The perspective of herbal mineral formulation Kaalamega Narayana Chendhooram (KMNC) as an anti-microbiological, anti-oral carcinoma, anti-tumor evaluations (A Siddha medicine for oral cancer, lung cancer) in in-vitro studies

Satheesh C¹, Balasubramanian S², Kalaiselvi D³, Abinaya R²

¹Agash Siddha Wellness Centre and Lab, Chennai- 610 044, Tamil Nadu- India
²Shanmuga Siddha Clinic and Laboratories, Thanjavur- 613 006, Tamil Nadu –India
³Rasi Clinic, Thottiyam- 621 215, Trichy –Dist, Tamil Nadu- India

Abstract
Leukaemia, one of the most lethal illnesses of the twentieth century, is becoming one of the most common illnesses of the twenty-first. The most common carcinoma among those under the mandatory age of 20 is oral also tumor cancer. Siddha herbal mineral formulation’s perspective this study aims to assess the anti-microbiological, anti-oral cancer, and anti-tumor activities of Kaalamega Narayana Chendhooram in vitro experiments. As well as to measure anti-tumor activity against KB cell lines as well as anti-cancer activity (MTT assay) and validate the therapeutic effects of KMNC in order to evaluate the therapeutic effects of KMNC. In the modern medical setting, oral cancer must be effectively treated. The percentage of KB cell viability in the MTT experiment was 69.46%, 55.06%, 51.67%, 40.33%, and 35.34% at concentrations of 62.5 𝜇g/mL, 12.5 𝜇g/mL, 25 𝜇g/mL, 50 𝜇g/mL, and 100 𝜇g/mL, respectively. The results of the present study support the anti-cancer activity of the formulation KMNC. The results of the present study support the anti-cancer activity of the formulation KMNC. KMNC is demonstrating its effectiveness as a tumor and oral carcinoma cancer prevention. Numerous pharmacological actions, including as antibacterial and anticancer properties, are present. This cancer treatment is inexpensive and doesn’t have any negative side effects. The anticancer properties of KMNC have been documented in scientific journals. In this paper, the anticancer activity of KMNC is discussed in the context of several in-vitro researches.

INTRODUCTION
Any material or product that is employed to study biological processes or conditions that are pathological to improve the recipients’ health and their interactions with other living beings is considered a medication. Pharmacology is the branch of science associated with the investigation of medicines. A religious leader or holy man was frequently the one having experience with the use of drugs [1]. Numerous traditions, including the Chinese, Greek, Indian, Roman, Persian, European, also several more, assisted in the creation of medications in ancient times. It was believed that drugs had supernatural abilities. It was understood that the pro-
cess to acquire consistent pharmaceutical preparations needed to be standardized from the start of the first century BC. With the advancement of science also the creation of scientific research techniques. New medications are introduced carefully and after taking careful precautions. We chose therapy for cancer prevention because of the high rate of cancer and the sophisticated scientific study methodology. The phrase "anti-cancer" refers to holistic treatments that work to either prevent the onset of cancer or to supplement more traditional medical treatments [2].

**A bird view of oral cancer:**

The squamous epithelium, an uppermost layer of the mucous membrane, is where oral void cancer most frequently begins. The squamous epithelium is composed of flat, thin cells. Carcinoma that affects oral squamous cells is the medical term for this specific kind of cancer.

Cancer is a condition marked by unchecked cell growth, which can result in a mass of tissue. Any unusual cell proliferation in the mouth cavity is dubbed as oral cancer. Head and neck tumor can also refer to cancer of the oral cavity. The most significant risk factors for head and neck cancers include alcohol consumption also tobacco utilize, as well as smokeless tobacco, such as betel cigarettes, is tobacco for chewing and snuffing also a potential risk associated with oropharyngeal cancer includes the virus known as human papillomavirus (HPV), especially HPV-16.

Symptoms and warnings include lumps and nodules, red regions, ulcers, problems with swallowing, a white, soft-style scaly plate, with inflammation also additional symptoms, such as oral numbness, burning, or dryness, which do not have a clear cause and extraordinary complexity speaking or swallowing.

Oral squamous cell carcinoma, which accounts for almost 95% of all cancers and is ranked sixth globally, is the most prevalent type of cancer. For patients with head and neck cancer, OSCC continues to be the predominant origin of mortality as well as morbidity. Worldwide, it is anticipated that 14.1 million novel instances of cancer will be diagnosed in 2012, 8.2 million people will pass away from the disease each decade, and a cancer diagnosis will be made in 32.6 million people within five years.

Chemotherapy, radiation therapy, and surgical procedure are just a few of the medications and procedures available today. A number of the majority frequently prescribed medicines for oral cancer include carboplatin, bleomycin, methotrexate, and 5-fluorouracil (5-fu). A few side effects of the medication are brought on by prolonged use. Treatment for a number of tumours, including eye damage, neurological damage, neck and head cancer additionally, medications like cisplatin are used to treat abnormalities in electrolytes such as hypokalemia, hypomagnesemia, and hypocalcemia [3]. Contrarily, 5-fluorouracil results in diarrhoea, pain, reddishness, hand, and foot erythema, and peeling. Adverse effects of bleomycin include allergic reactions, dizziness, disorientation, and respiratory problems. Weakness, diarrhoea, and birth abnormalities are adverse effects of methotrexate. Pruritis, atypical GIT discomfort, constipation, and vomiting are side effects of cetuximab. Radiation therapy does, however, have some adverse effects, including xerostomia, oral candidiasis, mucositis, and osteoradionecrosis of bone inside the radiation field, primarily in the mandible [4].

**Healthcare approach based on Siddha/Siddha’s philosophy of medicine:**

Since there are more people choosing natural medical remedies as well as herbal health drugs rises daily, Indian healthcare systems are receiving recognition on a worldwide level. The Siddha method should be propagated across the globe right now. Older than the first civilization, Siddha medicine has a very antique source. The accurate time edge of it’s real cannot be masked because it is mentioned openly that it existed before the stone’s stand appeared.

Siddhars, eminent researchers, as well as spiritual masters chronicled their experiences for the benefit of humanity in palm leaf documents, scriptures, and other works, some of which have been studied and many of which have not. Siddhars are typically thought of as superhuman beings who have described ages also other natural laws. The earliest Tamil medicine included elements of the Siddha School of treatment. The people receive a low-cost, effective service from it. This system’s goal is to maintain healthy physical and mental health. Siddhars had thoroughly researched the causes of illnesses, their effects, and the effects of all treatments. As a result, they were able to determine what was essential to their survival and what was not.

Under the Siddha framework, oral cavity carcinoma usually refers to via either Kanna putru or Vaai putru [5]. The hallmarks of carcinoma as now understood by modern science are consistent with descriptions of the condition in Siddha texts under multiple titles like Vippurudhi, Putru noi, Pilavai, Kazhalai.
Numerous plants, herbals, metallic substances, toxins, minerals, salts, also other organic materials have been produced by nature. Metals are vital cellular components that the environment has specifically chosen to work in several essential biochemical practices for living cells.

Redox operation, variable interaction modes, and reactivity in the direction of organic substrates are only a few of the distinctive properties that metals have. Owing to their reactivity, metallic substances were subject to strict regulation in a healthy environment, and abnormal (aberrant) metal ion levels were linked to some pathological illnesses, including malignancy. The investigation of anticancer action is a result of the exceptionally dissimilar structural chemistry also interactions of metallic composites through biomolecules like as nucleic acids and proteins [6].

Based on this wealth, with the significance of the medicine of Siddha and its better efficacy, nanoparticles naturally occurred in our research medication, in order to lessen the negative effects of alternative pharmaceuticals, as well as cost-effectiveness, because of a rise of frequency throughout the worldwide community. It will be a wise decision to use “Kaalamega Narayana Chendooram” as a tool against cancer of the oral cavity with more effectively.

Oncology epidemiology:

Over 20 lakh people in India receive a new cancer diagnosis each year and nearly 11.5 lakh people die from the disease. In India, more women are receiving new cancer diagnoses each year; based on the more recent Worldwide Oncology Survey published by the WHO. In the year 2021, in India, 8.37 lakh women received diagnoses of carcinoma, compared to 7.62 lakh men. In India, the rate of death from cancer is nearly the same for both men also women. In 2020, 5.36 lakh males in India passed away from cancer, compared to 3.16 lakh women. Indians have a one in ten chance of developing cancer under the age of 75 and a one in one hundred chance of passing away from the disease.

