be conducted for the welfare of cancer patients in the management of cervical cancer.

Keywords: *Parangi Rasayanam*, hela cell lines; siddha medicine; cervical cancer; MTT assay

Introduction
SIDDHA – the science of life evolved as a comprehensive system of healthcare systematically through scientific experiments of high order backed by sound and reproducible evidence based and stood the test of time. Siddha system is one among the traditional systems of medicine indigenous to our country. [1] This system was nursed by eminent Siddhars supposed to have lived at a very early period. They were men of highly cultured intellectuals and spiritual faculties combined with super powers. They used herbs, metals, minerals and marine products for making medicines to treat various diseases. They listed as many as 4448 diseases with medicines. [2] *Putru noi* is one among the diseases which is correlated with cancer in modern science. According to WHO, Cancer is a large group of diseases that can start in almost any organ or tissue of the body when abnormal cells grow uncontrollably, go beyond their usual boundaries to invade adjoining parts of the body and/or spread to other organs. The latter process is called metastasizing and is a major cause of death from cancer. A neoplasm and malignant tumour are other common names for cancer. Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women.

Cervical cancer is the fourth most common cancer in women. In 2018, an estimated 5, 70, 000 women were diagnosed with Cervical cancer worldwide and about 3, 11, 000 women died from the disease. When diagnosed, cervical cancer is one of the most successfully treatable forms of cancer, as long as it is detected early and managed effectively. Cancers diagnosed in late stages can also be controlled with appropriate treatment and palliative care. With a comprehensive approach to prevent, screen and treat, cervical cancer can be eliminated as a public health problem within a generation. [4]

Hence *Parangi Rasayanam* (PRM), a herbo mineral Siddha formulation which has been indicated for *Penkuri Putru* (Cervical cancer) has been studied for its anti-cancer effect in HeLa cell lines using MTT Assay.

Materials and Methods
1. Parangi Rasayanam
Parangi Rasayanam is a herbo mineral siddha formulation which contains herbs and minerals listed in Table 1.

Table 1: Ingredients of *Parangi Rasayanam.*

S.no	Siddha name	Botanical name	Parts used	Composition
1.	*Parangi sakkai*	Smilax china	Root	16.85%
2.	*Ammukara kilangu*	Withania somnifera	Root	16.85%
3.	*Nilapanai kilangu*	Curculigo orchioides	Root	8.43%
4.	*Thaneervittan kilangu*	Asparagus recemosus	Root	3.37%
5.	*Nannari ver*	Hemidesmus indicus.	Root	1.68%
6.	*Sangam ver*	Azima tetracantha.	Stem bark	1.68%
7.	*Kadukkai*	Terminalia chebula	Fruit	1.68%
8.	*Nelli vatral*	Emblica officinalis	Fruit	1.68%
9.	*Thaandrikai*	Terminalia belerica	Fruit	1.68%
10.	*Ilavanga pathri*	Cinnamomum tamala	Fruit	1.68%
11.	*Sanna lavangapattai*	Cinnamomum verum	Fruit	1.68%
12.	*Val milagu*	Piper cubeba.	Fruit	1.68%
13.	*Vaaivilangam*	Embelia ribes	Seed	1.68%
14.	*Kothamalli*	Coriandum sativum	Seed	1.68%
15.	*Seeragam*	Cuminum cyminum	Seed	1.68%
16.	*Karunjeeragam*	Nigella sativa	Seed	1.68%
17.	*Omam*	Trachyspermum ammi	Seed	1.68%
18.	*Kurosani omam*	Hyoscyamus niger	Seed	1.68%
19.	*Chitharathai*	Alpinia calcarata	Root	1.68%
20.	*Sandhanam*	Santalum album.	Stem bark	1.68%
21.	*Siru thekku*	Pygmacopremna Herbaceae	Whole plant	1.68%
22.	*Kandangkathiri*	Solanum xanthocarpum	Fruit	1.68%
23.	*Kodiveli*	Plumbago indica.	Fruit	1.68%
24.	*Vettiver*	Vetriveria zizanioides	Fruit	1.68%
25.	*Thakkolam*	Illicium verum	Fruit	1.68%
26.	*Thirakchai*	Vitis vinifera	Fruit	1.68%
27.	*Pareechai*	Phoenix dactylifera	Fruit	1.68%
28.	*Vetpalai arisi*	Wrightia tinctoria.	Seed	1.68%
29.	*Thamarai*	Nelumbo nucifera	Flower	1.68%
30.	*Sathi pathri*	Myristrica fragrans	Flower	1.68%
31.	*Kariveppilai*	Murraya koenigii	Leaf	1.68%
32.	*Chukku*	Zingiber officinale	Root	1.68%
33.	*Milagu*	Piper nigrum	Dried fruit	1.68%
34.	*Thippili*	Piper longum	Dried fruit	1.68%
35.	*Ilavanga pattai*	Cinnamomum zeylancium.	Dried fruit	1.68%
36.	*Kumkumapoo*	Crocus sativus	Stamens	0.22%
37.	*Nei*	Clarified butter		0.22%
38.	*Pachai karpooram*	Cinnamomum camphora		0.22%
39.	*Sarkarai*	Saccharum officinarum		Q. S. %
40.	*Thaen*	Honey		Q. S. %
41.	*Paal*	Cow's milk		Q. S. %

Method of Preparation

Gently fry the items from 1 to 36 and make a fine powder. Boil items 39 and 41 and make syrup. Sprinkle the powdered drugs on the syrup and mix it well briskly. Remove the container from stove an add 37 and 40 into it and mix well. Finally add 36 and 38 and mix to homogenecity.

