

Original Research

View Article online



## Evaluation of the In Vitro Anticancer Potential of Vaan Mezhu against KB Oral Cancer Cell Line

Suresh Ramasamy<sup>1\*</sup>, Sanjana Subramaniam<sup>2</sup>, Sudha Revathy Senthilkumar<sup>1</sup>

**Received:** 25 March 2025

**Revised:** 21 April 2025

**Accepted:** 12 May 2025

**Available online:** 03 July 2025

Edited by Kannan RR Rengasamy

### KEYWORDS:

Internal medicine  
Oral cancer  
Anticancer  
Siddha medicine  
Vaan mezhu

Nat Resour Human Health 2025; 5 (3): 414–421  
eISSN: 2583-1194  
<https://doi.org/10.53365/nrfhh/204978>  
Copyright © 2025 Visagaa Publishing House

<sup>1</sup>Department of Gunapadam (Siddha Pharmacology), National Institute of Siddha (Affiliated with The Tamil Nadu Dr. M.G.R. Medical University), Ministry of Ayush, India

<sup>2</sup>Department of Pura Maruthuvam (Siddha External therapy), National Institute of Siddha (Affiliated with The Tamil Nadu Dr. M.G.R. Medical University), Ministry of Ayush, India

**ABSTRACT:** Background: “Oral squamous cell carcinoma (OSCC)” is a significant health concern in India, with a high incidence and low survival rate because of delayed diagnosis and limitations of current treatments. A survey in 2022 indicated that the incidence rate for oral cancer in India was 14.7 per 100,000 for men and 5.0 per 100,000 for women. Siddha medicine, an indigenous system of medicine in Southern India, has shown potential anticancer properties, including the formulation *vaan mezhu* (VM). Objective: To evaluate the cytotoxic and apoptosis-inducing effects of VM on the KB human oral cancer cell line. Methods: The cytotoxic and apoptosis-inducing effects of VM on the KB human oral cancer cell line was evaluated using an MTT assay, and apoptosis was evaluated using acridine orange and ethidium bromide staining, and annexin V flow cytometry. Results: VM exhibited dose-dependent cytotoxicity, with an LC<sub>50</sub> value of 21.69 µg/mL. In apoptotic assay, treated cells showed chromatin condensation and distinct apoptotic markers in fluorescence microscopy. Flow cytometry revealed an increase in late apoptotic cells from 5.54% (control) to 20.78% (treated), with total apoptosis rising to 23.30%. Conclusion: VM demonstrated significant cytotoxic effects and apoptosis induction in KB oral cancer cells. These findings suggest its potential as an alternative therapy for oral cancer, warranting further in vivo studies and clinical trials.

## 1. INTRODUCTION

The uncontrolled growth of cells that have outgrown the body's natural control systems is known as cancer (Krieghoff-Henning et al., 2017). Cancer is unique among diseases as it can be metabolic, inflammatory, genetic, or all of these at once (Gyamfi et al., 2022). Any tissue in the body can acquire cancer, but each one has its characteristics even

though the fundamental mechanism is the same. In India, oral cancer poses a serious public health concern because of its unchecked cellular growth and invasion of nearby tissues in the oropharyngeal cavity. In India, oral squamous cell carcinoma (OSCC) is the most prevalent cancer that poses a threat to public health (Pires et al., 2013). OSCC typically presents as a tongue ulcer that does not heal, accompanied by varied degrees of xerostomia and mucositis. In addition,

\*Corresponding author.

E-mail address: [suresh03051994@gmail.com](mailto:suresh03051994@gmail.com) (Suresh Ramasamy)

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

there could be fistula, limited mouth opening, ulcers, and lymphadenopathy (Minhas et al., 2017).

It is the sixth most common type of cancer worldwide. With an estimated one-third of all cases globally, India is disproportionately affected. A quarter of the world's mouth cancer cases occur in India, where there are an astounding 77,000 new cases diagnosed each year and 52,000 related fatalities. About 70% of cases in India are detected at an advanced stage, in contrast to Western countries where early diagnosis is more prevalent. This considerably lowers the 5-year survival rate, which in India is still alarmingly low at about 20%. India has a higher risk of oral cancer because of several reasons. Smoking and nonsmoking tobacco use, excessive alcohol intake, poor dental hygiene, malnutrition, and persistent viral infections are recognized risk factors. Also, chewing paan is a significant local risk factor (Borse et al., 2020). In addition, the risk of oral cancer in this population is further increased by periodontal disease, which is frequently linked to the use of paan (betel leaf). The most commonly used treatment modalities are typically chemotherapy and radiation therapy, either before or following surgical excision of the malignant tumor. These therapeutic approaches frequently result in side effects such as mucositis, infection, taste malfunction of the salivary glands, and pain, which lead to secondary problems like malnourishment, dehydration, and dysgeusia (Minhas et al., 2017).

Head and neck radiation can cause irreversible damage to the oral mucosa and its blood vessels, which can lead to trismus, xerostomia, and bone and soft tissue necrosis (Borse et al., 2020). Because of the metastases, it is frequently impossible to surgically remove malignant growths (Tohme et al., 2017). According to a study, drug-resistant opportunistic bacteria developed in the oral cavity of elderly patients in China following treatment for oral cancer. In addition, it has been proposed that the presence of bacteria in the mouth cavity raises the risk of illnesses such as bacteremia and pneumonia (Pan et al., 2014). Therefore, a medication that can inhibit tumor cell growth, reduce the severity of symptoms, and aid in pain management must be used in the entire care of oral cancer. Numerous formulations in the Siddha school of medicine, which is indigenous to Southern India, offer substantial anticancer properties.

