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**ORIGINAL ARTICLE** 



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### Pancha Paashaana Chendhooram: Standardization, Characterization, and Instrumental Analysis of a Potential Anti-Cancer Herbal Mineral Formulation

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#### Article History:

Abstract

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Keywords:

Pancha Paashaana Chendooram, Siddha, herbal leaves, Anti-cancer, Oral cancer Cancer is a condition marked by unchecked or unregulated cell proliferation that can spread to other organs via lymphatic and blood vessels. The leading cause of death for both men and women worldwide is cancer. Using traditional Siddha literature as our guide, we have established how to prepare Pancha Paashaana Chendooram (PPC), a Siddha compound herbal-mineral therapy for treating oral cancer. The physicochemical characterization of the Siddha herbal-mineral formulation PPC was the goal of the current investigation. The physicochemical properties were examined, including ash values, loss on drying, pH, and flame test, in addition to solubility. The entire cinder analysis was negative, and the pH assessment was 7.3 with a reduction of drying at  $102\pm2$  °C of 0.133% w/w. The sample PPC's SEM analysis revealed the presence of nano- also near-nanoparticles. According to the quantitative examination, the medicine contained nitrate, iron, mercury, arsenic, sulfur, and other minerals. FT-IR spectroscopy, XRF and EDX were studied in this work. The studies revealed that PPC has equal chemical opulence and mineral richness. For the cure for an array of illnesses and disorders, the Indian Siddha medicinal organization regularly used A. indica, P. betle, G. herbaceum, E. axillarem, O. sanctum, P.deamia, with P.nodiflora. The antioxidant action with antibacterial movements of water concentrates of the dried leaves was evaluated in this work using in vitro traditional techniques employing sparkling leaves of the cited herbals. This PPC is also having multiple actions in Anti-Rheumatic, Anti- Inflammatory, Analgesic, Anti- Histamine, and Anti- Pyretic illnesses.

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### **INTRODUCTION**

People have used medicinal plants with minerals to treat a wide variety of ailments since ancient

times. In Asia, degenerative and life-threatening illnesses, including cancer, have historically been treated using a range of medicinal herbs, minerals, and arsenic in low dosages. The Siddha System of Tamil Nadu is considered one of the oldest healthcare systems in this Asian civilization. The cultural tradition and history of South India, and more specifically Tamil Nadu, are deeply ingrained in this system. Food as Medicine and Medicine as Food is the central tenet of this philosophy. It means that it is part of a lifestyle for good health rather than just a medical procedure. It is a sophisticated medicinal system that extends back thousands of years to India. The Siddha system of medicine includes 64 different varieties. It divides each of the 64 medications into two groups: internal medicine

and external medicine. Chendooram is one of the inside medicines used in this. A crucial stage in making novel, risk-free, and efficient medicines is the pharmacological monitoring of herbals, mineral resources, also animals. Over 80% of people utilize plant-based medications at least once in their lives, and over 50,000 plants in the globe offer therapeutic benefits [1, 2]. The chemical diversity of medicinal plants and minerals is crucial for finding novel active compounds against a wide range of cancers.

Cytotoxic active components from several medicinal plants and minerals have been used to make anti-cancer drugs. In the USA, about 60% of anticancer drugs with a license are derived from natural sources [3, 4]. In India, a total of 14.61 lakh cancer cases were discovered in 2022. Out of this, ten lakhs Indians lose their lives to cancer each year, over fourteen lakhs people each year receive novel identifications of the illness. More Indian women are being identified with cancer year, according to WHO estimates. Those with immune systems in India have a higher lifetime risk of developing cancer. According to estimates, there will be 12.8 more cancer cases per 100,000 people in 2025 compared to 2020.

Only a few of the potentially fatal adverse effects of the highly effective, currently advised metallic anticancer therapy are alopecia, nausea, and vomiting. Anti-cancer medications have an effect on the quality of life (QOL) of tumor patients as a result, but they are expensive for the average person. Monitoring patients' quality of life while receiving cancer therapy is now required. It is wise to be aware that chemotherapeutic medicine treatment for cancer significantly reduces a patient's quality of life, even long after the drug has been stopped. So, the difficult task at hand is to develop quick and creative methods that can identify and generate compounds that would be efficient in treating human tumors. Testing for suspected cancer-causing characteristics of many compounds with plant origin is urgently required. In vitro in addition to in vitro models are used to test anticancer medications systematically. With an emphasis on their benefits and drawbacks, this work addresses the standardization, characterization, also instrumental analysis of anticancer drugs.

### **MATERIALS AND METHODS**

### **Ingredients and Processes Used in Drug PPC**

### **Drug selection**

The Pancha Paashaana Chendooram (PPC) stamens were used in this study as a compound medication for oral cancer, and instructions for its produce were acquired from the traditional Siddha text.

### Chemicals

Ascorbic acid, butylated hydroxytoluene, iron chloride, sodium carbonate, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4'-disulfonic acid, potassium ferricyanide, tannic acid, -carotene, quercetin, furthermore vanillin could be bought at Sigma-Aldrich. The solvents used for all other compounds had analytical status used without further refinement.

### Herbal Plant Substance

Acalypha indica (Kuppaimeni), Piper betle (Vetrilai), Gossypium herbaceum (Paruththi), Enicostemma axillare (Vellarugu), Ocimum sanctum (Thulasi), Pergularia deamia (Veliparuththi), and Phyla nodiflora (Poduthalai) herbal leaves were among the plants collected from the areas around the Thanjavur, Tamil Nadu were utilized for the herbal preparations. The species was subsequently identified, validated, and registered at the Tamil Nadu Agricultural University in Coimbatore, Tamil Nadu, under the accession number 114004.

