

Research Article

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## Exploration of Novel Phytochemicals of Siddha Formulation Pungampoo Chooranam by Qualitative, Quantitative and Fluorescent analysis

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### Abstract

The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals. In addition, some medicinal plants are still obscured within the plants which need to be scientifically evaluated. *Pongamia pinnata* is one such Indian traditional herb which offers numerous therapeutic benefits to mankind since several centuries. Siddha system of traditional medicine uses several valuable medicinal herbs for its formulations. *Pungampoo Chooranam* (PPC) is a novel siddha preparation which majorly comprises of *Pongamia pinnata*. The main aim of the present investigation is to carry out the qualitative, quantitative, fluorescent analysis and sterility evaluation of the formulation PPC. The result of the qualitative phytochemical analysis shows presence of alkaloids, steroids, triterpenoids, phenols, tannins, and carbohydrates. Results of quantitative analysis reveals that the total alkaloid level of the sample PPC was found to be  $0.48 \pm 0.05$  mg/gm and the level of total tannins was  $0.38 \pm 0.02$  mg/gm, whereas the level of Total phenols was found to be  $0.78 \pm 0.04$  (GAE mg/gm). Further the sample is of highly sterile and free from *E-coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. In conclusion the results of the present investigation render some evidence based information about the siddha formulation *Pungampoo Chooranam* with respect to the nature and quantity of phytochemical present in it and also about the sterility and pattern of fluorescent emitting nature of the formulation PPC to the future researchers.

### Keywords

Siddha system,  
Medicinal plant,  
*Pongamia pinnata*,  
Pungampoo Chooranam,  
Phytochemical analysis.

## 1. Introduction

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. It has been shown that *in vitro* screening methods could provide the needed

preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [2].

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening

techniques, traditional knowledge systems have given clues to the discovery of valuable drugs [3]. Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents [4].

Using plant based drugs as preventive and curative medicines is a rich heritage in India. In recent years, numbers of studies have been reported which screened the extracts of medicinal plants against several communicable and non-communicable diseases. This made applications in pharmaceuticals, alternative medicines and natural treatment. Some of the active principles of bioactive compounds are preferred for therapy either singly or in combination to treat several dreadful disorders [5].

New drug discoveries have shifted attention from synthetic models and compounds to natural products of plants origin. This is because scientists now believe that drug leads\hit molecule discovery would be more probable in plant and other natural sources like marine and animals which are yet to be fully explored. This drift has promoted, in recent time, researches in plants considered to be of little or no economic or ecological significance. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there have been an emphasis in standardization of medicinal plants of therapeutic potential. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [6].

*Pongamia pinnata* (Fabaceae) is popularly known as Indian beech in English [7]. Commonly known by its vernacular names karanj (Hindi), honge/karajata (Kannada), pungai (Tamil). As per the literature the extract of stem bark of *P. pinnata* (L.) showed antihyperglycaemic activity in diabetic mice [8]. Further, reports available that concomitant administration of synthetic oral hypoglycemic drugs along with *P. pinnata* produced synergistic effect in diabetic mice [9]. The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, triterpenes, flavonoids, steroids, and volatile oils [10]. It has been identified that Cycloart-23-ene-3 , 25-diol

(B2) isolated from the stem bark of *P. pinnata* possesses antidiabetic activity in diabetic animals [11]. Cycloart-23-ene-3 , 25-diol improved the abnormalities of diabetic conditions in diabetic mice due to increased glucagon-like peptide 1 (GLP-1) insulin secretion [12] and has a protective effect on vital organs like heart and kidney [13].

The main aim of the present investigation is to carry out the qualitative, quantitative, fluorescent analysis and sterility evaluation of the formulation PPC and to create a monograph of nature and quantity of the phytochemicals present in the formulation by systematic procedures.

## 2. Materials and Methods

### 2.1. Source of raw drugs

The herb is collected from southern zone of Tamil Nadu, and other required ingredient is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India .Herb were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India

### 2.2. Ingredients

The siddha formulation *Pungampoo Chooranam*(PPC) comprises of two main ingredients as listed below

1. Pungam flowers (*Pongamia pinnata*)
2. Cow's Ghee

### 2.3.Preparation [14]

The shade dried flowers of *Pongamia pinnata* were roasted slowly by adding little bit of cow's ghee. Then it is powdered and sieved using cloth.

Dosage	: 2 gm twice a day
Adjuvant	: Warm water
Duration	: 48 Days

### 2.4. Preliminary phytochemical Evaluation [15]

*Pungampoo Chooranam* was subjected to class of preliminary phytochemical screening of the following components.

#### Test for flavonoid

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Shows the presence of yellow color indicates the presence of Flavonoids.