One such formulation is *vaan mezhugu* (VM), a Siddha herbo-mineral drug indicated for conditions such as *Kandamaalai* (Cervical lymphadenitis), *Kiranthi* (Glandular swelling), *Purai* (Sinus), and various other ailments (Kuppusamy, 1993; Sambasivampillai, 1991). VM is clinically used in the management of oral cancer by Siddha physicians, but currently, there is no scientific evidence for it. In addition, VM contains the following ingredients: *Thaalagam* (Yellow arsenic trisulfide), *Veeram* (Hydrargyrum perchloride),

*Pooram* (Hydrargyrum subchloride), *Lingam* (Red sulfide of mercury), and *Rasam* (Hydrargyrum). Numerous scientific studies have already indicated the enormous potential of these ingredients in curing cancer. Therefore, this study aimed to evaluate VM's growth-inhibiting impact in the human oral cancer KB cell line.

## 2. MATERIALS AND METHODS

### 2.1 Selection of the experimental drug

Siddha Vaithiya Thirattu written by Kuppusamy (1993), which is part of Siddha literature, indicates that the trial drug, VM, is one of the herbo-mineral formulations for *Kaandamalai* (Cervical lymphadenitis), *Kiranthi* (Glandular swelling), *Purai* (Sinus), *Powthiram* (Fistula), *Manjal Kaamaalai* (Jaundice), *Gunmam* (Peptic ulcer), and *Kaasam* (Bronchitis). The drug was procured from a GMP-certified pharma company.

### 2.2 Ingredients of VM

The components of VM are detailed in Table 1.

### 2.3 Preparation of VM

First, *Gandhagam* and *Rasam* were ground together into a fine black powder and separated. Subsequently, the other ingredients were added and mixed with the previously

**Table 1**  
Ingredients of *vaan mezhugu*.

S. No.	Vernacular Name	Botanical Name/Chemical Name
1.	<i>Rasam</i>	Hydrargyrum
2.	<i>Rasachenduram</i>	Red sulfide of mercury
3.	<i>Gandhagam</i>	Sulfur
4.	<i>Veeram</i>	Hydrargyrum perchloride
5.	<i>Gowripaadanam</i>	Arsenic oxide
6.	<i>Vellaipaadanam</i>	White arsenic
7.	<i>Mirutharsingi</i>	Galena sulfide of lead
8.	<i>Pooram</i>	Hydrargyrum subchloride
9.	<i>Lingam</i>	Cinnabar
10.	<i>Gandham</i>	Magnetic oxide of iron
11.	<i>Thaalagam</i>	Yellow arsenic trisulfide
12.	<i>Abin</i>	<i>Papaver somniferum</i> . L
13.	<i>Saambirani</i>	<i>Styrax benzoin</i> . Drynad
14.	<i>Karpooram</i>	<i>Cinnamomum camphora</i> . Linn

prepared *Gandhagam* and *Rasam* powder. *Anda Thylam* was then incorporated, and the mixture was ground thoroughly for 4 hours until it reached a semisolid, wax-like texture. The final product (VM) was stored in an airtight container.

Dosage: *Ulundhalavu* (65 mg)

Adjuvant: Palm jaggary

## 2.4 In vitro evaluation of anticancer activity

### 2.4.1 Cytotoxic effect determination by MTT assay

The KB human oral cancer cell line, sourced from the “National Centre for Cell Sciences (NCCS), Pune, India, was cultured in 25 cm<sup>2</sup> flasks using Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and antibiotics (penicillin, streptomycin, and amphotericin B).” Cells were maintained at 37°C in a humidified incubator with 5% CO<sub>2</sub>. Viability was assessed visually using an inverted phase-contrast microscope and through the MTT assay.

After trypsinization, 5×10<sup>3</sup> cells/well were seeded in 96-well plates and incubated under standard conditions. The test compound was prepared by dissolving 1 mg in 1 mL of 0.1% DMSO, mixed with a cyclomixer, and filtered through a 0.22 μm filter. Cells were treated with the compound at concentrations of 100, 50, 25, 12.5, and 6.25 μg/mL in triplicates, while untreated cells served as controls.

Cytotoxic effects, including cell rounding, shrinking, granulation, and vacuolization, were observed microscopically. For the MTT assay, 30 μl of MTT solution was added after 24 hours of incubation. Following a 4-hour incubation, the supernatant was removed, formazan crystals were dissolved in 100 μl DMSO, and absorbance was measured at 540 nm using a microplate reader (Jerard et al., 2020; Mosmann et al., 1983).

### 2.4.2 Assessment of apoptosis using acridine orange (AO) and ethidium bromide (EtBr) dual staining

KB human oral cancer cells were cultured following standard protocols and treated with the lethal concentration (LC<sub>50</sub>) of sample (21.69 μg/mL) for 24 hours, alongside untreated controls. After incubation at 37°C in a 5% CO<sub>2</sub> humidified environment, the cells were washed with cold PBS and stained with a mixture of AO (100 μg/mL) and EtBr (100 μg/mL) for 10 minutes at room temperature. The stained cells were rinsed twice with PBS and examined under a fluorescence microscope “(Olympus CKX41 with Optika Pro5 camera)” using a blue filter. Cells were classified into four categories: live, early apoptotic, late apoptotic, and necrotic (Zhang et al., 1998).