### Constituents made up of minerals and metals include: Orpiment

Saathi Lingam (Cinnabar), Manosilai (Realgar), Kaantham (Magnetic Oxide of Iron), Aritharam (Orpiment), Gandhinagar (Sulphur), Pooram (Calomel), and Vellai Paashaanam (White Arsenic) were purchased from M/s. Gopalan Asian Siddha and Ayurvedic Medicals, (Govt. approved wholesale agencies), Nagercoil, Tamil Nadu. The minerals and metals were evaluated as per IP grade standard at recognized laboratories.

### **Bacteria materials**

S.aureus and B.cereus were two of the five Grampositive bacteria that were isolated from the Institute of Microbial Technology Sector's (IMTS) Microbial Sort Culture Collection Center and Gene Bank in Chandigarh, Haryana, India. The other 3 Gramnegative microbes were E. coli, also P. aeruginosa, with K. pneumonia.

### **Cleansing of Raw Materials**

Cinnabar (50 mg) was cleaned with air to get rid of impurities. Cleaned cinnabar was put on a freshly cleaned mud plate and given 24 hours to soak in 50 ml of fresh cow's milk (specific gravity: 1.03 g/ml). It was then placed in the same mud plate and immersed for an additional 24 hours in fresh lemon juice (50 ml; specific gravity, 1.006gm/lit) after the initial soaking time. It was thoroughly cleansed with clean water and then dried using an air dryer in the dark to produce purified cinnabar. Realgar (50 grams) was cleaned with air to remove impurities. 50 ml of freshly squeezed ginger juice (Gingiber officinalis; specific gravity: 1.03 g/ml) was added to recently cleaned Realgar and left to soak for 24 hours. Dry the Realgar undercover with an air dryer after the soaking period has ended to get cleansed Realgar.

To remove contaminants, 50 g of magnetic oxide of iron was cleaned with water first and subsequently with deionized water. Clean Magnetic Oxide of Iron was placed in a 300 ml (specific gravity 1.018 gm/ml) solution of fresh root juice from Ponnavarai Cassia auriculata (Ponnavarai) and positioned in a freshly cleaned mud plate. It was then isolated from morning to evening for ten days without interruption. After the soaking period, it was dried in an air dryer in a shaded area for an additional two days without adding juice. This method was repeated twice, followed by a deionized water wash, to produce pure magnetic iron oxide.

To eliminate any contaminants, the 50 grams of orpiment were cleansed with air. It was then covered with purified limestone in the recently cleaned mud plate for five days. The limestone was treated with palm toddy ten times before being washed in fresh water and dried by air.

A stainless steel spoon contained 50 grams of sulfur. The spoon was heated while a small quantity (10gm) of fresh cow's butter was added, and the butter melted. The fresh cow's milk was then dipped at an angle into the concoction. This technique was repeated thirty times over a period of five days to generate pure sulfur. Each time, fresh milk was used.

A poultice made of pepper (Piper nigrum) and betel leaf (Piper betle) was dissolved in 1.3 liters of deionized water. Calomel (50gm) was then immersed in heated liquid from the crossbar after being tied with a new, clean towel. The water then shrinks to half its original size. The Calomel was taken out, washed with water, and dried in the shade of an air dryer to obtain pure Calomel.

A spotless, brand-new mud pot was used to triturate 50g of white arsenic with fresh lemon juice for 48 hours. It was then shaped into tiny cakes and dried in a shaded air dryer. This process was carried out seven times to produce pure White Arsenic.

## Pancha Paashana Chendooram (PPC) processing method

40grams of each of the following, pure Cinnabar, purified Realgar, uncontaminated Magnetic oxide of Iron, purified Orpiment, cleaned Sulphur, spotless Calomel, and dirt-free white Arsenic was taken for

processing of PPC. Extract from A. indica, P. betle, G. herbaceum, E. axillare, O. sanctum, P. daemia, and P. nodiflora was used.

### Extraction process of herbal leaves

The medicinal leaves were individually weighed in deionized water at room temperature after being continuously rinsed in water. For 48 hours, 200 grams of thoroughly cleaned plant leaves were submerged in 1 liter at a time of deionized water in an orbiting vibrator. Number 1, a Whatman filtration sheet with a Buckner cone, was used to sieve the concentrates. The temperature of each filtrate was held constant. 96.5% of the overall output came from the leaf yield. Each extract was stored in an airtight glass container.

### Procedure

Before being turned into powder, identical dosages (40 grams) of refined mineral medications were consumed. All the minerals were ground and then pulverized after that sieved in separately. One by one, the components from the sieve were thoroughly combined. The extract from A. indica was then added to this substance, and the mixture was ground for 12 hours. They were then given piper betle juice and crushed for 12 hours. Also added and ground for 12 hours each was extracted from the leaves of G. herbaceum, E. axillare, O. sanctum, P. daemia, and P. nodiflora in individually.

The cakes were created and then dried in an air dryer in the shade. These cakes were covered in betel leaf, put in a clean, new mud pot (the bottom pot), and covered with another clean, new mud pot (the top pot), whose rim perfectly matched the bottom pot's. The junction between the two pots was sealed with seven layers of clay-pasted cloth, the sole objective of which was to produce airtight containers. The pots were heated slightly for 12 hours at 50–60 °C. After that, the air was used to cool it down. With the help of the brush, the dark color sublimation (chendooram), which had been discovered deposited on the upper section, was gathered. The PPC was then finely crushed and placed in an airtight glass jar for storage. The combined weight of the PPC was 61 gm, while the waste was 219 grams. Pana Edai (488 mg) was the recommended dosage, and honey can be used as a softener.

### Establishment of the Drugs PPC:

Drug identification and potency and purity evaluation were both made possible by standardization.

The herbal-mineral combination was standardized and supported by qualitative also quantitative research based on physicochemical research, instrument analysis, also biological activities.

### **Physico-Chemical analysis**

According to the recommendations of the WHO, physical also chemical examinations such as pH value, loss on drying at  $102\pm2$  °C, action on heat, flame test, and ash test had been carried out at the Sairam Advanced Centre for Research.