During 2020, 8.6 million people were born to 17.7 million people. In 2020, there were 11.2 million fewer people overall than there were in 2020, the International Association for Study in Cancer (IARC) 2019, according to estimates, there will be 21.8 million new cases of cancer and 9.2 million fatalities due to cancer during 2019. The three most often reported malignancies worldwide were lung, breast, and colorectal (1.4 million, 9.7%), then mammary (1.7 million, 11.9%), as well as lung (1.8 million, 13% of the total).

The bulk of cancer-related deaths were caused by lung cancer (1.8 million, 20.8% of all cases), liver cancer (1.2 million, 10.8%), and stomach cancer (0.9 million, 10.2%). The greatest number of carcinoma-related fatalities were caused by lung carcinoma (2.8 million, 20.7% of all cases), cancer of the liver (0.9 million, 9.8%), well as tumors in the stomach (0.78 million, 10.2%).

According to estimates derived from IARC 2012, the ageing and growing world population would result in a significant increase to 19.3 million new carcinoma cases annually by 2025. Poorly developed regions of the globe reported more than half of the worldwide cases of cancer (56.8%) and cancer deaths (64.9%) in 2012, and these proportions will rise even more by 2025 [7].

Causes:

90% to 95% of cancer diagnoses are linked to environmental factors, with genetics only accounting for 5% to 10% of instances. The term “environmental,” as used by oncology investigators, refers to any element other than pollution, including habits, economic, and behavioral components. The consumption of tobacco (28-32%), dietary habits and overweight (32-37%), viruses (18-22%), irradiation (both of which irradiation and not ionizing up to 10%), nervousness, inactivity, and pollutants are some of the factors that might cause radiation sickness. Since the majority of cancers possess multiple potential origins, it is practically hard to determine what triggers carcinoma for a specific individual. For instance, if a heavy smoker gets cancer of the lung, this likely came from smoking. However, while everybody has a slight likelihood of getting lung cancer from exposure to radiation or air pollution, there is a tiny opportunity that the cancer was brought on by these factors [8].

A Medical Evaluation / Medical Evaluation

Definition of Chendooram:

A family medication known as chendooram is made using either metal or mineral (As, Hg etc.) in subjecting them to sublimation and grinding them with par-
ticular liquids, distillates, or concentrative, or calcinations, nor burning, nor frying, nor insolation process until the product develops its distinctive redness.

**Method of Formulation:**
With a few exceptions and variations, two preparation procedures are often used in their processing. Examples include:

1. Sublimation is achieved by making use of a bath of sand.
2. Calcinations.

**Employing a sandy bathtub for sublimation technique (Kuppi Erippu):**
Since the chendooram contains both sulphur and mercury in it, the sulfur should be pulverized to a fine powder in the mortar before adding the specified amount of mercury. Grinding should continue until a black, impalpable mobile fine particle is acquired. It shouldn’t come before any other ingredients.

The medicinal ingredients are kept in a heat-challenging glass container with an extended neck in the standard configuration of the sand-bath sublimation device. Ceramic dishes have also been utilized. These containers are wrapped in seven layers of cloth ribbons that have been covered in clay before being used, leaving the flask’s mouth exposed. The glass flask has to have been maintained securely stored in its freshly encased form to ensure its covering dries correctly.

It has lately come to light that enameled iron basins were used as an alternative of glass containers. When utilizing enameled iron basins, it’s crucial to choose two similar basins that are the appropriate size and capacity, inspect the rims to provide a tidy finish when stacked together. A bonding wire (metal) can then be used to connect the two basins by inserting tiny holes around the edges. The middle of the bottom of one of the basins is then pierced. The bowls should be bound and secured after being constructed this way by glueing the binding wire through the margin holes. The consequence would be the formation of a capsule with a top orifice.

The central opening is left exposed by the fabric tape covered with clay, just like a glass container. A probe is introduced through this opening to look inside and observe the internal response. The sand bath is constructed by filling an enormous earthen trough with two centimeters of coarse or fine sand or gravel at the bottom.

The capsule containing the medication ingredients is carefully cantered and set on neither gravel nor sand. The sand was then stuffed into the sidewalls, leaving the peak two centimeters empty and the capsule exposed. The neck of glass containers should be barely visible above the sand when being used. Heat is applied to this configuration by burn up firewood in the oven.

Their gradations are discernible when heat is applied. The only way to comprehend and grasp these three stages—mild, moderate, and intense—is to have some knowledge. According to Deepakkini, once the fire’s flames converge to form just one tongue from blazes, like the light of a lamp. Once numerous of such fire tongues lick on the vessel before dispersing like a lotus flower, it is mild (Kamalakkini). If the oven is filled to the brim with flames, increase the sandy pool. The Katakkini stage represents the fire’s most fierce one.

The preparatory technique calls for control as well as adherence to these fire stages. The heating typically lasts for three days straight. The light, medium, and strong stages remain under these conditions for an additional day daily.

The mixture of drugs included within the sachet will eventually disintegrate, based on the ingredients as well as the level of sulphur in the formulation. Yellow vapor emerges through the aperture first, which is sulfur. Later, a jet of blue flame will be released as it begins to burn. A long steel probe is pushed into the aperture just as the blue light goes out, and the component that goes into the container is going to have a white covering when it is brought out. Once the investigation is not blackened and a whitish coat, it is not recommended to close the probe and continue heating it for either one nor two hours before turning off the heat and letting the setup cool naturally. If the sulfur is still present and hasn’t the probe’s tip is going to be entirely burned and will be covered in a black, viscous covering.

The mud strip winding is removed after the setup has chilled, along with the pill capsule holding the medication. To release of the chemical, it is lightly tapped using the proper mixer or elevated using a spatula that has sublimed in the top portion of basin. In a mortar, thoroughly grind the sublimate you’ve acquired. It shouldn’t come before any other ingredients.

**Calcination (Pudam)**
The fine particles are pulverized in a kalvam for a predetermined amount of time with predetermined fluids. Small discs of the paste are formed, and then dried. They are placed in earthen cups (mann agal), each of which is wrapped by another and has a well-
sealed edge made of earthen fabric. It is getting time to dry. The cups are set at top cakes of cow dung, where they are burned. For pudams, earthen pits of varied depths and sizes are commonly dug. Cakes of cow manure cover half of the pan. The mud cups are enclosed once more with dessert prepared using dung from cows. In each of the four faces of a system, the blaze is positioned near the middle to provide even heat from all angles.

All the metallic substances and other constituents are removed following standard purification. Only in particular situations is specific purification (Sudhi) recommended; otherwise, simply mentioned in the Materia-Medica publications, it should be considered as a general purifying procedure for medicine.

Another preparatory strategy:
1. Unheated preparation (Araippu Chendooram)
2. Unguarded -heating (neither Erippu nor Varuppu Chendooram) preparation
3. (Lagu Puda Chendooram) is produced by employing heat in an area close to 100 °C.

Characterizations for Chendooram
1. Chendooram has a crimson color, fine-grained particles, and no flavor.
2. They provide therapeutic benefits when used with the proper adjuvant.
3. For 75 years, they are supposed to retain their potency [9].

Resources and Methods
Option of the Medication:
For the purposes of this research the complex medication formulation treating cancer of the oral cavity referred to within the primary "Athmarakshamirthham Ennum Vaithiya Saara Sangeraham" by Kandhasamy Mudhaliyaar is a text of the Siddha, First issue 1931 was a metallic-mineral formulation “Kaalamega Narayana Chendhooram” [10].

The components of the medicine / The drug’s ingredients
Potassium nitrate (vediuppu), Copper sulphate (thurusu), Aluminium potassium sulphate (padikaaram), Sodium bicarbonate ( Borax)(Vengaram), Ammonium Chloride (Navacharam), Sodium Carbonate (Pullers Earth) (Pooneuru), Red sulphate of mercury (Jaalthilingam), Sulphur (Gandhagam), Sodium chloride ( Kalluppu), Hydragryrum (Rasam), Tri sulphate of Arsenic (Yellow Orpiment) (Aritharam), Di sulphate of Mercury (Red Orpiment) (Manosilai).