### 2.4.3 Apoptosis by annexin V flow cytometry

KB human oral cancer cells were cultured as per standard procedures and treated with the LC<sub>50</sub> of the sample (21.69 μg/mL) from a 1 mg/mL stock solution, with untreated controls maintained. Cells were incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 24 hours. Following incubation, the cell sample was transferred to a 12×75-mm polystyrene tube, ensuring a minimum of 1×10<sup>6</sup> cells for fixation. Samples were centrifuged at 3000 rpm for 5 minutes, and the supernatant was carefully removed without disturbing the pellet.

Next, 100 μL of Muse™ Annexin V and a dead cell reagent was added to each tube. The mixture was gently vortexed or pipetted for 3–5 seconds and incubated for 20 minutes at room temperature in the dark. Finally, the cells were analyzed using a flow cytometer with Muse FCS 3.0 software, comparing them to untreated controls to assess apoptosis (Ciniglia et al., 2010).

## 3. RESULTS

### 3.1 Evaluation of in vitro cytotoxicity using the MTT assay

Cytotoxic effects of VM on cancer cells at different concentrations show a dose-dependent decrease in cell viability (Figure 1).

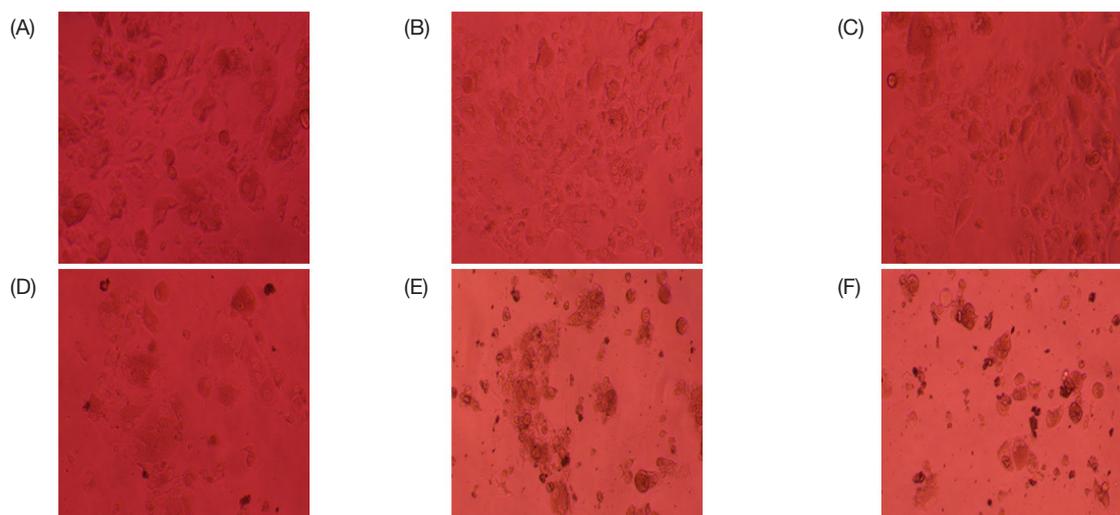
Cytotoxic effects of VM on cancer cells treated with various concentrations of cell viability percentage are presented in Table 2 and Figure 2.

The Y-axis represents percentage cell viability, while the X-axis shows different sample concentrations. All experiments were conducted in triplicates, and results are presented as Mean ± SE. Data were analyzed using one-way ANOVA followed by Dunnett’s test, with \*\*\*p < 0.001 considered significant compared to the control group.

### 3.2 Determination of apoptosis by AO and EtBr double staining

The cells were categorized into four groups:

- **Living cells:** Displayed a normal green nucleus.
- **Early apoptotic cells:** Exhibited a bright green nucleus with condensed or fragmented chromatin.
- **Late apoptotic cells:** Showed orange-stained nuclei with chromatin condensation or fragmentation.
- **Necrotic cells:** Had uniformly orange-stained nuclei.



**Figure 1.** Cancer cells exposed to varying concentrations of VM. (A) Control, (B) VM–6.25 µg/mL, (C) VM–12.5 µg/mL, (D) VM–25 µg/mL, (E) VM–50 µg/mL, and (F) VM–100 µg/mL.

**Table 2**

Percentage viability of *vaan mezhugu* at various concentrations.

VM Concentration µg/mL	Percentage Viability
Control	100
6.25	91.84***
12.5	71.72***
25	42.19***
50	30.97***
100	17.12***

\*\*\*p < 0.001

### 3.3 Apoptosis by annexin—V flowcytometry

VM treatment induces apoptosis in cancer cells, as evident from the significant differences observed between the untreated groups, as shown in Figure 4 and Table 3.

The percentage of early apoptotic cells was 4.26%, while the late apoptotic cells accounted for 5.54%. The overall apoptotic percentage was low, totaling 9.80%.

VM treatment induces apoptosis in cancer cells, as evident from the significant differences observed between treated groups, as shown in Figure 5 and Table 4.