### **Bacteriological test**

The Mokbel in addition to the Hashinaga method was used to measure an antibacterial assay. The nutrition hard agar was utilized to keep the bacteria store culture medium at 4 degrees centigrade (peptone 2.0 gm, beef concentrate 1.0 gram, NaCl 0.50 gm, with agar 2.0 gram per 200 ml H<sub>2</sub>O). The bacteriological media made using nutritious broth (peptone 1.0 gram, meat extracts 0.5 gram, NaCl 0.5 gram, with agar 0.4 gram each 100 ml H<sub>2</sub>O), which was pH-adjusted to 6.6, and soft agar medium (peptone 1.0 gram, meat extracts 0.50 gram, NaCl 0.25 gram, with agar 0.4 gram each 100 ml H<sub>2</sub>O and autoclaved at about120 degree centigrade for 30 minutes.

A minimum inhibitory concentration (MIC) was identified since the least attentiveness of methanol (mg/ml) on a leaf in an agar Petri plate did not result in any observable bacterial growth (MIC). The squashy nutrition agar was blended with the 10 ml of hard agar in the Petri plate. The 50 ml of plant methanol concentrates were then put one at a time to soft agar at the attention of 0.1, 0.5, 1.0, 2.0, 5.0, also 10.0 mg/lit, mixed thoroughly, and then transferred to sterile Petri dishes having 10 ml of rigid agar. The cultures (5  $\mu$ l) were added to three locations on the medium facade, collected from the nutrient broth, and kept warm at 37 °C. Chloramphenicol with Kanamycin was used as the benchmark to evaluate the performance with that of common antibiotics.

### **Fungal Test**

PPC was suspended in DM. The latest studies looked at the antibacterial and antifungal abilities of E. coli, C. albicans, S. aureus, and P. aeruginosa. Ketoconazole with amikacin was frequently combined in antibacterial also antifungal investigations. PPC was dissolved in DMF to get attentiveness of 100, 200, or 400  $\mu$ gm per ml. The opening of the No. 1 Whatman filter sheet was five millimeters, and it was spotless for an hour at 140 °C utilizing dehydrated warmth. The sterilized discs were inserted using the inoculant pine needle once the fungus had colonized the whole surface of the medium. The dishware was incubated at 27 °C for 72 hours while being turned upside down. Checks were made on the inhibitory zones 72 hours later. Fluconazole was the gold standard that was used, and DMF was the control.

The colony's diameter was used to measure the fungus's growth. The following equation had to be used according to custom in order to calculate the suppression of fungal development in the presence of composites.

Percentage of covered fungus development = 100 \* (C- T) / C ...... (1)

Where C denotes the size of the fungus colony on the positively regulated plate and T indicates the number of organisms on the trial surface.

### Evaluation of antioxidant activity by DPPH\* open radical scavenging technique

As an element that safeguards the body's health, antioxidants are crucial. The antioxidants may lower your risk of developing chronic diseases like cancer and heart ailment according to scientific research. Whole grains, fruits, with vegetables are important sources of antioxidants that occur naturally. It is well known that plant-based antioxidants like vitamin C, E, carotenes, phenolic acids, etc., may reduce the risk of disease. The bulk of the antioxidant compounds integrated into a typical diet is derived from herbal plant origins and fit into various substance groups with a wide range of combined physically with chemical features [5].A fast, convenient, and inexpensive technique to evaluate the antioxidant capability of chemicals was to use the open essential compound 2, 2-Diphenyl-1picrylhydrazyl (DPPH\*), which was frequently used to test a compound's potential to function as a free vital scavenger or hydrogen donor. The DPPH test method was based on the shrinkage of DPPH, a reliable open essential.

Several extracts from the above mentioned leaves were tested for their capability to search open essentials 1, 1- diphenyl-2-picryl hydrazyl (DPPH\*). In essence, ethanol was used to create a 0.1 mM DPPH solution. This fluid (1 ml) was blended using 3 ml of different concentrates at dissimilar concentrations (10,20,30, 40,50,60,70,80,90 also 100 $\mu$ l) with ethanol. In this scenario, only ethanol-soluble extracts were used, and varying concentrations of those extracts were created by diluting them. After giving the item a good shake, it was allowed to rest at room hotness for 60 minutes. An absorbance was established on 517 nanometers. 60 minutes of resting at room temperature were then permitted.

Employing a spectrophotometer (UV-VIS Shimadzu makes) [6], the experiment was run in triplicate, with ascorbic acid employed as the reference standard substance. The sample's IC 50 value, or the concentration of the sample necessary to slow down 50% of the DPPH open radical, was ascertained utilizing the log dosage inhibition curvature. The reaction mixture's decreased absorbance signaled increased free radical action [7].

The percentage DPPH searching effect was computed with the following equation:

Percent inhibition or DPPH searching action (%) =  $[\rm A_0$  -  $\rm A_1]$  /  $\rm A_0~X~100$ 

Where  $A_0$  represented the absorbance of the regulated reaction also  $A_1$  was the absorbance when an analysis or reference sample was present [8]. Around 517 nm, the open vital DPPH\* absorbs most effectively in addition to with an odd electron (purple color) [9]. By combining the stable free radical DPPH with such hydrogen donor, antioxidants reduce it to the less reactive DPPHH (like an antioxidant that searches open vitals). The DPPH absorbance decreased as a result. The primary DPPH-H variant displays decolorization concerning the number of electrons gathered (yellow color) [10].

The probability to reduction was greater the more coloration there was. The most often used method for assessing a novel drug's capacity to scavenge free radicals was this test [11]. When a DPPH solution was combined with a chemical that may donate an atom of hydrogen, the resultant reduced form (Diphenyl picrylhydrazine; non-radical) was produced with the loss of this violet hue (even though there would be anticipated to be a lingering pale yellow shade from the picryl cluster still present)<sup>•</sup> [12].It was alleged that this plant contains antioxidant properties.