Collection of the unprocessed supplies:
The raw supplies were obtained at M/s. R.N. Rajan rural medicine stores in Parrys Corner, Chennai.

Drug recognition as well as verification:
The government-authorized laboratory experts verified and approved the raw materials. For future usage, each raw materials test specimen has been kept separate.

Drugs are cleaned up:
According to the traditional Siddha literature, the purifying procedure was carried out.

Refining of Potassium nitrate (Vediuppu)
100 grams of potassium nitrate, 400 grams of aqua, 100 grams of effervescences buttermilk, and 100 grams of lime fruit juice are required. The potassium nitrate was mixed with water and slowly boiled over a low flame. For every 1400 gram of potassium nitrate, four white yolks of eggs were mixed, and any bubbles that formed from impure substances were then scooped out using a spoon made of timber. Then another pot was used to transfer all of the supplies, closed through clay-glued substance, maintained in a space without aeration, and then filtered. The water had been filtered, and the salt had been exposed to sunlight the following day. These steps were replicated seven times to cleanse it.

Padikaaram’s refinement (Aluminium Potassium Sulphate (Alum)):
Before filtering, alum-containing water was dissolving within it and heated. After cooling, a refined version was created.

Thurusu’s refinement (Coppersulphate):
Sulphate of copper was burned till it turned yellow.

Vengaram’s refinement (Sodiumbiorate):
The mineral borax remained controlled as well as suspend in a solution made from buffalo dung, and then boiled. It was washed through clean water also isolated in order to obtain the bundle in a pure state.

Refining of Ammonium chloride, or Navacharam:
It was purified by being dissolved in warm water and followed by filtering. The salt was created in a pure state after cooling and being put into a container with a wide opening. It was kept in a glass bottle with a small amount of jequirity root.

Refining of Kalluppu (sodium chloride):
It was cleaned with fabric material and purified by being liquefied in vinegar before being dehydrated within a shaded area.
Pooneeru refines (impure sodium carbonate):
1.3 liters of Fuller’s earth were soaked in 5.2 liters of dew water and subsequently permitted to settle. The top unguent layer was eradicated the following morning after it had been thoroughly churned. A pure version was created by isolating the leftover mixture on ceramic plates. Ten times each, after which it was placed into a glass bottle.

Rasam’s refinement (Mercury)
Mercury weighs 35 grams, 100 grams of while brick powder, 100 grams of turmeric powder and 1.3 liters of acalypha juice (Acalypha indica). Mercury was triturated for one hour each with brick flour also turmeric fine particles, and then rinsed by means of water. After adding the Indian Acalypha juice, the mixture was simmered until the concentrate had fully evaporated.

Lingam (Cinnabar) purification:
Cinnabar was purified by fusing it with lime juice, cow’s milk, as well as Acalypha indica sap in identical parts. It gave Cinnabar its refined, potent form.

Thaalagam (Yellow Orpiment) process:
35 grams of Arsenic trisulfide, the ingredients include 1 lit of cow urine, 0.3 lit of Indian acalypha fluid, also 300 grams of limestone. To purify arsenic trisulfide, it was wrapped up and boiled while submerged in a concoction of urine from cows, limestone, and Acalypha indica extract.

Refining of Gandhagam (sulfur)
35 grams of Sodium, 35 grams of Butter, and 150 mL of cow’s milk are required. A spoon made of iron was filled with sulfur. The substance was layered in cow’s milk at an angle after being topped with butter and heated on the spoon until the butter had dissolved. Sulfur was refined by repeating the process roughly seven

Refining of Manosilai (Red Orpiment)
Red Orpiment 35 grams, 125 ml of butter milk from cows are needed. Three hours were spent triturating red orpiment in cow’s buttermilk. To get an improved form, it was dried [10].

The trial specimen medicine “Kaalamega Narayana Chendhooram” is being prepared.

Process:
1. 842 grams of the eighth potassium nitrate and aluminum potassium sulfate solution were used.
2. 211 grams each of sodium bicarbonate, ammonium chloride, sodium chloride, and copper sulfate were carried out and then combined with 105 grams of sodium carbonate.
3. The previously mentioned ingredients have been divided into three parts and then pulverized finely.
4. After being put through a distillation process, the first portion of the fine particles were combined with the second portion and dried.
5. The third part of the fine particles was combined with the second part after being dried. Then a distillation process was performed on the remaining powder.
6. After distilling the third portion of the powder, the finished product was removed and stored in a locked bottle.
7. The following ingredients were blended with the end product of distilling over a period of twelve hours (4 saamam): 525 grams of Red Sulfate of Mercury, 350 grams of Tri Sulfate of Arsenic, Yellow Orpiment, 420 grams of Sulphur, as well as 140 grams of Di Sulfate of Mercury, Red Orpiment.
8. The dehydrated tiny particles were preserved in a clay pot that was plastered with seven layers of mud paste to seal it.
9. The earlier preparation was kept in another clay pot that had been filled with a tiny amount of sand, and the plaster that had been clay-glued sealed the lid.
10. The mud pot was light with an Aavarai stick for a period of thirty hours about (10 saamam), after which “Chendhooram” was obtained.

The description of a medicine
1. Pharmaceutical labeling : Kaalamega Narayana Chendhooram (KMNC)
2. Recommended quantity :Chendhooram 244 mg [1/2Panavedai]
3. Course :Oral
4. Adjuvant :Honey-infused thipili chooranam (bd for48 days– 1 mandalam)
5. Symptoms : Kannaputru [Oralcarcinoma], Elaippu [Tuberculosis], Kuttam 18 [Hansen’sailment]
Modern aspect of the Disease

In-vitro techniques

In-vitro cytotoxicity investigations on cancer cell lines use an assortment of cell labeling techniques to inadvertently count the number of live cells that remain after treatment. The primary feature of ideal in-vitro tests for determining cell proliferation and cytotoxicity should be that they are quick, easy, affordable, repeatable, sensitive, safe, and effective for a wide range of viable cell populations without interfering with the compound’s evaluation.

Benefits

1. Lessen the use of animals and time using
2. Exploiting several aspects of the cell to test the compound’s capacity to destroy the target cell
3. Capable of processing a lot of chemicals quickly with a small amount
4. The concentration range employed is comparable to that anticipated for in-vivo research [11].

Negative aspects include:

1. A challenge to preserving the culture
2. Show negative outcomes for substances that are stimulated subsequent to metabolism also vice versa. Pharmacokinetics cannot be determined.

Desirable traits for an in-vitro assessment approach:

The ultimate in-vitro examination process would include the following qualities: simplicity, economy, reproducibility, speed, and sensitivity. The assay must be adaptable to many different tumor varieties and test substances. The selection of the lines cell should be as close as possible to a perfect representation of the clinical scenario. It should be reasonable to expect treatments in vivo to use an array of pharmaceutical levels similar to those used in vitro. An enormous number of samples could be swiftly and automatically processed by the assessment.

Data collection should be straightforward, understandable, and practical. A screening assay’s objective is to determine a compound’s capacity to kill cells while also being able to distinguish involving replicating cells

Screening techniques:

A crucial tool for creating novel, risk-free, and potent medications involves the pharmacology evaluation of substances from plants, minerals, and wildlife. Approximately eighty percent of people utilize medications made from plants and salts a minimum of once in their lifetime, and there are over 50,000 plants in the globe that offer therapeutic benefits; the chemical diversity of medicinal plants and minerals is crucial for the identification of novel active compounds against a variety of cancers.

Anti-cancer medications have been created using cytotoxic active ingredients from a variety of medicinal plants with minerals. Monitoring patients’ standard of life while receiving cancer therapy is now required. It is wise to be aware that chemotherapeutic drug treatment for cancer significantly reduces the patient’s standard of life, even long after the drug has been stopped.

Therefore, at this time, the difficult issue is to create quick and innovative ways that might identify and produce compounds that may be useful in treating human carcinomas.

One of the focus topics for which affordable, effective treatments are not yet accessible is cancer. This is likely because the pathophysiology of cancer is not well understood. For such a dreadful disease, anti-cancer drugs were developed using an array of sources that include organic compounds (vegetation as well as microbes) and chemical compounds.

Among the root causes of treatment failure is the development of anticancer medication resistance.