In the VM-treated cells, the early apoptotic percentage was 2.52%, while the late apoptotic percentage was 20.78%. In addition, 9.72% of the cells were debris, leading to a total apoptosis rate of 23.30%.

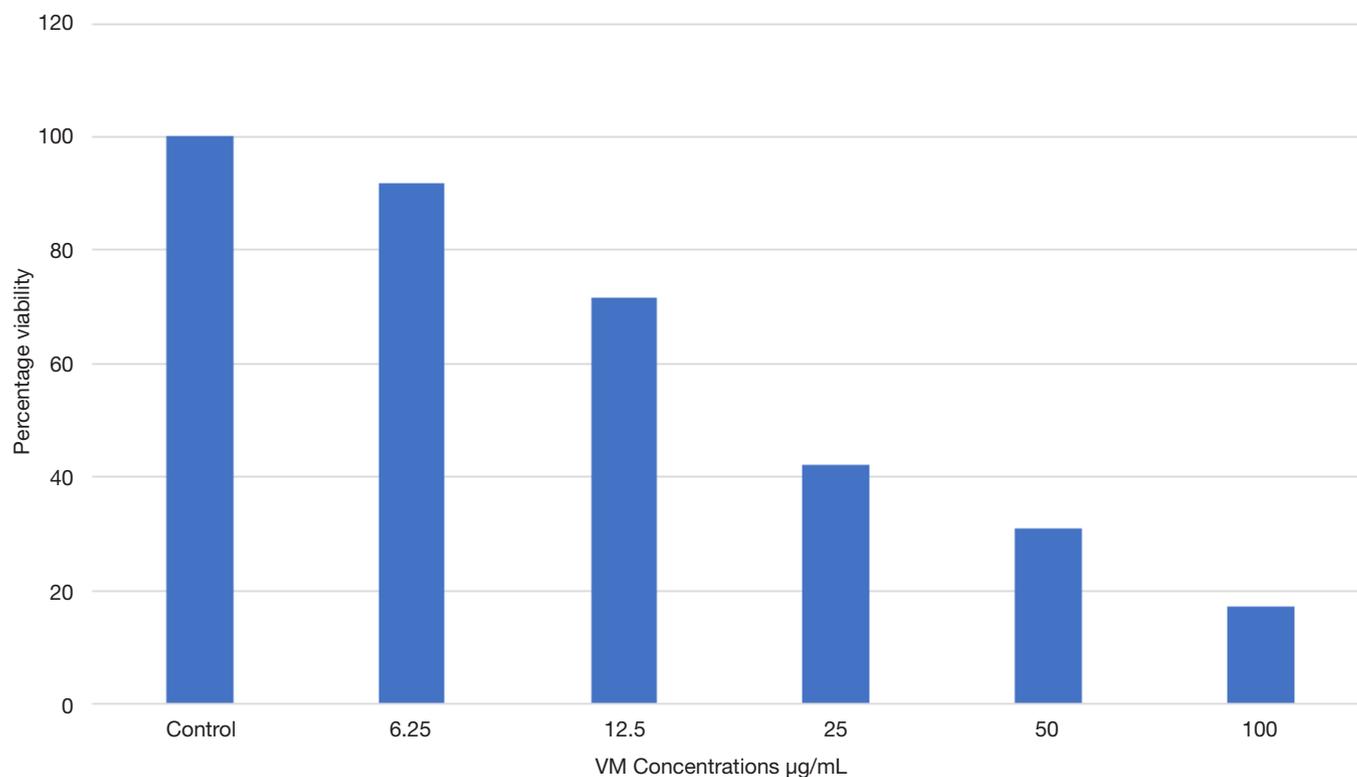
## 4. DISCUSSION

Chemotherapy and radiotherapy play vital roles in the management of oral cancer. Such treatment results in adverse

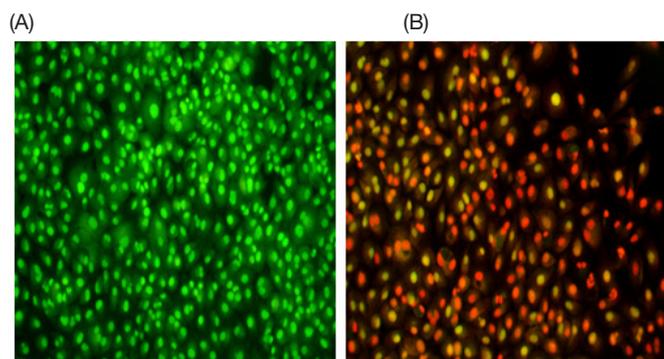
physical, psychological, and economic effects (Bjordal et al., 1994). Common physical effects such as nausea, vomiting, diarrhea, fatigue, and generalized weakness are persistent and affect daily activities (Karthikeyan et al., 2012). Cognitive impairments such as concentration and memory loss are not uncommon. Functionally, post-treatment causes changes in swallowing and chewing (Gunn et al., 2013). Consequences of surgery and radiotherapy such as facial impairment, disfigurement, and less lip mobility drastically lower the self-esteem of the patient (Bhandari et al., 2024; Mishra et al., 2024).