### **DNA binding studies**

We investigated the antioxidant activity of various substances utilizing the DPPH and essential scavenging technique. The stock solution of DNA-containing calf thymus (CT) in water with a pH of 7.2, 5 mM Tris-HCl, also 50 mM sodium chloride was used to conduct the DNA-binding experiments. By employing a UV absorbance measurement of 260 nm and a molar absorption value of 6600 M<sup>-1</sup> cm<sup>-1</sup>, it was possible to calculate the amount of DNA in a liquid. The outcoming metal composites weren't naturally luminous. Hence, ethidium bromide (EtBr) was added to DNA to cause fluorescence (since the calf thymus). A metallic composite was added to this amalgamation, which decreased the fluorescence.

### Determination of ascorbic acid

A different methodology was employed to calculate the ascorbic acid substance. Using a cold pestle with mortar and a 1% phosphate-citrate buffer solution with a pH of 3.5, the extract was conducted on powdered fresh leaf samples. Whatman No. 1 filter sheet was used to filter the homogenate. A 3 ml curette with 1 ml of 2, 6-dichloroindophenol (2, 6-DCPIP) at a concentration of 1.7 mM was filled with the filtrate. The absorbance of 520 nm was measured ten minutes after the reagents through the extraction buffer employed due to a void were combined and per gram of fresh weight, ascorbic acid was calculated to be mg.

### Statistic evaluation

The experimental results were expressed using the three repetitions average and standard error (SE). ANOVA (SAS, Edition 8.1) was used to examine the statistics at P 0.05 (Fisher's guarded slightest major dissimilarity).

### **Biological Activities**

The generated PPC examines the two properties of antibacterial Action and antifungal activity.

### Antimicrobial activities

Antibacterial effects Microorganisms like bacteria, fungi, viruses, and protozoa can often be killed by or prevented from growing by a substance known as an antimicrobial agent. Antimicrobial drugs either eliminate germs or inhibit their development. Exercises in agar-well dispersion examine the antibacterial activity of each compound against a selection of microorganisms. The created composites include the PPC evaluation in favor of in vitro antibacterial action utilizing an ideal dispersal technique, using agar nourishment as the testing medium.

Amikacin was used as a benchmark for comparing the PPC's antibacterial abilities to strains cultured on potato dextrose agar. According to standard procedure, the bacteria were immunized using a finely produced agar medium [13]. A micro-pipette was employed to put the test liquid spot into each well, and the guard was kept at 35 °C for a minimum of one day in microorganisms and three days in fungi.

Subsequent to some time, the channel's approximated diameter and the number of zones that prevent growth, measured in millimeters (mm), were determined. Recent studies looked at the antibacterial with antifungal abilities of E. coli, C. albicans, S. aureus, also P. aeruginosa, in addition to on antibacterial along with antifungal medications typically pairs ketoconazole also amikacin. DMSO was the solvent employed.

### Anti-fungal effects

Fungi were highly sophisticated multicellular or single-celled creatures. They could be found practically anywhere, the majority of them like land over the sea or clear water, especially in the soil or plant substance. The rotation of carbon and other components depends on a group of organisms known as the decomposers, which thrive in the ground or on dead plant matter. A few were plant parasites that disperse diseases like canker, rust, scabs, and mildew. Crop illnesses caused by fungi might cost the farmer more money. Animals could become ill from fungus in relatively tiny quantities. These consist of ailments including athlete's foot, ringworm, and thrush that damaged people's skin.

Antifungals are drugs that either kill or stop the development of fungi. They are used in medicine to treat illnesses like ringworm, athlete's foot, and thrush, and they work by utilizing the differences between mammalian and fungal elements. Without endangering the host, they got rid of the fungus. Eukaryotes, as opposed to bacteria, included both fungi and humans. Due to the molecular similarities linking human and fungal units, it was more difficult to recognize an antifungal drug target that did not also exist in the pathological organism.

The microbes (microorganisms) were the fungi chosen for the study: Aspergillus flavus (MTCC 8790), Candida albicans (MTCC 7253), Aspergillus niger (MTCC 8652), Aspergillus oryzae (MTCC 8023), Aspergillus sojae (MTCC 8779). They were kept on potato dextrose agar also intermediate nutrition agar slants.

The effectiveness of PPC's antifungal activities was evaluated using the agar plate method.

### Antioxidant properties

Reactive oxygen species (ROS) of various types are generated as an outcome of the oxygen usage required for cell development [14]. The body's normal oxygen consumption, which includes breathing with several cell-mediated immunological practices, continuously creates it. The free radicals that makeup ROS include bonded essential species like hydrogen peroxide  $(H_2O_2)$  with solitary oxygen, superoxide anion essentials  $(0_2$ -), hydroxyl activists (OH), and furthers  $(0_2)$ . Peroxidation of casing PPC may begin quickly because physiological practices constantly produce ROS, which feeds the formation of lipid peroxides. In addition to carbohydrates, reactive oxygen sorts could also obliterate vital macromolecules like lipids, proteins, and nucleic acids. It can also break DNA, which could outcome in alterations. When biological elements couldn't really exclude ROS, the disease started to occur. ROS was connected to vast numbers of illnesses (100 or more) [15].

Antioxidant defenses, including antioxidant food

ingredients and antioxidant enzymes, were present in all aerobic organisms and were utilized to eradicate or revamp damaged molecules. Foods and medications degrade during preparation and storage for a number of reasons, one of which is lipid peroxidation. By delaying this process, antioxidant molecules could look for open radicals with a longer shelf life. In addition to reactive oxygen sorts' antioxidants also guard against the negative effects of free radicals. The progression of various chronic ailments slowed down, as does lipid peroxidation [16].

Finding an alternative, reliable, and secure supply of food antioxidants was thus necessary. Normal antioxidants, especially those derived from plants, to become more significant in recent years. Antioxidants were frequently used as food additives to prevent foods from oxidative deterioration [17]. Currently: propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, also tert-butyl hydroquinone were used. Nonetheless, it claims equally BHA and BHT were responsible for liver damage and carcinogenesis. As a result, there was growing interest in inexpensive and reliable antioxidants [18].