The often prescribed medications known as cancer chemotherapy medications have a variety of adverse reactions, including hair loss, nausea, and vomiting as well as bone marrow suppression. This calls for the screening of many different chemicals. The systematic testing of anticancer medications is done for this aim using both in-vitro models [12].

Tetrazolium Salt Assessment (also known as the MTT otherwise micro-culture tetrazolium analysis)

Tetrazolium Salt Evaluation (commonly referred to as the microculture tetrazolium analysis or simply MTT):

A popular in-vitro technique for evaluating anticancer medicines is the MTT evaluation. Despite the fact that there are numerous approaches to measuring living cells, additional staining techniques are also employed; however, these have the drawback of requiring washing stages, which lengthens processing period and introduces sample variation. The multi-well disc scanning spectrophotoscopes are capable of swiftly and accurately measuring a large number of samples.

The colorimetric examination of living cells should ideally use the colorless substrate that can only be
altered by living cells—neither non-viable nor dead cells nor culture medium—to produce a colored result. Conversely the MTT evaluation uses a shade reaction to gauge the viability of cells.

The assay requires mitochondria dehydrogenating enzyme in living tissues or cells with metabolic activity cells to reduce the tetrazolium salt 3-(45-dimethylthiaizol-2-yl)-25-diphenyl tetrazolium bromide and generate a bright blue Formosan result. The viability of the cells has a direct relationship to the quantity of blue formazan produced. During the log phase of incubation, trypsinized cell lines from a particular line of cells are measured using a hemocytometer, also before being injected into various multi-well discs (typically 96-well discs), the material is diluted to the correct density using a suitable environment.

For a particular amount of time (often 1–4 days), the cells are processed with different drug concentrations (in duplicates). Then, every well receives MTT staining, and dishes are placed using an incubator filled with CO2 at around 37°C over 4 hours.

Now remove the discs from their incubators, also at room temperature, dark navy with isopropanol or DMSO, the Formosan crystalline form is completely liquefied. An ELISA scanner then uses 570 nm to inspect the dishes. The calculation below is used to compute the proportion of viable cells relative to a control group.

Percentage of viability cell lines = (OD of the cell lines treated / OD for the unaltered cell lines) x 100

We have utilized this assay successfully. When used as a solvent, DMSO quickly dissolves formazan and serum, and using spectrophotometric- status DMSO results in steady "background" absorbance stages. Although these compounds are employed, other solvents such as dimethyl formaldehyde, C₃H₆O, C₆H₁₄, also others serum shouldn’t be solubilized in levels greater than 0.0625 %.

The advantage of this approach is the fact that hundreds of lines of cell specimens in microtiter plates can be tested simultaneously allowing researchers to compare the effects of different drug doses on each drug under investigation. As an outcome, this examination may be used to determine the medication’s IC50 (the amount of medicine needed to cause 50% of cells to proliferate) level.

Additionally, this assay is straightforward to perform. Both adherent as well as suspension lines of cells can utilize it. With this procedure, many medications may be promptly tested for anti-proliferative action; it is cheap, only a small number of cells are needed, and it is manageable. The assay, however, has the flaw of producing erroneous results since it includes cells that may be metabolically active but are not liable to divide (non-replicating). Additionally, medications whose mode of action may additional mitochondria cannot produce favorable outcomes in this evaluation, particularly for brief incubation durations. In addition, the handling of DMSO by laboratory staff must be safe.

Contemporary Feature for Anticancer Drugs:
Anticancer medications are those that work to prevent cancer. Another name for them is antineoplastic medications. Cycle-specific-specific and non-cyclic-specific-specific might be used to categorize them.

Figure 1: a. E.coli precise medium in a culture dish; b. K. pneumonia –precise media on a culture dish

Figure 2: c. S. aureus precise medium on a culture dish; d. P. aeruginosa precise media on a culture dish

Figure 3: e. B. cereus precise media on a culture dish; f. C. albicans precise media on a culture dish

Typical adverse reactions for anti-cancer medications:

1. Because anti-cancer drugs do not specifically target one type of cell, they frequently induce negative side effects.
2. Hair follicle damage causes frequent cases of hair loss.
3. Anaemia
4. Immune system disorders
Table 1: Following are some examples of how various assays exploit distinct cell characteristics

<table>
<thead>
<tr>
<th>S.No</th>
<th>Tissue Characters</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9348</td>
<td>Study with a tetrazolium salt (MTT)</td>
</tr>
<tr>
<td>2</td>
<td>Creation of proteins as well as composition</td>
<td>Sulphorhodamine Binvestigation</td>
</tr>
<tr>
<td>3</td>
<td>Creation of DNA as well as composition</td>
<td>Uptake of H-Thymidine Using flow cytometry, more recent fluorescent analogs a clonogenic test</td>
</tr>
<tr>
<td>4</td>
<td>Tissue consistency</td>
<td>Color sensitivity studies</td>
</tr>
<tr>
<td>5</td>
<td>Clonogenic Characters</td>
<td>Clonogenic analysis</td>
</tr>
<tr>
<td>6</td>
<td>Tissues separation</td>
<td>Counting the analysis of the cell</td>
</tr>
</tbody>
</table>

Table 2: Here is a chart with the information

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Outcome</th>
<th>Pattern</th>
<th>As instructed by AYUSH / WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbes at work</td>
<td>Insufficient</td>
<td>NMT105CFU/g</td>
<td>As instructed by AYUSH norms</td>
</tr>
<tr>
<td>Fungi at work</td>
<td>Insufficient</td>
<td>NMT103CFU/g</td>
<td></td>
</tr>
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Table 3: Results from Specified Infectious organisms on KMNC

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<thead>
<tr>
<th>Microbes</th>
<th>Structure</th>
<th>Findings</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-coli</td>
<td>Insufficient</td>
<td>Insufficient</td>
<td>As instructed by AYUSH norms</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>Inactive</td>
<td>Inactive</td>
<td></td>
</tr>
<tr>
<td>B. cereus</td>
<td>Insufficient</td>
<td>Insufficient</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>Insufficient</td>
<td>Insufficient</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Shows the area of immobility in mm as well as the proportion of conventional medications

<table>
<thead>
<tr>
<th>S. No</th>
<th>Experimental Cells</th>
<th>Stimulation area width in millimeters</th>
<th>A proportion of the mean KMNC-inhibition area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin Standardization Control samples (Bacteria)</td>
<td>KMNC</td>
</tr>
<tr>
<td>1</td>
<td>E. coli</td>
<td>26-34</td>
<td>15-21</td>
</tr>
<tr>
<td>2</td>
<td>P. aeruginosa</td>
<td>32-36</td>
<td>14-18</td>
</tr>
<tr>
<td>3</td>
<td>S. aureus</td>
<td>29-33</td>
<td>14-19</td>
</tr>
<tr>
<td>4</td>
<td>K. pneumonia</td>
<td>28-33</td>
<td>12-15</td>
</tr>
<tr>
<td>5</td>
<td>B. cereus</td>
<td>27-30</td>
<td>10-12</td>
</tr>
<tr>
<td>6</td>
<td>C. albicans</td>
<td>15-19(Clotrimazole)</td>
<td>11-15</td>
</tr>
</tbody>
</table>
Table 5: An explanation of an individual medium together with its abbreviation

<table>
<thead>
<tr>
<th>Experimental Cells</th>
<th>Symbol</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-coli</td>
<td>EC</td>
<td>EMB Agar</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>KP</td>
<td>Deoxycholate agar</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>PA</td>
<td>CetrimideAgar</td>
</tr>
<tr>
<td>S. aureus</td>
<td>SA</td>
<td>Mannitolsalt agar</td>
</tr>
<tr>
<td>B. cereus</td>
<td>BC</td>
<td>Deoxycholate agar</td>
</tr>
<tr>
<td>C. albicans</td>
<td>CA</td>
<td>EMB Agar</td>
</tr>
</tbody>
</table>

Table 6: Microbial and fungus diluting

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Dilution</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbes</td>
<td>$10^{-4}$</td>
<td>6</td>
</tr>
<tr>
<td>Microbes</td>
<td>$10^{-6}$</td>
<td>5</td>
</tr>
<tr>
<td>Fungus</td>
<td>$10^{-3}$</td>
<td>zero</td>
</tr>
<tr>
<td>Fungus</td>
<td>$10^{-2}$</td>
<td>zero</td>
</tr>
</tbody>
</table>

