

## Phytochemical analysis and Invitro Anticancer study of a Siddha formulation KKPN against Cervical Cancer

Thomas M. Walter<sup>1</sup>, C. Samuel Justin Raj<sup>2</sup>, K. Swathi<sup>2</sup>, V.S. Nandini<sup>2</sup>,  
G. Sabari Devi<sup>2</sup>, G. Sanjana<sup>2</sup>, S. Merish<sup>3</sup>

<sup>1</sup> Asst. Professor, Government Siddha Medical College, Palayamkottai, Tirunelveli.

[walter@siddhawalter.org](mailto:walter@siddhawalter.org)

<sup>2</sup>Third Year BSMS, Government Siddha Medical College, Palayamkottai, Tirunelveli.

[samuel16jan@gmail.com](mailto:samuel16jan@gmail.com)

<sup>3</sup>Consultant, Walters' Siddha Research Centre, Tirunelveli. [merish@siddhawalter.org](mailto:merish@siddhawalter.org)

### Abstract

*Karuppai Kazhunthu putru noi* (Cervical cancer) is the second most common cancer among women worldwide and also in India. It is getting more prevalent nowadays with increasing morbidity and mortality. A current estimate shows that approximately 1,32,000 new cases are diagnosed every year in India and 74,000 deaths are annually recorded accounting to one third of the total deaths worldwide. So, it is the need of the hour to find a proper remedy and also to reduce the occurrence of the disease. Siddhars have dealt in the field of gynaecology and oncology. They have also mentioned many specific herbs and drugs for the management and treatment of cervical cancer. Our formulation KKPN has proved its effectiveness against Hela Cell lines in invitro studies. In this paper, the outcomes of the invitro anti-proliferative studies of the formulation using the MTT assay and the preliminary phytochemical analysis of the formulation are briefly described.

### Keywords

KKPN, Cervical cancer, Phytochemical assay, In-vitro assay

## Introduction

Cancer is a life threatening and dreadful disease with unexploring treatment modalities in the conventional system of medicine. Among many cancers, the most common one that threatens the women is that Cervical cancer. Many formulations that are meant for cancer are mentioned in the Siddha literatures. Some of them include *Puttru pathangam*, *Nandhi mai* etc. With the help of Siddha literatures that are dealt with Gynaecology and Oncology, We have formulated a new combination. In this study, we have tested our rug for invitro anticancer activity using MTT assay in Hela cell lines after a preliminary phytochemical analysis.

## Phytochemical analysis

Using Thin Layer Chromatography, we have identified the Rf values as 0.92, 0.85, 0.75. This reveals that there may be the presence of Flavanoids, Saponins respectively. The Saponins are found specifically against Cervical cancer.

## In-vitro anti-proliferative effect determination by MTT assay

HeLa (Cervical cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen).

The cell line was cultured in 25 cm<sup>2</sup> 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 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany).

The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

## Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10<sup>4</sup> cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

## Preparation of compound stock:

The compound solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

### **Anti-proliferative Evaluation:**

After 24 hours the growth medium was removed, freshly prepared compound in 5% DMEM was taken from this 6.25 $\mu$ l, 12.5 $\mu$ l, 25 $\mu$ l, 50 $\mu$ l, 100 $\mu$ l taken and make up to 250 $\mu$ l using 5% MEM and were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

### **Anti-proliferative Assay by Direct Microscopic observation:**

Entire plate was observed after 24 hours of incubation in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation 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.

### **Anti-proliferative 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.

After 24 hours of incubation period, the sample content in wells were removed and 30 $\mu$ l of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After the incubation period, the supernatant was removed and 100 $\mu$ l of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

Sample Concentration ( $\mu\text{g}/\text{mL}$ )	Average OD at 540nm	Percentage Viability
Control	0.9881	100
<b>SAMPLE CODE : KKPN</b>		
6.25	0.8869	89.76
12.5	0.8318	84.18
25	0.7266	73.54
50	0.698	70.64
100	0.6731	68.12

**LD50 value –KKPN- 173.995 $\mu\text{g}/\text{ml}$  (calculated using ED50 PLUS V1.0 software)**

#### **Conclusion:**

There is a huge need in finding a permanent cure for cervical cancer. Hence we have derived a new formulation based on the basic principles of Siddha system of medicine. Our formulation has proved its efficacy in preliminary phytochemical analysis and in-vitro anti-cancer assays. More advanced studies are yet to be done in this formulation to confirm its efficacy and safety profiles. This drug will be beneficial to mankind in eradicating Cervical cancer.