A decline in “health-related quality of life (HRQL)” is seen in long-term cancer patients (Nordgren et al., 2003). Psychologically, extreme levels of depression and anxiety are observed, but fear of recurrence is consistent and, in turn, affects mental health (Ryan et al., 2005; Reyes et al., 2023). Economically, patients are affected because of treatment costs which results in financial strain and thoughts of committing suicide (Wajid et al., 2021). It is therefore essential to explore alternative therapies that are effective while minimizing side effects. Presently, cancer research emphasizes both modern medical treatments and traditional healing practices. A holistic approach that integrates conventional therapies with traditional remedies could prove vital in tackling this complex disease.

The Siddha system of medicine is grounded in three core principles: *vali* (movement), *azhal* (transformation), and *iyam* (structure). Specifically, *azhal* plays a crucial role in cellular digestion and metabolism. An imbalance where *azhal* dominates over *iyam* (fire surpassing water) is a hallmark of tumors, resulting in unchecked tissue growth. Conversely, a deficiency in *azhal* can cause an imbalance favoring *vali* and *iyam*, potentially leading to issues such as tissue migration



**Figure 2.** A graph illustrating the anticancer effect of the sample on KB cells using the MTT assay was plotted.



**Figure 3.** (A, B) The phase contrast images captured during the double staining procedure.

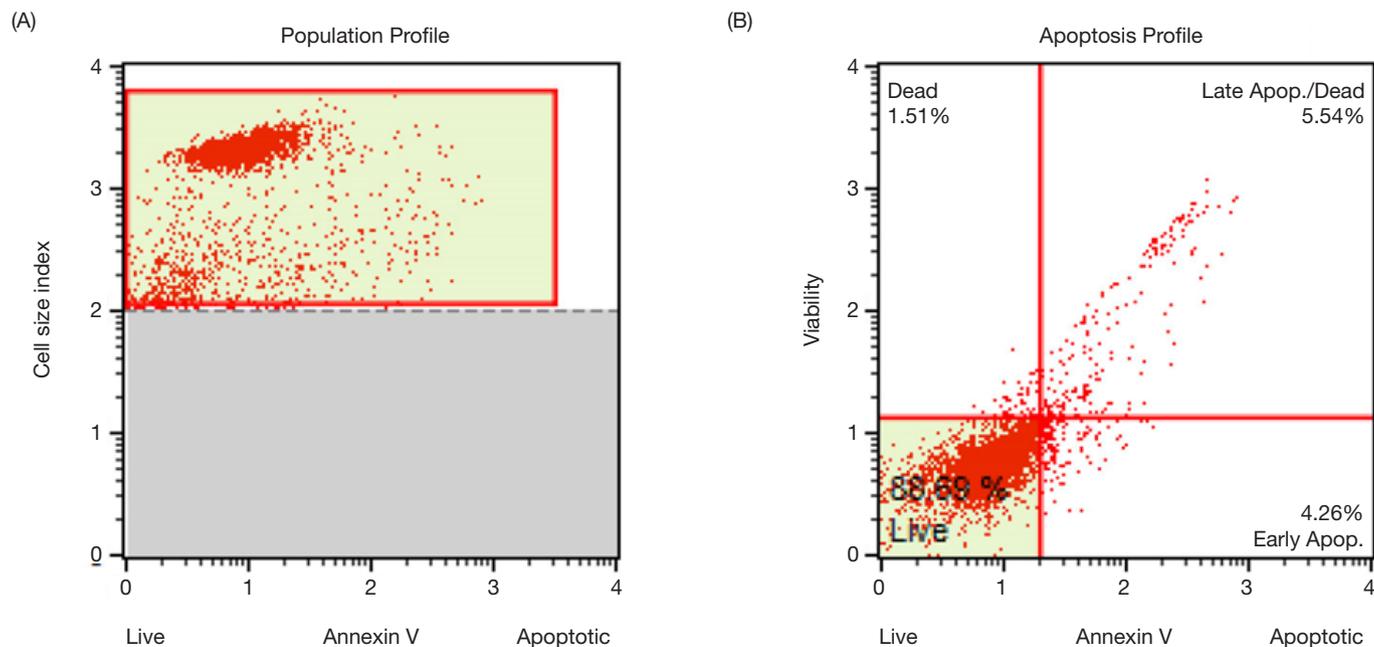
because of disruptions in *Viyana vaayu*, *Samana vaayu*, and *Suzhumunai naadi*.

Each person has a unique constitution, referred to as *Thegi*, which is influenced by the dominant dosham “(*Vaatham*, *Pitham*, *Kapham*, or combinations thereof)” and the individual’s body time. This constitution determines how a person interacts with their environment, their susceptibility to diseases, and their response to treatments. To maintain health, it is essential to keep the dosham in balance. In the context of cancer, the most affected doshas, *Kapham* and *Vaatham*, often exacerbate tumor growth and spread (Subathra et al.,

2022). Various compound formulations have been suggested for the treatment of oral cancer, including VM, a preparation made from multiple ingredients. This formulation works by addressing imbalanced vital forces, restoring harmony through its inherent heat-based potency.

The results of the MTT assay demonstrated that cell viability decreases as drug concentration increases, exhibiting an inverse relationship. At a concentration of 6.25 µg/mL, cell viability was 91.84%, whereas at 100 µg/mL, it dropped significantly to 17.12%. These findings confirm that the cytotoxic effect of the drug is dose-dependent.  $LC_{50}$ , where 80% of cells were inhibited, was determined to be 21.6945 µg/mL.