Table 7: Antimicrobial property: Antimicrobial activity results of KNMC microbes

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Bacteria</th>
<th>Specimen</th>
<th>Level ($\mu$g/mL)</th>
<th>Region of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative</td>
<td>E. coli</td>
<td>KMNC</td>
<td>Streptomycin(100$\mu$g)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td></td>
<td>Streptomycin(100$\mu$g)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td></td>
<td>Streptomycin(100$\mu$g)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>23</td>
</tr>
<tr>
<td>Gram positive</td>
<td>S. aureus</td>
<td>KMNC</td>
<td>Streptomycin(100$\mu$g)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>S. mutans</td>
<td></td>
<td>Streptomycin(100$\mu$g)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>24</td>
</tr>
</tbody>
</table>

14mm–Minimal susceptible, 15mm –Modest, beyond 16mm– Extreme susceptible

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Table 8: Survivability of percentage KB Cell

<table>
<thead>
<tr>
<th>Sample Concentration (μg/ml)</th>
<th>Average O Dat 540nm</th>
<th>Percentage Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9348</td>
<td>—</td>
</tr>
<tr>
<td>6.25</td>
<td>0.6245</td>
<td>69.46</td>
</tr>
<tr>
<td>12.5</td>
<td>0.5372</td>
<td>55.06</td>
</tr>
<tr>
<td>25</td>
<td>0.5049</td>
<td>51.67</td>
</tr>
<tr>
<td>50</td>
<td>0.4105</td>
<td>40.33</td>
</tr>
<tr>
<td>100</td>
<td>0.3547</td>
<td>35.34</td>
</tr>
</tbody>
</table>

5. Clotting issue brought on by the death of certain blood-forming tissues, which results in fewer red, white, and platelet tissues.

6. Depression related to the marrow in the bones

7. Esophagitis, glossitis, with GIT-stomatitis.

8. Amenorrhea in females and decreased sperm production in males

9. Vomiting and nausea are instant negative effects.

10. Renal failure is brought on by hyperuricemia (boosted plasma uric acid levels).

11. Carcinogenicity (cancer-causing potential).

Anti-Microbes Activity

Due to the increasing occurrence of microbe isolates that have become immune to the pharmaceuticals used in conventional anti-cancer treatments, there is a rush in the development of new medicines that do not rely upon synthetic anti-microbial agents for controlling the signs of recurring cervix carcinoma illness. The antibacterial activity on the MTCC strains using E. coli, K. pneumonia, P. aeruginosa, S. aureus, B. cereus, as well as C. albicans was noticed using the Hole plate diffusing methodology in this study as a portion using the efficacy of presentation for KMNC in lung, oral, tumor, bench and benign carcinoma conditions [13].

In the evaluation of therapy against them, most of the mentioned infections showed 50% or alternatively increased responsiveness with KMNC as compared to Ciprofloxacin control. During experiments with the microbes’ P. aeruginosa, E. coli, as well as K. pneumonia, the study medication had better results.

A typical drug was used in this study to serve as a reference. Ciprofloxacin was utilized for the antibacterial experiment; while Candid-BTM cream ( clotrimazole-betamethasone), a brand-name medication, was used for the antifungal research. KMNC demonstrated a roughly equal impact on C. albicans.
to that of Beclomethasone (Candid-BTM) and regular Clotrimazole. The study demonstrates the antimicrobial effectiveness of the Siddha preparations KMNC used in the treatment of lung, oral, tumor, bench, and benign carcinoma against a variety of bacteria that cause infections as a helpful supplement.

With respectable wide-spectrum antimicrobial properties versus all gram-negative bacteria also bacteria-positive microbes, the Siddha Products KMNC was tested (Figures 1, 2 and 3), using E. coli microbes, showing an improved adaptability area of approximately 70 in comparison to the conventional beneficial regulation Ciprofloxacin (Ranbaxy). The sensitivity of the trial medicine for the additional species listed in Table 1 was also nearly fifty percent greater than that of ciprofloxacin. The experimental drug, KMNC, has a C. albicans fungal sensitivity to Clotrimazole as well as Beclomethasone, which is quite comparable and approximates 88% (Candid-BTM). As a result, due to the antibacterial effect of this medicine, it may also be helpful in treating pelvic inflammatory illnesses when taken to treat lung, oral, tumor, bench, benign cancer concerns.

An antibacterial investigation was conducted at IIT Madras in Tamil Nadu.

Microbiological Stack Sterility Appraisal Using the Pour Plate Method

The plate counting approach was one of the most popular techniques since it permitted the recording of living cells. It additionally helps to confirm the sterility of the product. After the necessary incubation period, the organism’s spread was controlled through a distinct arrangement of cells observed in the infected or unsterile specimen (formulation), which encouraged the growth of the living thing whenever it came into contact with a rich in nutrients environment. Colonies are identified using colony-forming units (CFUs) [14].

Essential Substances Desired:

Medium Muller Hinton Agar (1 L)
A Medium Muller Hinton Agar (MHI Agar Medium), the medium, a commercially viable product, had been dispersed in 1000 cc pure distilled water. The soluble media was sterilized at around 121 °degrees Celsius with 15 psi for fifteen minutes. While still molten, the autoclaved medium was thoroughly mixed also transferred (25–30 ml per plate) onto 100-mm Petri plates.

Nutrient broth (1L)
13.0 gram of marketable available nutritional media (HI Media) was dissolved in 1000 ml of distillate water and then heated to complete dissolution to create 1000 ml of nutrient broth. To sterilize the solution as necessary, it was then sterilized for fifteen minutes at around 121 degrees Celsius and 15 lbs under pressure. Streptomycin (10 mg/ml), a common antibacterial drug.

Test organisms cultured; culture growth adjusted to McFard’s norm, 0.5%

a. E. coli, S. aureus, P. aeroginosa, and b. S. mutans, withK. pneumoniae

Process
E. coli, S. mutans, P. aeroginosa, K. pneumoniae, and S. aureus microbe’s cultures were planted into Petri dishes having 20 ml of Medium Muller Hinton Agar (incubate of the culture was controlled by McFards Standard, 0.5%). Using a reliable cutter, wells of about 10mm were bored, and various sample concentrations, additionally, concentrations of 250 µg/mL, 500 µg/mL, also 1000 µg/mL had been injected. The plates were then incubated for 24 hours at around 37 degrees Celsius. The effectiveness of antibacterial agents was measured by estimating the diameter of the inhibitory area that was formed surrounding the hole (NCCLS, 1993). Streptomycin served as the beneficial reference.

Development of microorganisms

The microbial species used for the efficiency evaluation were K. pneumoniae, E. coli, S. aureus, B. cereus, P. aeruginosa, as well as C. albicans. These have been obtained through the MTCC in accordance with the National Committee for Clinical Laboratory Standards’ regulations and SOPs, Chandigarh, India, as well as subcultures. Stock cultures of bacteria have been kept at a temperature of around 4 °C on nutritional agar gradients. (Hello, media from Mumbai)

Disinfection and cleansing
Prior to being sterilized over a period of three hours in an oven with hot air at a temperature of 180 °C, the glassware utilized in this experiment had been rinsed with a cleaning liquid.

The whole nutritional medium was autoclave disinfected at 121°C and 15 psi for more than 15 minutes.

Development of test medication specimens
The experimental material, KMNC squat, was blended with two milliliters of pure water obtained through distillation to make it more viscous and manageable using a pipette.

The specimen being tested was then processed in around thirty minutes to establish an equivalent solution.
Screening for sterility with KMNC

Employing the disc plate method, the experimental composition passed sterility testing. The utilized sterile disc was filled with freshly organized nutritional agar material. Clean up or different kinds of streaking are not feasible because of the colloidal environment of the experimental combination. The experimental formulation was applied to the disc in a 200 \( \mu l \) concentration, and it was allowed to incubate for approximately 48 hours with intermittent monitoring. The incubation sheets were observed for 12, 24, and 48 hours after incubation; however, neither a single creature nor a community could be observed developing within the preparation.