Dosage

5 grams; twice a day.

Adjuvant

Milk, Hot water.

Indications

Penkuri putru (Cervical cancer); *Aankuri putru* (Penile cancer); *Megam* (Veneral diseases); *Parangipun* (Veneral sore); *Kandamalai* (Nodular swelling of neck) and *Kiranthi* (Nodules).

The test drug *Parangi Rasayanam* was procured from SKM Siddha and Ayurveda Company, Erode, Tamil nadu, India.

2. Anti-Cancer Activity

Preparation of test solutions

Serial dilutions of test formulation (10, 50, 100 and 200 µg/ml) were prepared using Distilled water.

HeLa (Cervical adenocarcinoma) cells culture and media

HeLa cell lines were procured from National Center for Cell Science, Pune. Stock cells were cultured in a medium which was supplemented with Dulbecco's Modified Eagle Medium (DMEM), penicillin (100 IU/ml) and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells were checked and centrifuged. Further 50,000 cells / well were seeded in a 96 well plate and incubated for 24 hours at 37ºC in 5% CO_2 incubator. [7]

Source of reagents

All the chemicals and reagents required like DMEM, FBS, Pen strip, Trypsin were procured with standard quality from

Himedia Labs, Chennai.

Anti- proliferation assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% Foetal Bovine Serum (FBS). To each well of the 96 well micro titre plates, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off and the monolayer was washed once with supplement medium. Then 100µl of different test concentrations of test drug was added on to the partial monolayer in micro titre plates. The plates were then incubated at 37ºC for 48 hours in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100µl of MTT (5 mg/10 ml of MTT in Phosphate Buffered Saline) was added to each well. The plates were then incubated for 4hours at 37ºC in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) value was generated from the dose-response curves for each cell line. Figure 1 shows the Anti-Cancer activity of *Parangi Rasayanam* in HeLa cell lines at different concentrations.

$$\text{Survival rate (\%)} = \frac{A_{sample} - A_b}{A_c - A_b} \times 100$$

IC_{50} Value

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve. [8] The IC_{50} value of *Parangi Rasayanam* was given in Table 2.

MTT Assay

In vitro determinations of anti-proliferative effects of *Parangi Rasayanam* have been performed by counting viable cells after staining with a vital dye. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide or MTT, which is a water soluble tetrazolium salt. Upon incubation, MTT is converted into an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. The Resulting coloured solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material. [9] The effect of *Parangi Rasayanam* on cell viability and cell death has been illustrated in chart 1 and 2.

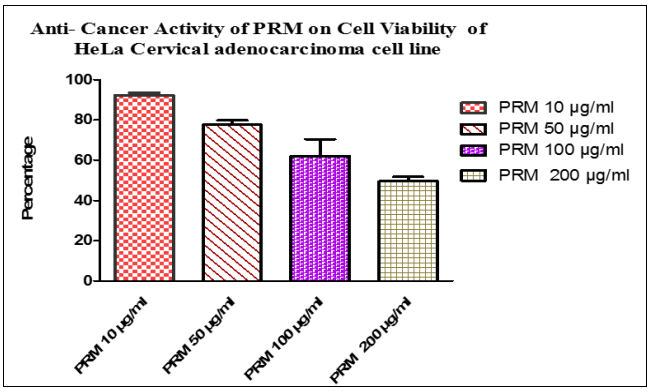

Fig 1: Effect of *Parangi Rasayanam* on Cell viability of HeLa (Cervical adenocarcinoma) cell line

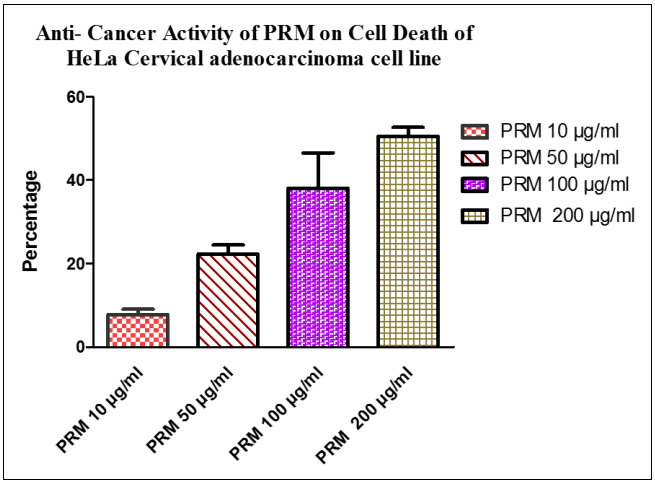

Fig 2: Effect of *Parangi Rasayanam* on Cell death of HeLa (Cervical adenocarcinoma) cell line