#### **Reference**

1. Uthamaraayan, H.P.I.M., *Thotrakirama Aaraaychiyum Siddha Maruthuva Varalaaarum*
2. Uthamaraayan, H.P.I.M., *Siddha Maruthuvaanga Surukkam*
3. Murugesu Muthaliar, DIM, *Gunapadam Mooligai Vaguppu* (Vegetable section), 1<sup>st</sup> Edition, Published by D I M & H ,Chennai
4. Shanmughavelu, H.P.I.M., *Noi naadal Noi mudhal naadal thirattu* Part 1, First Edition, Published by D I M& H , Chennai
5. Shanmughavelu, H.P.I.M., *Noi naadal Noi mudhal naadal thirattu* Part 2, Second Edition, Published by D I M& H , Chennai

6. Thomas M.Walter, 'Screening of Anti-cancer activity of Pappili – a Traditional Siddha Medicine' Conference Paper · June 2013, Conference: International Conference on Emerging Trends in Chemical and Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Anantapur, Andhrapradesh. [https://www.researchgate.net/publication/243458218\\_Screening\\_of\\_Anti-cancer\\_activity\\_of\\_Pappili\\_-\\_a\\_Traditional\\_Siddha\\_Medicine](https://www.researchgate.net/publication/243458218_Screening_of_Anti-cancer_activity_of_Pappili_-_a_Traditional_Siddha_Medicine)
7. T.R. Mahadeva Pandithar, *Roganirnaya saaram ennum roga nidhaanam*, published by *Thaamarai Noolagam*, Chennai
8. S. Kannusaami pillai, *Sigitchaarathna dheebam* Part 2, Eighth Edition, Published by B. Rathna Naicker and Sons, Chennai
9. S. Merish Thomas M. Walter, Anticancer Efficacy of A Poly Herbal Siddha Formulation on 4549- Human Lung Carcinoma Cells (Feb.2017) Asian Journal of Pharmaceutical and Clinical Research.
10. C.N. Kuppuswami, H.P.I.M., V.S. Parvathi, L.I.M., *Anubhava Vaidiyya Murai*, Published by Government Oriental Manuscripts Library, Madras.
11. R. Thiagarajan, L.I.M., *Yugi Munivar Vaithiya Sindhamani Perunool* 800, Part 2.
12. D. Amirtharajan, *Putru Noigalai Velvom*.
13. S. Venkatarajan, L.I.M., *Agasthiar* 2000, Published by Saraswathi Mahal Nool Nilaiyam, Thanjavur.
14. L. Mahadevan, BAMS, MD, J. Sriram, BSMS, MD, C. Mary Sharmila, BSMS, M.R. Twinkle Pon. Seenu, BSMS, *Yugi Muni Vaadha Noigal Matrum Kanyakumari maavatta vaadha noi thogudhi* published by Saradha Mahadeva Iyer Ayurvedic Educational and Charitable trust, Kanyakumari.
15. S.Chidambaradhaanu Pillai, *Udhara noi maruthuvam*, published by Siddha Medical Literature Research Centre, Chennai.
16. S.P. Ramachandran, *Agastiyar Aayul Vedham* 1200, Published by *Thaamarai Noolagam*, Chennai.
17. S.P. Ramachandran, *Agastiyar vaithya kaaviyam* 1500, Published by *Thaamarai Noolagam*, Chennai.
18. C. Samuel Justin Raj, K. Swathi, G. Sabari Devi, G. Sanjana, V. S. Nandini, S. Merish, Thomas M. Walter. 'An insight into the Siddha aspects of Karupai Kazhunthu Putru Noi (Cervical Cancer)' *Siddha Papers*, ISSN 0g74-2522. World Siddha Day Special, Aprll 20L7. [www.siddhapapers.org](http://www.siddhapapers.org).
19. S. Venkatrajan, LIM, *Agastiyar* 2000, Published by *Thanjavur Maharaja Saraboji's Saraswathi mahal nool nilayam*, Thanjavur.

20. S. Chidambaraddhaanu Pillai, *Aan Pen Ina Uruppu maruthuvam*, Published by Siddha Medical Literature Research Centre, Chennai.
21. S. Chidambaradhaanu Pillai, *Siddha Maruthuva Noi Vakaigalum Thanmaigalum*, Published by Siddha Medical Literature Research Centre, Chennai.
22. S.Venkatarajan, L.I.M., *Dhanvanthiri Vaithiyam Part 1*, Published by *Thanjavur Maharaja Saraboji's Saraswathi mahal nool nilayam*, Thanjavur.
23. Kanthasami Mudhaliyar, *Aathmaratchaamirtham*, published by B. Rathnanayakar and sons, Chennai.
24. P.M. Venugopal, *Magalir Maruthuvam*, 2013, Fifth Edition, Published by Directorate of Indian Medicine and Homeopathy.