## RESULTS AND DISCUSSIONS FOR EVALUATING PPC

## Infrared Spectroscopy Using Fourier Transforms (FT-IR)

FT-IR spectroscopy led to the identification of functional clusters in organic also inorganic compounds (Figure 1) (Table 1) [19]. The use of FTIR to detect the functional clusters of biomolecules could shed light on their structures and establish the presence of the active chemicals that give Siddha medicines their therapeutic effects. This spectroscopic method used lower energy emission to generate vibration as well as rational excitation of individual atoms and clusters of atoms inside molecules. Due to the symmetry of the various atomic groups, the variations in atomic mass, and the electrical configuration of these clusters, the absorption models of each species would be distinct, allowing for species identification.



Figure 1: Image of FT-IR investigation

Peak Values	Functional Groups	
	Alcohols and alcohols	
3628.4	Phenols and Alcohols	
3407.1	Phenols, amines, alcohols	
2927.2	Alkanes	
2369.0	Carboxylic acid	
2345.2	Alkyl	
1652.7	Alkenes	
1569.9	Aromatics and amines	
1419.5	Nitro	
1110.9	Polysaccharides and amines	

Table 1: Peak FT-IR values and the proper functional groupings

Table 2: Element in oxide form

Formula	Concentration (%)
S03	30.89
Hg	21.01
CaO	20.12
Cl	7.21
MgO	7.10
K20	4.09
SiO2	2.85
Na2O	2.40
PbO	1.85
Al2O3	1.30
MoO3	0.73
CuO	0.26
P205	0.19

### Table 3: Element form

Formula	Concentration (%)
Hg	27.22
S	26.58
Са	19.23
Cl	10.21
Mg	6.98
K	3.89
Na	1.78
Si	1.77
Pb	0.88
Al	0.69
Мо	0.48
Си	0.21
Р	0.08

1 Solubility Insoluble	
2 pH 7.3	
3 Analysis for Iron (Ferrous) +ve	
4 Analysis For Mercury +ve	
5 Analysis for Arsenic +ve	
6 Analysis for Sulphate +ve	
7 Analysis For Nitrate +ve	

Table 4: The physical characteristics and outcomes of investigations on basic with acidic radicals

#### Table 5: Quantitative Findings Base (420)

Element	Net Counts	Weight %	Atom %
С	2899	18.74	51.31
0	4816	7.50	15.41
Si	3580	1.17	1.37
S	21803	9.05	9.29
Cl	6351	3.15	2.92
К	3734	2.05	1.72
Са	1873	1.15	0.95
Cr	122	0.13	0.08
Mn	59	0.08	0.05
Fe	127	0.20	0.11
Со	47	0.09	0.05
Cu	80	0.24	0.12
As	40665	26.71	11.72
Cd	243	0.21	0.06
Hg	793	29.54	4.84
Total		100.00	100.00

#### Table 6: Comparison of PPC's antibacterial characteristics

S. no.	Material	Growth inhibitory area (mm)					
		Gram +ve germs			Gram –ve microbes		bes
		S. aureus	B. cereus	E. coli	P.	aerugi-	K. pneumonia
					nos	sa	
1	PPC	+6	+10	+10		+6	+5

## Table 7: PPC antifungal activity at 400 mgm per ml concentration (diameter of the zone of inhibition in millimeters)

Fungus	Growth inhibitory area (mm)	
A. flavus	+34	
A .niger	+28	
A. oryzae	+16	
A. sojae	+32	
C. albicans	+23	

S.no	Fungus	Attentiveness		
		100 $\mu$ gm/lit	200 $\mu$ gm/lit	400 $\mu$ gm/lit
1	A. flavus	+9	+15	+34
2	A.niger	+11	+19	+28
3	A.oryzae	+0	+6	+16
4	A.sojae	+6	+10	+32
5	C.albicans	+7	+13	+23

### Table 8: Antifungal activities of PPC (Region of inhibition's diameter in millimeters)

### Table 9: Antioxidant action (IC<sub>50</sub>) of the PPC

S. No	Material	IC <sub>50</sub>
1	PPC	644.312/235.741



Figure 2: SEM study of the drug's image



Figure 3: Drug icon using energy-dispersive X-ray spectroscopy (EDX) research



Figure 4: Growth inhibitory areas for Bacillus cereus [PPC]



Figure 5: Growth inhibitory areas for E.coli [PPC]



Figure 6: Growth inhibitory area of PPC against A. Flavus



Figure 7: Growth inhibitory area of PPC against A. niger



Figure 8: DPPH\* the search for open essentials through PPC



Figure 9: Fluorescence Spectra for DNA binding with PPC (1, 2, 3, 4, 5, 6, 7, 8, 9 & 10 correspond to the concentration of PPC in 10, 20, 30, 40, 50, 60, 70, 80, 90, also  $100 \mu l$  respectively)



Figure 10: Fluorescence Spectra for DNA binding without PPC (1, 2, 3, 4, 5, 6, 7, 8, 9 also 10 correspond to the concentration of PPC in 10, 20, 30, 40, 50, 60, 70, 80, 90 also  $100\mu$ l respectively)

Because of their anti-oxidative properties and potential anti-carcinogenic effects, phenolics are now a topic of considerable awareness. It was simple for free radicals to remove the hydrogen atom from the OH group in phenols. Also acting as sinking agents, open essential searchers, also quenchers of solitary oxygen production were phenolic acids with flavonoids. The two most significant classes of secondary metabolites and bioactive substances were flavonoids also phenolics. They are also anti-aging and anti-cancer, in addition to being antioxidant compounds that may scavenge free superoxide radicals.

An omega-3 fatty acid is identified as docosahexaenoic acid (DHA). With six (hexa-) cis double bonds and a 22-carbon chain (docos- is Greek for 22), it has the structure of a carboxylic acid (-oic acid). DHA was discovered to increase the efficacy of chemotherapy with prostate tumour units, and the chemoprotective effects in a mouse model were also revealed [20]. As a non-toxic additive, it can further be used to increase the efficacy of chemotherapy. DHA was discovered to slow the development of human colon cancer cells in mice. The reduction in cell growth regulators was what led to DHA's cytotoxic action [21].