Using the EtBr and AO double-staining method, the nuclei in the control group appeared green under epifluorescence, showing a uniform spherical structure and organized chromatin. In contrast, cells treated with the  $LC_{50}$  displayed distinct nuclear apoptotic bodies with fragmented DNA, visible as green fluorescent patches indicative of early apoptosis. In advanced stages of apoptosis, these apoptotic bodies disintegrated, resulting in fragmented DNA typical of late apoptosis, observed as orange to red-stained nuclei. Necrotic cells were identified by uniformly orange-stained nuclei.



**Figure 4.** Apoptosis profile of untreated cells. (A) Population and (B) Control.

**Table 3**

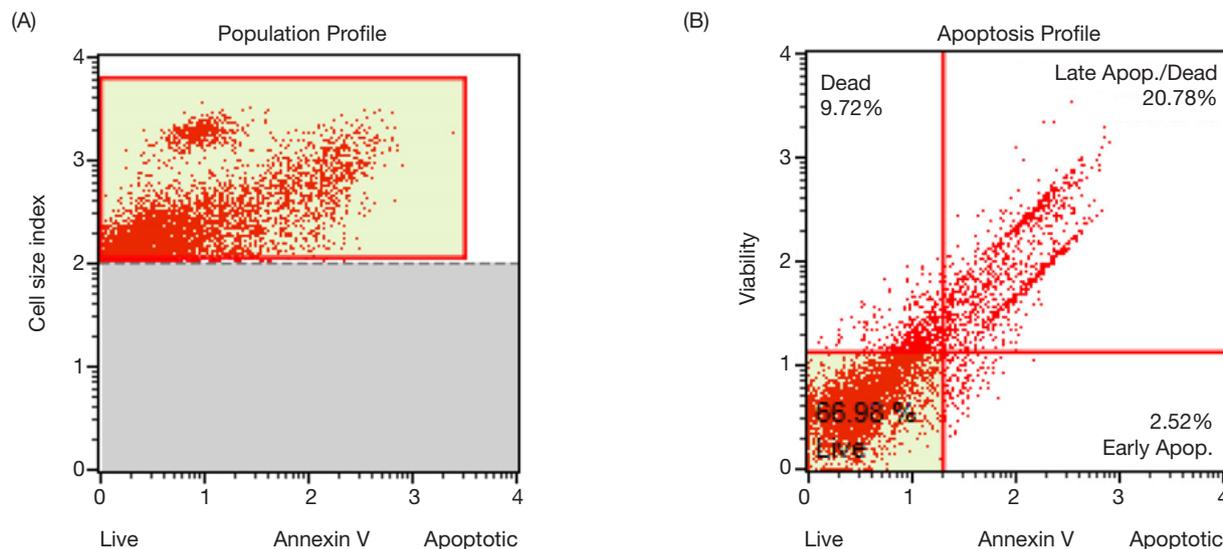
Cell distribution of cells in control cells.

Live (LL)	2.44E+05	88.69
Early Apoptotic (LR)	1.17E+04	4.26
Late Apoptotic/Dead (UR)	1.52E+04	5.54
Debris (UL)	4.16E+03	1.51
Total Apoptotic	2.69E+04	9.80

Annexin V binding assays, in combination with propidium iodide, were employed to differentiate apoptotic cells from necrotic cells, providing a reliable method for assessing apoptosis in testicular cancer cells. Quantification revealed that the fold increase in cell death compared to the control reflected the apoptotic response to treatment with the  $LC_{50}$ . Flow cytometry analysis using annexin V detailed the cellular response to therapy, highlighting the effectiveness of the treatment. Results showed that late apoptotic changes increased from 5.54% in the control group to 20.78% in the drug-treated group. The total percentage of apoptotic cells rose from 9.80% in the control to 23.30% in the treated group, underscoring the drug's significant role in inducing apoptosis and enhancing cell death.

Certain components in this formulation, including *Rasam* (Hydrargyrum), *Veeram* (Hydrargyrum perchloride), *Pooram*

(Hydrargyrum subchloride), *Thaalagam* (Yellow arsenic trisulfide), *Lingam* (Red sulfide of mercury), and *Gandbhagam* (Sulfur), have been shown to exhibit cytotoxic and anti-proliferative properties. [Manjari et al. \(2018\)](#) studied the anticancer effects of *Pancha paasana chendhuram* (PPC), which contains *Rasa chendoorum*, *Veeram*, *Vellai pasanam*, *Thalagam*, *Gaandham*, and *Gandbhagam* on MCF-7 cells, demonstrating dose-dependent cytotoxicity ( $IC_{50} = 59.26 \mu\text{g/mL}$ ), apoptosis induction, Bax and p53 upregulation, Bcl2 downregulation, and moderate antioxidant activity, suggesting apoptosis induction via mitochondrial pathways ([Manjari et al., 2018](#)). Similarly, [Abinaya \(2018\)](#) evaluated the in vitro anticancer activity of *Kalamega narayana chendoorum* (KNC), containing *Rasam*, *Lingam*, *Thalagam*, and *Gandbhagam*, against human KB oral cancer cells using cell viability and apoptosis assays ([Abinaya, 2024](#)). [Manjari et al. \(2016\)](#) investigated *Rasa karpooora kuligai* (RSK), with *Pooram* as the primary ingredient, on HeLa cells, showing reduced cell growth and dose-dependent regulation of apoptotic pathways ([Manjari et al., 2016](#)), while [Rajalakshmi et al. \(2014\)](#) demonstrated that VM, containing *Veeram*, exhibited 74.05% cytotoxicity against EAC cell lines at a concentration of 1000  $\mu\text{g/mL}$  ([Rajalakshmi, et al., 2013](#)). This study used MTT assay, apoptosis double staining, and annexin V flow cytometry to show that VM plays a major role in its anticancer effects.