### Test for Steroids

When the sample reacted with chloroform, acetic acid and conc.  $H_2SO_4$  and formed a blue and green colour. Which indicates confirmed the presence of steroids.

### Test for Alkaloid

Test drug was extracted with 2ml of HCl was added. To this acidic medium 1ml of dragendroffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids.

### Test for Phenol

To test drug a few drops of alcohol and ferric chloride solution was added. Bluish green or red indicates the presence of phenol.

### Test for tannins

Test drug was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

### Test for Glycosides

Test drug were mixed with a little anthrone reagent on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste, warmed gently over water bath. The presence of glycosides was identified by dark green coloration.

### Test for Starch

Test sample was added with iodine solution. Appearance of bluish black color denotes the presence of starch.

## 2.5. Quantitative Phytochemical Evaluation

### Estimation of Alkaloid [16]

5g of the sample (PPC) was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

### Estimation of Tannin [17]

The tannin content was determined using Folin Ciocalteu assay. Aliquot of sample PPC 100  $\mu$ L was added to 750  $\mu$ L of distilled water, 500  $\mu$ L Folin-Ciocalteu reagent and 1000  $\mu$ L of 35 % sodium carbonate ( $Na_2CO_3$ ). The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm. Distilled water was used as blank. Tannic acid standard solutions were prepared and standard calibration curve was plotted with varying concentration. The total tannins content were expressed as Tannic acid mg/gm, as calculated from the prepared standard curve.

### Estimation of total phenol by Folin-reagent method [18].

Folin Ciocalteu reagent was used for the analysis of total phenolic content of the sample PPC. Stock solution of sample in methanol (10 mg/ml) were prepared and 0.02 ml of each stock solution was added to 1.58 ml of distilled water in a test tube. Then, 0.1 ml of diluted Folin Ciocalteu reagent was added to the test tube. The mixture was kept at room temperature for 3 min and then, 0.3 ml  $Na_2CO_3$  7.5 % solution was added. After 30 min, absorbance of the mixture was measured at 765 nm by UV-spectrophotometer (Multispec-1501 Shimadzu). A standard curve was prepared using gallic acid (Merck, Germany). The determinations were carried out in triplicate and the total phenolic content was expressed as gallic acid equivalents (mg of GAE/g of sample).

## 2.6. Fluorescence analysis of PPC[19]

A small quantity of test sample PPC was treated with freshly prepared acids, alkaline solutions and different solvents. The drug powders were treated with acids *viz.*, Conc. HCl, Conc.  $H_2SO_4$ , Conc.  $HNO_3$ , and acetic acid. Similarly the sample treated with alkaline solution aqueous NaOH. The sample then treated with solvent like water and methanol. They were subjected to fluorescence analysis in visible light and in short UV-light (254 nm) and long UV-light (365 nm).

## 2.7. Sterility Test [20, 21]

About 1ml of the test sample PPC was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37° C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU.

### 3. Results

#### 3.1. Preliminary Phytochemical Evaluation of PPC

Bioactive phytocomponents are majorly responsible for the therapeutic activity of the sample PPC which may render multiple mechanisms and exert the

stipulated pharmacological activity. The results obtained from the Preliminary phytochemical analysis of the sample PPC reveals the presence of alkaloids, steroids, triterpenoids, phenols, tannins, and carbohydrates. The results were depicted in table 1.

**Table 1: Preliminary phytochemical analysis of *Pungampoo Chooranam***

S.No	Name of the Phytocomponents	PPC
1	Alkaloids	Presence
2	Flavonoids	Absence
3	Glycosides	Absence
4	Steroids	Presence
5	Triterpenoids	Presence
6	Coumarins	Absence
7	Phenols	Presence
8	Tannins	Presence
9	Saponins	Absence
10	Proteins	Absence
11	Carbohydrates	Presence
12	AnthoCyanins	Absence
13	Beta Cyanins	Absence

#### 3.2. Quantitative Phytochemical Analysis of PPC

Preliminary phytochemical is a qualitative methods usually carried out to identify the nature of phytocomponents present within the formulation where the qualitative analysis offers information of the quantity of each bioactive components present per

gram of the crude drug. The results obtained from the quantitative analysis shown that the total alkaloid level of the sample PPC was found to be  $0.48 \pm 0.05$  mg/gm and the level of total tannins was  $0.38 \pm 0.02$  mg/gm, whereas the level of Total phenols was found to be  $0.78 \pm 0.04$  (GAE mg/gm). The results were listed in table 2.

**Table 2: Quantitative Phytochemical Analysis of *Pungampoo Chooranam***

S.No	Phytoconstituents	Sample PPC
1.	Total phenols (GAE mg/gm)	$0.78 \pm 0.04$
2.	Total alkaloids(mg/gm)	$0.48 \pm 0.05$
3.	Total tannins(mg/gm)	$0.38 \pm 0.02$

Mean with 3 replicates  $\pm$  SD.