In reality, many polysaccharides undergo extensive research as drug delivery systems. Polysaccharides could be utilized to make polysaccharide-drug conjugates, coating materials, or nanoparticles. In addition to enabling the successful delivery of chemotherapy medications to specific tumor cells, polysaccharide-based nanoparticles had several other advantageous characteristics relating to the water solubility, biocompatibility, and biodegradability qualities of such polymers.

### Fluorescence X-ray Spectrometer (XRF)

The drug's XRF examination reveals the presence of calcium, sulfur, and elemental mercury at their maximal levels. Only a carbon peak could be detected at the very top, with oxygen, sulfur, and arsenic appearing in the peak value that followed, according to the results of the EDX investigation. Copper, Chromium, Calcium, Magnesium, and Cobalt were all found in trace amounts, while Mercury and Silica were found in extremely tiny quantities. (Tables 2 and 3)

When exposed to powerful liveliness, and simplewavelength radiation, samples can ionize (like Xrays). If the emission is lively enough to liberate an inner electron that is tightly bound, the atom becomes unsteady and gains a new electron in its place. The weaker fastening energy of the inner electron orbital than the exterior one caused the discharge of energy. The emission that was released had less energy than the X-rays that were emitted. The resultant fluorescence X-rays could be used to determine how many element(s) were present in the sample because the liveliness of a released photon indicated a transformation involving a specific electron orbiting in a particular ingredient [22].

During the XRF examination of the herbal-mineral medicine PPC, peak values of elemental calcium, sulfur, and mercury were discovered. In the level below, potassium, magnesium, with chloride were stated. Silica and sodium were barely visible. Finally, trace levels of lead, aluminum, molybdenum, copper, also phosphorus were shown.

In Asia, sulfur is frequently used in traditional medication to treat cancer-related inflammation. Many in vivo also in vitro investigations demonstrated sulfur-containing complexes derived from naturally occurring compounds have significant anti-carcinogenic effects. In immortalized human oral keratinocytes with oral cancer units, extremely pure sulfur has been expressed to exhibit actions associated with apoptosis and growth inhibition. The human body uses molecules containing sulfur as powerful antioxidants. The disclosure of the drug's antioxidant properties was supported by our research. The potential anti-carcinogenic advantages of vitamin D—which have been found to have an independent, optimistic effect from calcium alone on tumour risk-were taken into consideration in a recent study that was optimistic about its findings.

The potential anti-carcinogenic advantages of vitamin D—which have been found to have an independent, optimistic effect from calcium alone on tumour risk—were taken into consideration in a recent study that was optimistic about its findings. Other subsequent studies were inconsistent in this regard. In a randomized controlled experiment, 1450–1550 mg of additional calcium also 1100 IU of vitamin D3 were observed to minimize the relative risk of aggregated malignancies.

With the use of the molecule oxygen in both myoglobin and haemoglobin, iron contributed a vital role in the formation of composite materials. In vertebrates, these dual molecules were often oxygentransporting proteins. Cellular respiration and oxidation also needed iron [23]. There was apparent mercury toxicity after 200 ml of a 1:500 perchloride of mercury liquid was used so anti-cancer medication during kidney surgery. Mercury levels in the blood have always been much lower than the harmful level. Studies on urine production verified the safety of this method. Hence, mercury perchloride administration during mass bowel surgery was a safe anti-cancer treatment [24]. (Table 4)

Across the 18th -19th centuries, multiplicities of arsenic composites were exploited as medications [25]. Arsenic trioxide has been used therapeutically for more than 500 years, with cancer treatment being the most common application. This medicine acquired FDA approval in 2000 [26] as a treatment for sharp promyelocytic leukaemia resistant to ATRA.

### SEM and EDX

The SEM and EDX methods were the most wellknown and regularly used by all facade investigation techniques. The surface topography was imaged with high resolution and excellent deepness of field employing a highly focused scanning (primary) electron ray. The key electrons perforated a facade with a lively array of 0.5 - 30 kV, generating a variety of low-energy minor electrons. A material's planar texture regularly controlled the strength of these minor electrons. To obtain a picture of the sample façade, secondary electron strength could be measured as a role of the scanning primary electron beam location. The significant electron ray was found to be an extremely minute dot (<10 nm), permitting elevated granularity resolution. It was possible to get increased sensitivity to topographic features on the outmost surface (< 5 nm) while using a principal electron ray using a liveliness of about 1 kV. (Figures 2 and 3) (Table 5)

Drug delivery could advance thanks to the significant features of nanoparticles. Cells absorbed these nanoparticles because of their small size, whereas the body would have rejected larger particles. In addition to the ability to carry medications across cell membranes and into the cytoplasm of cells, complex drug delivery methods were now being developed. Effectiveness was crucial since different diseases depend on cellular functions that could only be hampered by medications that enter the cell. Arsenic trioxide has been used in numerous therapeutic procedures throughout the past 500 years, with cancer treatment being the most common. For the treatment of sensitive promyelocytic leukaemia/ leukemia, this medication received FDA approval in 2000. Sulfur is often used as a natural treatment for cancer and inflammation in Asia. Oral cancer cells and immortalized human keratinocytes equally exhibited growth-inhibitory and apoptosis-related responses to highly pure sulfur.

### **DPPH\* open radical searching activity**

It was discovered that the leaf concentrates a higher concentration of DPPH\* open vital-seeking activity. The leaf extracts were shown to have an upper level of DPPH\* radical searching activity. The scavenging action of the leaves peaked at 87.3% at 0.1 mg/ml concentration, whereas at a similar concentration. The DPPH\* vital was rapidly and effectively neutralized by the leaf extracts in methanol. This activity was better than BHT's (54.6%) but less effective than ascorbic acid's (98.6% at 0.1 mg/ml) performance.