**Figure 5.** Apoptosis profile of treated cells. (A) Population and (B) *vaan mezhugu* profile

**Table 4**

Cell distribution in cells treated with *vaan mezhugu*.

Parameter_Cell Concentration (Cells/mL)_% Gated_		
Live (LL)	3.68E+05	66.98
Early Apoptotic (LR)	1.39E+04	2.52
Late Apoptotic/Dead (UR)	1.14E+05	20.78
Debris (UL)	5.35E+04	9.72
Total Apoptotic	1.28E+05	23.30

## 5. CONCLUSIONS

The test drug VM significantly reduced cell viability in a dose-dependent manner, indicating its cytotoxic effects. Images showed chromatin condensation and orange staining, confirming that apoptosis had occurred. Flow cytometry results revealed a notable percentage of late apoptosis in the treated group, with the  $LC_{50}$  value highlighting its ability to induce apoptosis to some extent. Furthermore, in vivo studies and clinical trials are needed to confirm its potential as a treatment for oral cancer.

## ACKNOWLEDGEMENT

The authors are grateful for the support and facilities provided by the National Institute of Siddha, Tambaram sanatorium, and the Center for Research on Molecular Biology and Applied Science, Trivandrum, for their support in this study.

## AUTHOR CONTRIBUTIONS

Conceptualization, data collection, and compilation were done by SR; manuscript writing and proofreading and editing were done by SR, SS, and SRS.

## CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

## ORCID

Suresh Ramasamy [0009-0004-9403-0629](https://orcid.org/0009-0004-9403-0629)  
 Sanjana Subramaniam [0009-0009-9671-9063](https://orcid.org/0009-0009-9671-9063)  
 Sudha Revathy Senthilkumar [0009-0003-7496-0792](https://orcid.org/0009-0003-7496-0792)

## REFERENCES

Abinaya, R. 2018. Scientific validation of anti-oral cancer, anti-tumour, antimicrobial activities of siddha metallo-mineral formulation kaalamega narayana chendhooram in cell line studies