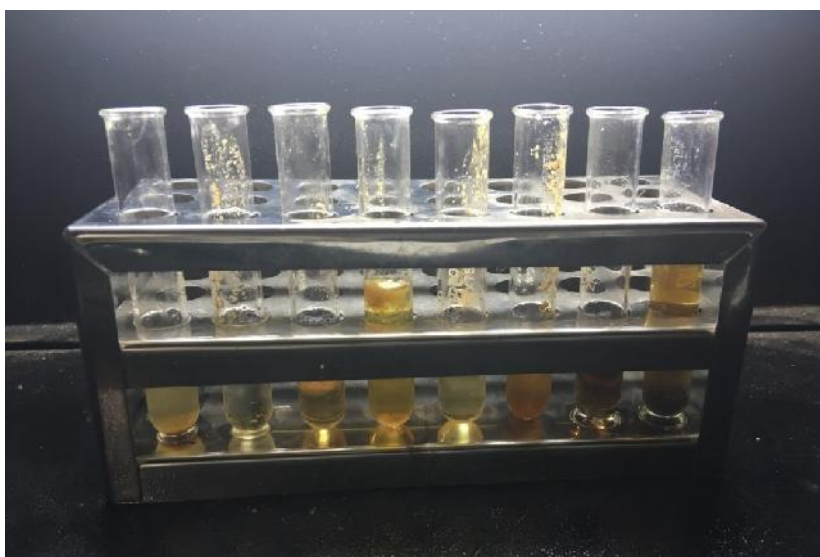
#### 3.3. Fluorescence analysis of PPC

Fluorescence analysis is adequately sensitive and enables the precise and accurate determination of the components over a satisfactory concentration range without several time consuming dilution steps prior to other analyses of pharmaceutical samples. The fluorescent analysis of the sample provides beneficial information about the nature and presence of fluorescent emitting components. The sample PPC was subjected to fluorescent analysis under day light and UV light by treatment with different chemical reagents showed different color. Results of fluorescent

analysis of the sample PPC showed Crimson red, Yellowish, Milky White, Yellowish Brown, Pinkish red and Greenish Brown colors. Similarly in UV the sample PPC Exerts Fluorescent Yellow, Fluorescent Green, Yellowish white, Green and Greenish Brown with solvents such as acetic acid, sodium hydroxide, Conc. HCl, Conc. H<sub>2</sub>SO<sub>4</sub> and Conc. HNO<sub>3</sub>. This analysis suggests that the siddha formulation PPC probably contain active agent(s) with fluorescent emitting capacity and this provides the basis for their traditional use for some human ailments. The results were projected in table 3 and and illustrated in figure 1 to 3.

**Table 3: Fluorescence analysis of *Pungampoo Chooranam***

S.No	Experiment	Visible light	Short UV – Light 254 nm	Long UV – Light 365 nm
1.	Sample+ Distilled Water	Pale White	Yellow	Yellow
2.	Sample+ Methanol	Yellow	Cream white	Cream brown
3.	Sample + glacial acetic acid	Crimson red	Fluorescent Yellow	Crimson red
4.	Sample + Sodium hydroxide in water	Yellowish	Fluorescent Green	Crimson green
5.	Sample + Conc. Nitric acid	Milky White	Yellowish white	Fluorescent yellow
6.	Sample + Conc. Hcl	Yellowish Brown	Green	Yellowish
7.	Sample + Conc. Sulphuric Acid	Pinkish red	Greenish Brown	Dark Green
8.	Sample + Ferric chloride	Greenish Brown	Greenish Brown	Dark Green



**Figure 1: Fluorescence analysis of *Pungampoo Chooranam* Observation under visible light**



**Figure 2: Fluorescence analysis of *Pungampoo Chooranam* Observation under Short UV light**



**Figure 3: Fluorescence analysis of Pungampoo Chooranam Observation under Long UV light (365 nm)**

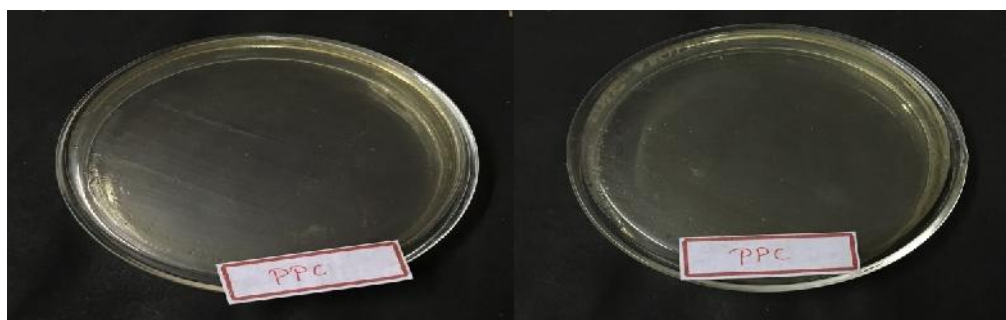
### 3.4. Sterility Test of formulation PPC