A DPPH searching evaluation technique revealed that the concentrate of this herbal plant has higher antioxidant activity. By employing a UV visible spectrophotometer, it was discovered that the water concentrate had an absorbance at 517 nm of 0.0989 and an IC 50 value of 24.8 g/ml. It indicates that more free radicals produced by DPPH\* were absorbed by the plant extract at greater concentrations, causing a fall in absorbance and a rise in the IC 50 value.

### Ascorbic acid with $\beta$ -carotene substances

Vitamin C, usually referred to as ascorbic acid, is among the most prevalent antioxidants in crops. Vegetation's defence against oxidative stress is ascorbate. It is an effective water-soluble antioxidant with a well-known application in preventing scurvy. The basic building blocks of antioxidant action are carotenoids, and beta-carotene is just a pioneer in the vitamin A family and is crucial for human vision along with the protection of numerous malignancies. In South India, new fresh leaves were frequently used to make extracts, the conventional functional medicines having febrifuge, stomachic, and anti-inflammatory qualities. The significant amounts of ascorbic acid also beta-carotene present in new fresh above mentioned leaves of may be responsible for health-promoting qualities. The findings showed that the ascorbic acid level was substantially higher in leaves, but -the carotene quantity between leaves varied very slightly.

### **Bacterial resistance**

Water extracts from the above mentioned leaves have significantly higher antibacterial action. The MIC was set at 10 mg/ml, which was incredibly high, yet it still showed that the in vitro plant extracts have wide-band antibacterial action. Gramnegative germs are believed to be more resilient than Gram-positive ones. Even at a high MIC above 10 mg/mL, this concentrate was effective against the three Gram-negative microbes used during the experiment. S. aureus with P. aeruginosa have recognized pathogens of respiratory disorders and are identified to cause respiratory infections. The two extracts' suppression of it suggested a potential application for them in the management of chest with respiratory illnesses. Additionally, the leaf concentrates reduced the growth of E. coli. Although enter hemorrhagic injury of E. coli is naturally present in humans, it has caused significant food poisoning, necessitating the application of preservatives to stop its growth. Hence, it may be beneficial to utilize the concentrates of above said herbals and all have much higher antibacterial activity in their leaf extracts. With a MIC of 10 mg/ml, the leaf concentrate was effective versus all investigated bacterial species. A list of PPC's bactericidal characteristics can be seen in Table 6.

The results demonstrate that the PPC was highly aggressive against the investigating microorganisms while being somewhat active adjacent to the microorganism's value (Figures 4 and 5)

### **Overall antioxidant property**

The skill of antioxidant potential of the leaf extracts from above mentioned herbals was assessed using PPC. The number of polyphenols boosted antioxidant activity proportionately. Recent studies suggested that too many plant species showed a highly constructive correlation linking total phenols also antioxidant activity (FRAP).

Likewise, there was an optimistic correlation linking entire antioxidant performance (FRAP) also whole phenolics with such a coefficient of  $R^2 = 0.76$  in the current investigation, involving the antioxidative action of the concentrates of above revealed herbals mainly due to the existence of phenolic composites in the concentrate.

The concentrates of above cited herbals have total antioxidant activity ranging from 362.18 to 106.53 m Fe (II)/gm Distilled water. The FRAP ratings for leaf concentrations were significantly higher than those for BHT.

### **Antifungal Activity**

On the other hand, 400 gm per ml Pancha Paashaana Chendhooram (PPC) was examined for its antifungal properties. Concerning A. flavus, A. niger, A. oryzae, A. sojae, as well as C. albicans, the combination showed more antifungal efficacy than pure PPC.

A. oryzae elicited a weak antifungal response from the PPC. Antifungal activity at a dosage of 400 gm per ml was visually represented for each PPC listed in Tables 7 and 8. The results of standard PPC and antifungal therapies were shown in Figures 6 and 7.

The PPC showed low antifungal efficacy against Aspergillus sojae (32 mm), moderate antifungal action vs. Aspergillus niger (28 mm), as well as C. albicans (23 mm). Tables 7 and 8 clearly summarize the results.

# Antioxidant in addition to antibacterial qualities of the leafs

Moreover, the 1-Diphenyl-2-picrylhydrazyl assessment revealed that the leaf concentrate also illustrated enhanced essential scavenging activity. The results of the antibacterial assay revealed that equally Gram-positive also Gram-negative bacteria were significantly sensitive to the leaf water concentrates. With minimum inhibitory values of 10 mg/ml for three bacterial species, the leaf concentrate's action was more evidence against several bacterial species. According to the findings, there was a considerable increase in the antioxidant activities of the leaf concentrates which would be suitable candidates for pharmaceutical plant-based merchandise and functional foodstuffs.

Anti-oxidants are hypothesized to affect DPPH\* because of their capacity to donate hydrogen. The study discovered that the concentrates could donate protons and function as open-loop critical inhibitors or searchers, potentially acting as primary antioxidants, even if their capacity to scavenge DPPH radicals was significantly diminished. The extracts of above stated herbals may therefore be effective therapeutic agents for treating pathological damage caused by radicals.

### Advantages of the DPPH assay

The DPPH test has the benefit of being quick and effortless to perform, just requiring a UV-visible spectrophotometer, which is why its use in antioxidant monitoring has grown dramatically. DPPH was a lasting nitrogen-free radical in contrast to the very hasty and transient peroxyl fundamentals engaged in the oxidation of lipids. Because of steric restrictions in accessibility, several antioxidants that reacted promptly through peroxyl principles may react slowly or even not at all in DPPH. Also, hydrogen transfer decreased marketers' capacity to decolorize DPPH, resulting in inaccurate assessments of antioxidant potentials [27].