- (Doctoral dissertation, Government Siddha Medical College, Chennai).
- Bhandari, S., Singh, R., Mehndiratta, S., Dadhich, M. 2024. The impact of facial disfigurement and self-image anxiety on the quality of life of head and neck cancer patients. *Oral Oncology Reports* 10, 100518. <https://doi.org/10.1016/j.oor.2024.100518>
- Bjordal, K., Kaasa, S., Mastekaasa, A. 1994. Quality of life in patients treated for head and neck cancer: a follow-up study 7 to 11 years after radiotherapy. *International Journal of Radiation Oncology, Biology, Physics* 28(4), 847–56. [https://doi.org/10.1016/0360-3016\(94\)90104-x](https://doi.org/10.1016/0360-3016(94)90104-x)
- Borse, V., Konwar, A.N., Buragohain, P. 2020. Oral cancer diagnosis and perspectives in India. *Sensors International* 1, 100046. <https://doi.org/10.1016/j.sintl.2020.100046>. Epub 2020 Sep 24. PMID: 34766046; PMCID: PMC7515567.
- Ciniglia, C., Pinto, G., Sansone, C., Pollio, A. 2010. Acridine orange/ethidium bromide double staining test: a simple in-vitro assay to detect apoptosis induced by phenolic compounds in plant cells. *Allelopathy Journal* 26(2), 301–8.
- Gunn, G.B., Mendoza, T.R., Fuller, C.D., Gning, I., Frank, S.J., Beadle, B.M., et al. 2013. High symptom burden prior to radiation therapy for head and neck cancer: a patient-reported outcomes study. *Head Neck* 35, 1490–8. <https://doi.org/10.1002/hed.23181>
- Gyamfi, J., Kim, J., Choi, J. 2022. Jan. Cancer as a metabolic disorder. *International Journal of Molecular Sciences* 21;23(3), 1155. <https://doi.org/10.3390/ijms23031155>. PMID: 35163079; PMCID: PMC8835572.
- Jerard, C., Michael, B.P., Chenicheri, S., Vijayakumar, N., Ramachandran, R. 2020. Sep. Rosmarinic acid-rich fraction from menthaarvensis synchronizes Bcl/Bax expression and induces G0/G1 arrest in hepatocarcinoma cells. *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences* 90(3), 515–22. <https://doi.org/10.1007/s40011-019-01122-9>
- Karthikeyan, G., Jumrani, D., Prabhu, R., Manoor, U.K., Supe, S.S. 2012. Prevalence of fatigue among cancer patients receiving various anticancer therapies and its impact on quality of life: a cross-sectional study. *Indian Journal of Palliative Care* 18(3), 165–75. <https://doi.org/10.4103/0973-1075.105686>
- Kriehoff-Henning, E., Folkerts, J., Penzkofer, A., Weg-Remers, S. 2017. Feb. Cancer—an overview. *Med Monatsschr Pharm* 40(2), 48–54. English, German. PMID: 29952494.
- Kuppusamy, M. 1933. Siddha Vaithiya Thirattu. *Indian Medicine and Homeopathy*, First edition—1933, Pg. No. 197.
- Manjari, V., Murugesan, M., Saravanababu, C. 2016. A preliminary investigation on the cytotoxic and antiproliferative effect of rasakarpoora kuligai (rsk). *World Journal of Pharmaceutical Research* 5(9), 1684–1717, ISSN 2277–7105. <https://doi.org/10.20959/wjpr20169-7035>
- Manjari, V., Murugesan, S., Banumathi, V., Pattarayan, R. 2018. Anticancer activity of pancha paasana chendhuram on MCF-7 Cells. *International Journal of Nutrition, Pharmacology, and Neurological Diseases* 8, 143–52. [https://doi.org/10.4103/ijnpnd.ijnpnd\\_5218](https://doi.org/10.4103/ijnpnd.ijnpnd_5218)
- Minhas, S., Kashif, M., Altaf, W., Afzal, N., Nagi, A.H. 2017 May. Concomitant-chemoradiotherapy-associated oral lesions in patients with oral squamous-cell carcinoma. *Cancer Biology & Medicine* 14(2), 176–182. <https://doi.org/10.20892/j.issn.2095-3941.2016.0096>. PMID: 28607808; PMCID: PMC5444929.
- Mishra, A., Singh, R., Baluni, P., Bhutani, R. 2024. Bottleneck issues and conceptual barriers of oral cancer rehabilitation. *Oral Oncology Reports* 11, 100603, <https://doi.org/10.1016/j.oor.2024.100603>.
- Mosmann, T. 1983. Dec. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 16;65(1–2), 55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4). PMID: 6606682.
- Nordgren, M., Abendstein, H., Jannert, M., et al. 2003. Health-related quality of life five years after diagnosis of laryngeal carcinoma. *International Journal of Radiation OncologyBiologyPhysics* 56(5), 1333–43. [https://doi.org/10.1016/s0360-3016\(03\)00292-x](https://doi.org/10.1016/s0360-3016(03)00292-x).
- Rajalakshmi, P., Savariraj Sagayam, C., Brindha, P. 2014. Preparation and standardization studies on veera mezhugu—a siddha anticancer formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*, ISSN-0975-1491.
- Pan, J., Zhao, J., Jiang, N. 2014. Jul–Aug. Oral cavity infection: an adverse effect after the treatment of oral cancer in aged individuals. *Journal of Applied Oral Science* 22(4), 261–7. <https://doi.org/10.1590/1678-775720130546>. PMID: 25141196; PMCID: PMC4126820.
- Pires, F.R., Ramos, A.B., Oliveira, J.B., Tavares, A.S., Luz, P.S., Santos, T.C. 2013. Sep–Oct. Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single oral pathology service during an 8-year period. *Journal of Applied Oral Science* 21(5), 460–7. <https://doi.org/10.1590/1679-775720130317>. PMID: 24212993; PMCID: PMC3881836.
- Reyes, I.M., Arenilla, M.J., Alarcon, D., Jaenes, J.C., Trujillo, M. 2023. Psychological impact after treatment in patients with head and neck cancer. *Med Oral Patol Oral Cirugía Bucal* 28(5), e467–73. <https://doi.org/10.4317/medoral.25878>. Published 2023 Sep. 1.
- Ryan, H., Schofield, P., Cockburn, J., et al. 2005. How to recognize and manage psychological distress in cancer patients. *European Journal of Cancer Care* 14(1), 7–15. <https://doi.org/10.1111/j.1365-2354.2005.00482.x>
- Sambasivampillai, T.V., 1991. Tamil-English dictionary of medicine, chemistry, botany, and allied science, In: G.D. Naidu Charities, second and five editions (Eds.). *Indian Medicine and Homeopathy, Chennai*.
- Subathra, T., Gomathi, R., Shanmugapriya, P., Ramamurthy, M., Meenakumari, R. 2002. A review on chithiramoola kuligai—therapeutic approach for cervical cancer in accordance with siddha principles. *Journal of Population Therapeutics and Clinical Pharmacology* 29(04), 3919–3926. <https://doi.org/10.53555/jptcp.v29i04.6366>
- Tohme, S., Simmons, R.L., Tsung, A. 2017. Apr. Surgery for cancer: a trigger for metastases. *Cancer Research* 1;77(7), 1548–1552. <https://doi.org/10.1158/0008-5472.CAN-16-1536>. Epub 2017 Mar 22. PMID: 28330928; PMCID: PMC5380551.
- Wajid, M., Rajkumar, E., Romate, J., George, A.J., Lakshmi, R. 2021. Exploring the problems faced by patients living with advanced cancer in Bengaluru, India. *Heliyon* 7(4), e06686. <https://doi.org/10.1016/j.heliyon.2021.e06686>
- Zhang, J.H., Yu, J., Li, W.X., Cheng, C.P. 1998. Jun. Evaluation of Mn<sup>2+</sup> stimulated and Zn<sup>2+</sup> inhibited apoptosis in rat corpus luteal cells by flow cytometry and fluorochromes staining. *Chinese Journal of Physiology* 30;41(2), 121–6. PMID: 9801843.