The results of the sterility test of PPC by pour plate technique reveals that the formulation PPC is of high sterile and the culture after incubation shown the

absence of pathogens such as *E-coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. The results were tabulated in Table 04 and illustrated in Figure 4

**Table 4: Sterility Evaluation of Pungampoo Chooranam**

Test	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus aureus</i>	Absent	Absent	
<i>Pseudomonas aeruginosa</i>	Absent	Absent	



**Figure 4: Sterility Evaluation of PPC**

## 4. Discussion

Plants play important roles in discovery associated with new beneficial therapeutic agents and have received significant focus because of their bio- active substances like antioxidants, hypoglycemic and hypolipidemic factors. India has a prosperous record associated with applying different potent natural herbs and plant based components regarding management of different diseases. Plants have invariably been exemplary source of drugs and a number of currently

available drugs happen to be derived directly or indirectly from them. Flavonoids tend to be most commonly known with regards to antioxidant nature. They are transformers which alter the body biochemical reactions to carcinogenic chemicals, viruses, and things that trigger allergies. Many plants display their characters for anticancer, anti-inflammatory, antibacterial and anti-allergic nature [22], and could be useful in therapeutic roles [23].

Medical plants are plants containing built in active ingredients familiarized to cure disease and relieve from pain [24]. The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed [25]. Modern-day pharmacopoeia however contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated from plants. Involvement in medicinal plants as a re-budding health assistance has been fuelled with the rising charges of prescription drugs in the safeguarding of personalized health and wellbeing and the bio prospecting of new plant derived drugs [26]. The ongoing development recognition regarding medicinal plants is due to various reasons; include increasing faith in herbal medicine [27]. On the top of that, an increasing dependence on the use of these medicinal plants in the industrialized organizations has been traced towards the extraction and development of drugs and chemotherapeutics from these plants as well as from conventionally used herbal remedies [28].

The therapeutic properties of plants could be based on their anti-oxidant, anti-microbial, antipyretic effects of the phytochemicals constituents in them [29]. According to World Health Organization, medicinal plants would be the greatest source to obtain an array of drugs. Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness [30]. In the present study the preliminary phytochemical analysis of the sample PPC reveals the presence of alkaloids, steroids, triterpenoids, phenols, tannins, and carbohydrates. The results obtained from the quantitative analysis shown that the total alkaloid level of the sample PPC was found to be  $0.48 \pm 0.05$  mg/gm and the level of total tannins was  $0.38 \pm 0.02$  mg/gm, whereas the level of Total phenols was found to be  $0.78 \pm 0.04$  (GAE mg/gm)

Crude drug of different parts of plant may exert different fluorescence under ultraviolet radiation. Each fluorescence characteristic of the treated sample was observed under ordinary light and then under UV light of both long and short wave lengths [31]. Therefore fluorescence evaluation is used for identification of significant phytocomponents present in the crude drug as well as in formulation drug [32]. In some siddha formulation it is often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [33]. Results of fluorescent analysis of the sample PPC showed Crimson red, Yellowish, Milky White, Yellowish

Brown, Pinkish red and Greenish Brown colors. Similarly in UV the sample PPC Exerts Fluorescent Yellow, Fluorescent Green, Yellowish white, Green and Greenish Brown with solvents such as acetic acid, sodium hydroxide, Conc. HCl, Conc. H<sub>2</sub>SO<sub>4</sub> and Conc. HNO<sub>3</sub>.

Safety is of the highest concern for the siddha preparation intended for internal usage in this regard the formulation should satisfy the sterility parameters before its usage in patients. The results of the sterility test of PPC by pour plate technique reveals that the formulation PPC is of high sterile and the culture after incubation shown the absence of pathogens such as *E-coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

## 5. Conclusion

The active principles responsible for the therapeutic effects of medicinal plants are phytochemicals, usually secondary metabolites, including but not limited to alkaloids, steroids, flavonoids, terpenoids and tannins. Out of 500 000 reported species of higher plants, only about 6 and 15 % have been evaluated for biological activity and phytochemical analysis respectively. Hence identifying the nature of phytotherapeutics present in medicinal plants like *Pongamia pinnata* would benefit the scientific community in better understanding the role of siddha formulations like *Pungampoo Chooranam*.

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