Many researchers have employed the influence of a chemical's hunt on the DPPH vital exercise to swiftly and consistently confine their investigation interest in the in vitro antioxidant. It was clear from the results that openness significant scavenging activity of these composites is awareness responsibility. The IC50 price was 136 gm, with nutrition C being notable for its antioxidant effects during DPPH essential scavenging activities. By employing the DPPH\* radical scavenging technique, this study found that water extract from the leaves of the plant species of above stated herbals showed better antioxidant potential. The water extract's IC 50 assessment was discovered to be 24.8 g/ml. We can infer that this plant possesses antioxidant qualities as a result.

The antioxidant abilities of PPC, evaluation of the compounds, and the capability of constant DPPH open radicals were significant to the elements of the substance for open radicals. The quantity of focus needed for the scheme to detect 50% of the DPPH open was known as the IC50 charge [28, 29]. The activity of scavenging free radicals would increase as the reaction complex's attention level decreased [30]. Table 9 lists the IC<sub>50</sub> (inhibition concentration) evaluations for every complex.

Complexes' capacity to function as antioxidants depend on their structural composition, yet composites produced by metallic ions may have less appealing chemical characteristics due to altered PPC molecular belongings. Antioxidant undertaking data for PPC are presented visually in Figure 8.

### **Research on DNA Binding**

According to cell biology, the mass of anticancer with antiviral drugs employed DNA as their primary target molecule. The hunt for microscopic compounds with DNA-binding and DNA-chopping abilities was now gaining steam. It has performed how they were employed in the determination of DNA structure in addition to the creation and production of artificial restriction enzymes, novel medications, instruments for DNA fingerprinting, etc.

Since PPC exhibit a wide range of biological effects, including anti-carcinoma, anti-mycobacterial, with anti-bacterial activity, in addition to anti-Candida, they were of great interest. Moreover, the usage of nicotinic acid hydrazide composites as synthetic nucleases; and DNA intercalating mediums has garnered a considerable amount of interest. The dual functions of PPC as a pharmacological mediator and a significant performer in biological systems were well known.

PPC was active in equally in vitro also in-vivo environments, and it has been hypothesized that it functions as a likely anticancer and carcinoma-inhibiting drug. The PPC successfully self-triggered DNA cleaves and has cytotoxic effects on the cell lines L1210 murine leukaemia and A2780 human ovarian cancer; it was recently discovered.

There has been a bunch of investigation on the interactions between coordination compounds and DNA over the last few decades. Coordination chemicals offered promising prospects for DNA minor structure probes, photo cleavers, and also anti-carcinoma drugs. Due to its significance in the development of novel medicines and nucleic acid configuration probes, PPC has received a lot of attention.

Synthetic metallonucleases have potential use in the treatment of cancer, the manipulation of genes, the mapping of protein-DNA interactions, and the research of DNA-precise patterns. Hence, the development of synthetic nucleases was vital for mutual biotechnology also medicine.

The acridine pigment family and its derivatives, also further heterocyclic aromatic stains, exhibit extraordinary attraction for DNA. Intercalation has recently received a lot of attention from scientists as the reason for this attraction with this technique of DNA binding. Because they can stop the synthesis of nucleic acids in vivo, organic intercalates are presently among the most often utilized anticarcinoma treatments in scientific treatment.

Under constraints of extensive utilization of amikacin also DMSO as a solvent, the PPC replaced the DNA-bound EtBr and the fluorescent Grampositive germs (S. aureus, also B. cereus) in addition to the three Gram-negative microbes (E. coli, P. aeruginosa, with K. pneumonia). (Figures 9 and 10)

### **Proposed Approach**

Based on the analytical results, the amounts of carbon, hydrogen, nitrogen, sulfur, and metallic ions were calculated, which included elemental analysis and metal estimate, from which the molecular formulae for the PPC were developed. These were nonelectrolytes, as evidenced by the PPC's electrical conductance measurements.

PPC was examined for its DNA-binding, antioxidant, also antimicrobial (antibacterial and antifungal) qualities. A PPC demonstrated effective antibacterial action, according to antimicrobial research. All of the microorganisms used were subject to moderate PPC activity. S. aureus, B. cereus, E. coli, K. pneumonia, also P. aeruginosa were microorganisms employed. The technique for screening for ACKNOWLEDGMENT microbes was the agar-well diffusion process.

It demonstrated greater antifungal action against A. flavus, A. niger, A. oryzae, and A.sojae than against C. albicans compared to pure PPC. Nonetheless, PPC's antifungal effects were inferior to those of fluconazole (standard).

The antioxidant capabilities of PPC were tested using the DPPH\* essential seeking procedure. According to the findings, PPC performed well as an antioxidant as compared to the industry norm. According to a PPC's DNA binding study, they possessed more DNA binding character.

### **CONCLUSION**

Siddha is a multitalented method of medicine that uses minerals and plants. The PPC has developed the following Siddha teachings: The results demonstrated that PPC contains significant amounts of chemicals with minerals. Many state-of-the-art sophisticated instruments, including FT-IR, EDX, XRF, and SEM proved the same. Therefore, the PPC's potential for compounds rich in minerals was proved using contemporary characterization techniques, and extensive research was necessary to establish that it successfully treats oral cancer using a range of in vitro also in vivo methods. The methods listed above help us identify the various Siddha medicine-prepared drug parameters so that we can move forward.

We assessed the synthetic PPC's antibacterial capabilities using the Kirby Bauer disc dispersal tech-According to the study's findings, the nique. leaf concentrates of above mentioned herbals have antioxidant qualities and might operate as primary antioxidants or as open radical searchers or inhibitors. Also, water extracts have broadspectrum antibacterial action and are as effective as conventional medications.

Also, with time, bacteria develop antibiotic resis-Because recent research has placed a tance. strong emphasis on natural resources of antioxidant also antibacterial chemicals, the above-mentioned herbal leaves can be used as a reservoir of innate antioxidants with a wide-band antibacterial media. In an instance of emission spectral investigations, a change in the intensity of discharge was used to demonstrate the effectiveness of the complexes' DNA binding. According to the findings, the complex exhibits more significant stakeholder activity.

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There was no conflict of interest, according to the authors.

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