Table 2: IC $_{50}$ Value of *Parangi Rasayanam*

IC $_{50}$ Value of PRM	183.7 ± 17.88

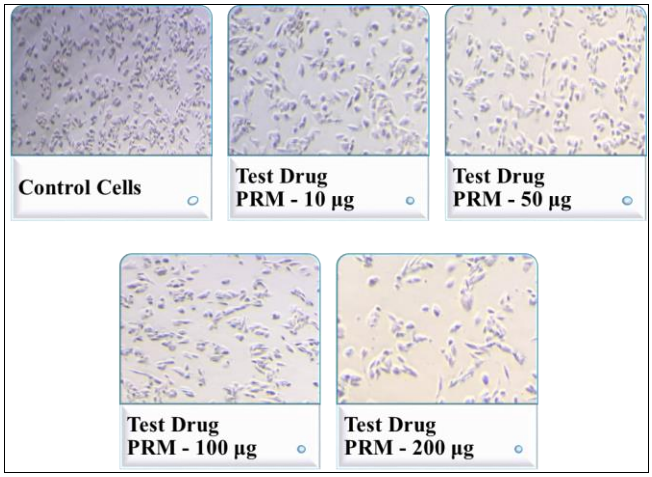

Fig 3: Images showing the Anti-cancer activity of *Parangi Rasayanam* against the HeLa cell lines at different concentrations.

Results

In-vitro anti-cancer evaluation of *Parangi Rasayanam* on the cell viability against HeLa (Cervical adenocarcinoma) cell line was performed at varying concentration ranging from 10 to 200 µg/ml.The reuslts obtained from the study revealed that the percentage of cell viability of HeLa cell line decreased with increase in concentration of the test drug PRM. Least viability of cell was observed at the concentration of 200µg/ml showing 49.53 ± 2.28%, followed by this at 100µg/ml and 50 µg/ml showed 61.98 ± 8.58 % and 77.62 ± 2.28 % respectively. Similarly 10 µg/ml showed 92.22 ± 1.32% cell viability in MTT assay. The corresponding IC_{50} value was found to be 183.7 ± 17.88 µg/ml.

Discussions

In recent years, many studies on the drugs obtained from herbal sources plays a major role in the treatment and managament of many life style and metabolic disorders. Cancer is one among these disorders which is influenced profoundly by dietary habits, smoking, alcohol consumption and infections. In Siddha system, cancer is addressed by many terms like Vipurudhi, Putru, Kazhalai, Katti, etc.., many medicines have been mentioned in old siddha literatures for the treatment and management of cancers. Parangi Rasayanam is one among the herbo mineral drug indicated for Penkuri putru (Cervical cancer); *Aankuri putru* (Penile cancer); *Megam* (Veneral diseases); *Parangipun* (Veneral sore); *Kandamalai* (Nodular swelling of neck) and *Kiranthi* (Nodules). Hence it was studied preliminarily for its anti-cancer potential in the management of cervical cancer in HeLa cells using MTT assay.

It showed a promising anti-cancer effect which is attributed with presence of many herbs like Smilax china, Withania somnifera, Asparagus recemosus, etc.., they are also mentioned as rejuvenating herbs which are referred as *Kaya Kara Mooligaigal* in Siddha system. They are said to be rich in anti-oxidants which helps in reducing the production of free radicals, so called chemical substances responsible for the formation of cancer cells by harming the natural cells of our body.

Kaempferol-7-*O*-β-d-glucoside (KG), a flavonoid glycoside found in the rhizome of *Smilax* china may be responsible for the anti-cancer activity of PRM on a panel of established cancer cells, of which, HeLa human cervix carcinoma cells were the most sensitive. Meanwhile, the cytotoxic effects of KG on normal human cells were also found to be much smaller than on cancer cells.

Same way the presence of active component Withaferin A, which is found to be anti-proliferative may be responsible for the anti-cancer potential of PRM.

Asparagus recemosus containing Shatavarin IV may be responsible for the anti-cancer potency of PRM which acts by reducing the lipid peroxidation induced by the oxidative stress produced by the free radicals.

Conclusion

Hence, from the above *in vitro* analysis, the drug *Parangi Rasayanam* is found to have anti-cancer potential and it can be used for the management of cervical cancer. Further *in vivo* studies have to be conducted to find the pharmcodynamic and pharmacokinetic mechanisms of action of PRM.

Conflicts of Interest

None declared.

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