Inoculums for cultivation

Organisms taken from the storage cultures were placed into a circular pattern for mildew testing employing Sabouraud dextrose broth (SDB) as well as twenty-four hours of growth at 37 degrees Celsius and 25 degrees Celsius, respectively, to form vigorous cells for testing utilizing tubes for testing with Mueller-Hinton broth (MHB) testing microbes. The colonies were suffering at 2.0x10\(^5\), the number of colonies per milliliter in newly created Mueller-Hinton broth containing Sabouraud dextrose to attain optically dense populations for microbes that are equivalent to 2.0x10\(^5\) colony forming elements [15, 16]. Method using a hole plate [17–19].

Dilution:

The diluents, sometimes referred to as the diluents unoccupied, are composed of a significant amount of sterilized water or saline and a smaller volume that has been precisely measured. Dilutions are often created in multiples of ten. The procedure was listed below with one dilution. Diluents are identical to dilution, and the sample's measurements are equal to the specimen's entire volume.

Procedure:

A sterilized Petri dish was first covered with 15 mL of boiling, 45°C-heated agar prior to the experiment's specimen being placed. By tilting and whirling the dish, the test sample also agar were well combined. Agar was given the freedom to develop into an adhesive material. Approximately ten minutes. To examine the growth of fungi, these discs were subsequently flipped also then maintained at a temperature of 37 degrees Celsius for a period of 24 to 48 hours. They were then left there for another 72-hour period. The CFU levels of the organism’s cells were then calculated [20].

1. The digits ranging from \( 10^{-1} \) via \( 10^{-7} \) have to be written on the samples used for dilution.

2. You can start diluting by reducing the original sample ten times and combining one milliliter of the actual specimen with nine milliliters of the diluted specimen \( (10^{-1}) \).

3. Rotate the tube forward and backward while the hands are connected to achieve an even dispersion of the microbes.

4. Transfer 1 ml of the sample to the dilute with empty \( 10^{-2} \), and beginning from the initial dilution, produce 100 dilutions using the initial sample employing a clean, new, one milliliter pipette.

5. The first sample needs to be 1000 times reduced using one brand-new, disinfected pipette by adding 1 cc of the \( 10^{-3} \) dispersion to the \( 10^{-3} \) dilute empty.

6. Continue this step if necessary, employing a fresh, clean pipette every single time, until 10,000,000 copies of the original sample have been dissolved.

7. Adjusting the pipettes as necessary; pour 1 ml of the solution into the relevant dilute solutions on sterilized Petri plates. Three Petri plates need to be employed for every dilution.

8. The nourishing media should be dissolved as well as refrigerated at 45 degrees Celsius in 15 cc for every Petri dish with the reduced specimen. Swirl the substance on every plate lightly to distribute the living cells across the medium.

9. These dishes have to be maintained for between 24 and 48 hours at 37 degrees Celsius while turning the plates often to allow the dishes to solidify.

Outcomes of contamination from microbes:

The microbial contamination of KMNC findings are shown in Table 2. The chart mentioned earlier proves that there were no living microbes present in the test ingredient, KMNC. Therefore, sterilized medication maintains the potency and effectiveness of the test substance.

Inspection

Keep an eye out for the development of microbe colonies on each plate. Use the following formula to get the bacterial density of the baseline suspension:

There are colonies (on average across 5 replicates).

Plate volume x dilution = organisms per millimeter.
RESULTS

There were no colonies or other indications of development on any of the plates where the KMNC was present.

Results for Specific Infectious organisms

The test medication KMNC was specifically checked for E. coli, S. aureus, also P. aeruginosa, but no signs of any of the four viruses were discovered. Figures 1, 2 and 3 display the plates of culture on which the results given in Table 3 were obtained.

Analytical evaluation

A culture dish containing media designed especially for K. pneumonia. Every study was repeated at least six times using three separate experiments, as well as the results were presented as mean SD. The statistical analysis made use of the Sigma Stat 3.5 programs (Origin Pro 8.0) by Systat Program, Inc. also a univariate ANOVA. Statistics deemed P values under 0.05 were considered significant in statistical terms. Three tests—one for every composition and organism—resulted in the value displayed in the data (Table 4). A series of millimetre amounts, ranging from minimum to maximum, is used to specify the area’s width.

Approach for Specific Infectious organisms

Residues of pesticides were collected [21, 22] and there was an analysis for aflatoxins [23]. Antimicrobials (both microbial as well as fungus burdens) are readily available in (KMNC) Table 5.

Investigation

1. It is the sole substance used to make Siddha herbal-mineral treatments that could potentially be tainted.

2. The microbe contamination in medicinal products not only speeds up degeneration but also reduces their efficacy.

3. An agar dish approach was used to count the number of microorganisms present in the KMNC.

4. The presence of microorganisms’ contamination has been checked in the load of microorganisms.

5. The harmful impacts created by bacteria renders the medications unfit for human ingestion because, rather than curing the illness, the tainted medication can lead contribute to the emergence of other illnesses.

6. Microorganisms can contaminate an experimental medicine, which not solely results in biodeterioration but also lessens its effectiveness. Here, both bacterial and fungal loads have been used to analyze the contamination of KMNC.

7. The total bacterial burden is one in a 10-4 dilution and zero in a 10-6 dilution.

8. The total fungal load is zero in a 10-2 dilution and two in a 10-3 dilution. Here, the level of KMNC pollution is within WHO guidelines. As a result, the medicine is gathered, ready, packed, and decontaminated before formulation.

Attention: Stock level approximately 10 mg/mL DMSO

1. Extreme susceptibility to S. mutans in 500 µg/mL

2. Extreme susceptibility to S. aureus in 250 µg/mL

3. Extreme susceptibility to E. coli in 250 µg/mL

4. Extreme susceptibility to K. pneumonia of 250 µg/mL

5. Extreme susceptibility to P. aeruginosa in 250 µg/mL

Comments:

A need to rediscover new antibacterial drugs in conventional medical systems emerges from the growing struggle against the currently accessible antibiotics. For the following microbes—E. coli, P. aeruginosa, K. pneumoniae, S. aureus, as well as S. mutans—different dosages of the test drug dissimilar amounts for the trial medication were contrasted with the standard medicine, streptomycin (100 µg/mL).

The findings show that KMNC may prevent all the earlier-mentioned organisms from growing in 250 µl, 500 µl, and 1000 µl/plate. Minimal susceptibility at 14 mm: modest susceptibility at 15 mm; also extreme susceptibility at or beyond 16 mm. The outcomes show that the Siddha medicine KMNC, which is used to cure ailments, has anti-microbial effectiveness against bacterial infections.

A difficult weapon will be developed using a medication’s clinical study findings in order to attain its antibacterial efficiency in malignancy.

Pharmaceutical activity of anti-carcinoma:
Determine an in vitro cancer prevention effect using an MTT evaluation. [24] Pune, India’s National Centre for Cell Sciences, KB (oral malignancy) lymphocytes were given, and DMEM (Sigma Aldrich, USA; DMEM) was used to cultivate them.

The line’s tissues were raised using DMEM enhanced by 10% FBS, L-glutamine, also sodium bicarbonate, as well as antibiotic fluid include penicillin (100 U/ml), streptomycin (100 µg g/ml), and ampicillin (100 µg/ml), amphotericin B (2.5 µg/ml) within a German company called Merck’s 25 cm² cell cultivation thermal container.

The growth media was eliminated after 24 hours, and there were five newly proposed complexes per 5% DMEM, progressively degraded by two-fold dispersion (in 100 µl of 5% DMEM, 100 µg, 50 µg, 25 µg, 12.5 µg, also 6.25 µg), as well as every level at 100 µl was triple-mixed and added to the correct wells. A mixture was then positioned into a humidified incubator containing five percent CO2 at around 37 degrees Celsius. Control cells that had not been treated were also remained alive.

After an initial direct examination of the cells under inverted contrast phase microscopy, the MTT evaluation technique was utilized to assess the health of the cells. These operate on a trypsinized, dual-day-old consolidated single-layer suspended over a ten percent incubation medium. The following two days saw the cells continue to proliferate at around 37 degrees Celsius in a humid environment using 5% CO2.

**Complex stocks preparation:**

1 mg liquid the specimen was measured and then dispersed 1mL liquid, utilizing a cyclomixer; DMEM. The test liquid passed through filtration via a 0.22-m Millipore syringe and an instrument screen to assure sterilization.

**Direct microscopic inspection of Anticancer Assay:**

This method was used to conduct an anticancer assay following a 24-hour treatment period. The microscopic observations were photographed using an Optika Pro5 CCD camera and an Olympus CKX41 inversion phase comparison culture tissue microscope. Cell morphological alterations like encircling or minimizing, graining, also the presence of vacuoles in the cytoplasm was considered a sign for cytotoxicity.

**Anticancer evaluation using the MTT technique:**

Fifteen milligrams in MTT (Sigma, M-5655) was added to three milliliters using PBS, and allowed to dissolve completely before filter sterilization. After the first day of the incubation process, the specimen material within each of the wells vanished also the solution containing MTT was reconstituted and 30 µl was transferred to every experiment as well as control cell sample. After a smooth shake and a 4-hour incubation period at 37 degrees Celsius, using moistened five percent carbon dioxide, the dish was finally removed.

After incubation, the extra liquid was withdrawn, 100 µl of MTT of the soluble phase solutions has been added (DMSO, Sigma Aldrich, United States), and the wells were softly stirred with pipeting motions up and down to disperse the crystals of formazan. The absorbance observations took place using a microplate reader with an effective wavelength of about 540 Angstroms.

The proportion of development anxiety was calculated using the following relationship:

Survivability proportion = (mean spectral density of specimens / average spectral density of control sample) x 100

Various amounts of KMNC (6.25–100 µg per 100 l of 5% MEM) were distributed throughout a single day. It turned out that as the amount of cells present decreased with increasing dosage, and at an amount of extracts roughly 50 µg/ml, 50% of the cells in question (KB cells) were lower than the typical control, shown in Figure 4. By comparing the O.D. of the treated cells to the control cells, it was possible to calculate the percentage of viable cells. With an emission wavelength approximately 540 nm, a spectrophotometer is a tool that measures the concentration of light. In order to compare values, inhibition of growth around 50% (IC50) in organisms subjected to specific medications is used (Tables 6 and 7).

The MTT evaluation’s findings indicate that the inhibitory concentration (IC) dosage for KMNC approximately 50 µg/ml. The vitality of KB cells declines with dosage. Using the basis of the KB cell line, it was discovered as the percentage inhibition of proliferation rose with higher levels with KMNC continuously reaching 6.25 µg/ml (Table 8), and the value of the inhibition coefficient (IC) was 50 and the coefficient of variation (R) was 0.9808.

**In-Vitro Anti-Tumor Activity In Cell development**

**Evaluation of the development of cells**

KB tissues were cultivated using three separate instances on 24-well dishes with a concentration of 1 x10⁵ bacteria/ml. The cultures received varying amounts of KMNC the next day (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml) and
were permitted to develop over 24, 48, and 72 hours. The living cells have been gathered, as well as the trypan blue stain rejection technique was employed to determine the number of cells that were viable.

**Evaluation of the growth of colonies**

Using 6-well dishes, the living things were placed with an average density of $1 \times 10^3$ organisms per milliliter. The cultures underwent exposure to KMNC at various levels over a total of a week: 6.25 $\mu g/ml$, 12.5 $\mu g/ml$, 25 $\mu g/ml$, 50 $\mu g/ml$, and 100 $\mu g/ml$. For a period of one week, dishes were incubated in an environment containing 5% CO2 at 37 degrees Celsius.

These colonies were then stained with 0.5 percent violet crystals after being fixed with a solution of 4% paraformaldehyde [25].

**Evaluation using soft agar plates**

The incubation medium of reference cell lines (5 $\times 10^3$ cells/ml) as well as cultures exposed to KMNC at different levels (6.25-100$\mu g/ml$) were blended around forty degrees Celsius using 0.35% agar (DNA status GIBCOBRL, United States) also 0.5% agar in the medium of culture had already gelled into 6-well dishes over twenty minutes at ambient temperature.

**Estimation of Apoptosis**

The cultures had been planted at an average density of 5 $\times 10^5$ organisms per well before being exposed to KMNC at various doses (6.25-100 $\mu g/ml$). The tissues have been taken away and given two PBS washes after being treated for twenty-four hours. According to the directions provided by the manufacturer, tissues had been stained using Annexin V-FITC (Annexin V-FITC apoptotic kit #3, in-vitro-gen), and the Cell Quest System was employed for analysing the outcomes of apoptosis by FACS.

**Estimation of Cell Apoptosis Using Flowing Cytometry**

The AnnexinV-FITC Apoptosis Screening Kit I (Clontech Laboratories, Inc., United States) was used to determine cellular apoptosis in accordance with the instructions provided by the manufacturer. In a 60-mm plate, cells taken from KB lines were seeded and then cultivated with a density of 6–10 units per ml. For 6, 12, also 24 hours, the cells were exposed to a free medium having different levels of ABC. Following trypsinization to extract the cells, they were once more rinsed with chilly PBS as well as centrifuged at around 1000 revolutions per minute. Re-suspending the cells in 100 $\mu l$ interaction buffers, centrifuging them at around 1000 rpm, after another five minutes, and collecting the resulting liquid were the next steps.5$\mu l$ pure annexin V-FITC also 10 $\mu l$ pure propidium iodides were added to the final mixture, followed by culturing at ambient temperature under darkness. Samples were examined using a flow cytometer at 488 nm. Within an hour of discoloration, the dispersion of cells was examined using an automated flow cytometer and the Becton-Dickinson Cell Quest programme tool. For every statistics file, 10,000 cells’ worth of information was gathered. Cells that were Annexin V-FITC positive and negative were known as apoptotic cells.

**Outcomes and Comments**

Among the Siddha metal-mineral compounds is called KMNC, a number of recent scientific tests have been conducted on it to establish its efficacy for both researchers and members of the public. To support the anticancer potential of KMNC against oral cancer, literary analysis, physicochemical also elemental investigation, toxicological research, with pharmacological studies were conducted. The positive effects are highlighted and addressed concerning their anticancer properties.

1. The effectiveness of the medication Gunapadam in treating carcinoma was discussed, along with Siddha and contemporary elements of the illness.
2. Reviewing the condition leads to knowledge of it.
3. Specific pharmaceutical evaluation describes the cancer prevention strategies and drugs used.
4. The medicine Chendhooram, as well as its qualities and effectiveness in treating carcinoma, are described in pharmaceutical reviews and lateral research, respectively.

**Discussion on the review of Gunapadam**

1. Padigaram could treat persistent ulcers.
2. Vengaram was employed to treat ulcers.
3. Thurusu was used to treat deep, persistent ulcers.
4. Lingam could treat deep ulcers
5. Navachaaram, a component of Veera mezhugu, exhibits powerful anticancer properties.

**A review of contemporary drugs discussion**

1. Alum exhibited anti-microbial properties, and microorganisms are an essential component in the growth of cancer [26].
2. Mercury aids in the destruction of cancer cells and slows the growth of tumors [27].

3. Cinnabar, orpiment, and copper sulfate all have antibacterial properties [28].

4. Borax contains healing and anti-inflammatory properties [29].

5. Blood malignancies can benefit from orpiment therapy.

6. Sulfur has anti-cancer properties.

7. Arsenic has anti-cancer properties [30].

**A review of pharmacology discussion**

1. The KB lines cell (oral carcinomadles cell) was used to create the cell lines for this anticancer properties assessment.

2. So, an innovative approach for validation is to analyze pharmacological properties using KB lines cell. They outlined KMNC’s potent anti-cancer properties.

**Discussion of the Drug Review**

1. Chendooram.

2. The product’s 75-year shelf life attests to its durability.

3. Its tiny particle size enhances its medicinal impact.

**Discussion of the tools and processes**

1. The Department of AYUSH approved the trial medication based on classical Siddha literature and the publication.

2. All of the constituents were obtained from an authorized vendor, and the experts identified and verified.

3. Therefore, the ingredients were both ideal and unique.

4. Our well-equipped lab was used to prepare the medication.

5. Only accredited and recognized labs performed the analytical parameters. As a result, KMNC’s performance in numerous analytical processes demonstrated its correctness.

6. Numerous investigations had been conducted to validate the Siddha metal-mineral formulation KMNC scientifically and to evaluate its safety.

7. To demonstrate its effectiveness, physicochemical also elemental analysis, toxicological tests, with pharmacological research are carried out.

**Anti-Tumor property:**

**Apoptosis evaluation**

Double labelling with the findings from the viability of cells tests can be used to measure apoptosis were verified employing annexin V as well as propidium iodide (PI). For 48 hours, KMNC at 500 nm was applied to AMOS III tissues. The Annexin V test set from Sigma, St. Louis, MO, tagged organisms with PI and Annexin V-FITC compound. The cells were then examined using the BD Cells Quest Pro programme. Additional evidence for these conclusions came from Western blot analysis of particular caspases with the poly (ADP-ribose) polymerase test.

**Untreated Ce1ss Vehicle Control KMNC:**

In contrast to untreated (4.2%) mortality as well as both Annexin V examinations, which are used vehicle-controlled tissues (7.4%) for oversight, KB cells exposed with KMNC showed an impressive rise at the start of treatment and thereafter (37.5%).

In this investigation, we investigated the antiproliferative as well as fatal outcomes of KMNC using KB lineages. A new investigation indicates that KMNC might possess a time-dependent also dose-dependent antiproliferative impact on KB cancer cell lines. The inhibitory concentration (IC) of KMNC on KB lines cells was found to be 37.5%. The results of flow cytometry analysis demonstrated that the percentage of the earliest apoptotic lines rose in parallel with the amount of KMNC. For this reason, the fundamental apoptotic pathway appears to be the root cause of line cell apoptosis.

The KB cells were exposed to KMNC at various levels (6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml also 100 μg/ml) and cultivated for 24, 48, and 72 hours in order to examine the impact on development kinetics. Trypan blue, which was employed to stain the cells at the conclusion of every therapy, and the live cells that had been spared the dye were then enumerated.

When contrasted with pretreated cells used as controls, the development kinetics of cells subjected to KMNC was reduced in a dose-dependent manner (Figure 5). Additionally, it was discovered that the development kinetics were substantially lowered (2-fold) when using an KMNC therapy level of...
roughly 50 g/ml relative to the development kinetics observed in the control group without treatment cells (p ≤ 0.05 for 24 h; p ≤ 0.001 for 48 h and 72 h).

It was reinforced with the colony formation evaluation, in which tissues were exposed to various KMNC levels over a week with a smaller inoculation density. In comparison with control tissues, these cells displayed considerably fewer colonies with a density. In comparison with control tissues, these cells displayed considerably fewer colonies with a 50 μg/ml level of KMNC (Figure 6).

It was shown that the KMNC extracts caused a dose-dependent reduction in the number of soft agar territories, which was in line with the slow development rate. Intriguingly, the number of soft gelatin territories was decreased by three times (p ≤ 0.001) after 50 μg/ml KMNC therapy relative to the untreated reference organisms (Figure 7). All of these findings suggested that KMNC significantly changed the development kinetics of KB tissues, suggesting that this compound may have anti-tumor effects on cancerous cells.

We carried out apoptotic tests to better understand the anti-cancer strategy with KMNC on carcinoma cells. The percentage of apoptotic tissue was determined with Annexin V-FITC as well as propidium iodide colouring, subsequent to flowing cytometric evaluation, following those cells having received different levels of KMNC (Figure 7). The proportion of cells that are experiencing apoptosis was shown to rise substantially with levels of between 25 and 50 μg/ml KMNC. Intriguingly, the number of cells that were undergoing apoptosis rose 2.6-fold (p ≤ 0.001) with a KMNC dosage of 50 g/ml compared with the pretreated cells in the control group.

We observed that the use of KMNC extracts substantially and dose-dependently affected the development rate of the KB line cells. The outcomes of the colony-building as well as the use of soft agar experiments, which revealed statistically important reductions in the total number of territories of KMNC-treated cultures relative to the untouched cells used as controls, added to the proof presented. As a result, it is possible to suggest that KMNC is a good candidate for limiting the proliferation of cancer cells.

Apoptosis is essential for maintaining the equilibrium of healthy tissues and for the removal of aberrant cells. Numerous anticancer medications work by activating the process of apoptosis that eliminates carcinoma cells. We performed apoptosis tests to see whether KMNC may cause the carcinoma line of cells, KB, to undergo apoptosis. In comparison with control tissues, a considerable number of cells have been demonstrated to undergo apoptosis with a successful KMNC level of 50 μg/ml. Since calcium flux constitutes one of the primary mediators underlying apoptosis, we examined when KMNC might change this so as to further comprehend the process of apoptosis.

**Summary**

KMNC is the experimental drug's medicinal effectiveness was verified using for anti-tumor, anti-bacterial, and anti-oral cancer (Kanna Putru) effects. The Siddha philosophy, the contemporary idea, the incidence of oral cancer globally, and the problems related to contemporary therapies have been determined to constitute the primary factor in human death, all of which are covered in the study's introduction, including the importance of test medicine for curing oral cavity cancer. Professionals identified and verified each ingredient.

To validate the sample's accuracy, anti-microbial action was also examined. Pharmacological research has been finished. It is now well-recognized that "KNMC" has anti-cancer, anti-tumor, as well as anti-bacterial effects. Outcomes also discussion provide the critical validations to demonstrate the drug's potency. The conclusion provides a compiled version of the research and describes how all the essential components and supporting actions work together to further the investigation.

**Future scope**

As a result, its anti-cancer properties against oral cancer were strengthened and guaranteed. Knowing the precise molecular mechanisms of action requires further detailed animal model investigations as well as human clinical trials. As a result, it might be employed on a global scale to treat oral cancer and fulfill the requirements for a painless and safe anti-neoplastic procedure.

**CONCLUSION**

One of the most terrible illnesses, Carcinoma, has been on the rise recently. The search for a long-lasting treatment for oral malignancy is urgently needed. So, using the foundational ideas of the Siddha School of Medicine, we came up with a brand-new formulation. Instead, by using the time-tested, higher-order Siddha medicine, which is full of benefits, the damaging cycle, may be stopped. Therefore, following Siddha's traditional literature, we have decided to select the higher-order medication "KMNC" for the therapy for tumor. In-vitro, anticancer assays, anti-tumor evaluations, and microbes evaluation, have all demonstrated the effectiveness of the Siddha formulation KMNC. In oral carcinoma cell lines, KMNC demonstrated positive anti-
cancer potential by inducing apoptosis in carcinoma cells. Additionally, in xenografts containing cancerous oral tumors, KMNC demonstrated outstanding anti-tumor action without severely affecting normal tissues, highlighting its preliminary research effectiveness as a possible oral cancer anti-cancer therapy. Additionally, KMNC exhibits resistance to the bacteria that cause cancer. From the vantage point above, I’d like to investigate how the experimental medicine KMNC participates in the treatment of cancerous cells of lineages of cells and has anti-tumor as well as anti-microbial activities. Oral carcinoma is the sixth most prevalent cancer worldwide, and certain forms are very radiation resistant. Additionally, chemotherapy medications cause unpleasant side effects that are more severe than the illness initially. It opened the door for a cutting-edge, non-invasive anticancer medicine that treats oral carcinoma. This study’s objective is to respond to the previously indicated need. The term “KMNC” refers to a non-violent anticancer treatment for oral cancer that was derived from Kandhasamy Mudhaliyar’s ancient Siddha literary works, Athmaraksha Mirtham Ennum Vaithiya Sarga Sangham, which the department of AYUSH recognized as both classical literature and experimental medicine. During the trial, both securities as well as efficacy were carefully scrutinized. The medicine production process and its standardization procedures revealed GMP. The AYUSH-assigned testing protocol for Chendhooram has been fully satisfied by the trial medication KMNC. The manufacture and effectiveness of “KMNC” were demonstrated. The anticancer impact on KB lines cell, the antitumor outcome on KB lines cell, and the anti-microbial actions support the pharmacological actions. The potential of the experimental medicine was also evaluated with respect to its anti-microbial characteristics. The primary perspective of this research is justified by factors such as efficacy, longevity, bioavailability, and the existence of important elements, anions and cations, and also minerals that favor the action. The anti-cancer causes of KMNC could be supported by science. The health community and everyone around the globe would profit from its non-toxic anti-cancer result.

Conflicts of Interest

There are certainly no conflicting activities.

Funding Support

No funding support for this study.

REFERENCES


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