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Evaluation of Anti-oxidant and Anti-inflammatory activities of Siddha polyherbal formulation *Panchadeepakini Ilagam* in Invitro method

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ABSTRACT:

Kayakarpam is a unique therapeutic division in the Siddha system of medicine that helps in rejuvenation, longevity, and spiritual well-being. The great saint *Thirumoolar* has explained the concept of *Kayakarpam* in his text *Thirumanthiram*. *Kayakarpam* can be correlated to antioxidants. And most human diseases are associated with inflammation. There is a dire demand for the development of novel, efficacious, and safe anti-inflammatory drugs preferably from natural sources. To explore the literature evidence scientifically, antioxidant and anti-inflammatory activities are carried out for Siddha polyherbal formulation *Panchadeepakini Ilagam* (PDI) using DPPH assay and Albumin Denaturation assay respectively. In *Siddhavaithiya thirattu*, PDI is indicated for the vatha disease, pithavayu, valli. The main ingredients of PDI are *Sukku (Zingiber officinale), Milagu (Piper nigrum), Thippilli (Piper longum), Elam (Elettaria cardamomum), Seeragam (Cuminum cyminum)*. The result indicates that the polyherbal Siddha formulation PDI decreased oxidative stress via its antioxidant properties and was found to be an effective scavenger of DPPH and it also possesses significant Anti–inflammatory activity. Further elaboration of this study with In Vivo screening will be more helpful for the drug for clinical application. Thus, it is concluded that *Panchadeepakini Ilagam* has Anti-oxidant activity and Anti-inflammatory activity.

KEYWORDS: Siddha, Kayakarpam, Panchadeepakini Ilagam (PDI), Anti-oxidant, Anti inflammatory.

INTRODUCTION

Siddha system of medicine is an archaistic medical system in South India. It provides a healthy life to the people through natural sources which are a treasure to mankind. Siddhars classified diseases into 4448 and they were treated by 32 internal and 32 external types of medicines. Siddhars contributed not only to medicine but also extended the knowledge of Alchemy, Eternity, and Yogic living. *Kaya karpam* is a distinctive therapeutic method in the Siddha system of medicine. It is a process of rejuvenation, decreasing morbidity, and increasing lifespan. According to *Thirumoolar* rejuvenation is about purifying the soul by preserving the body so as to attain salvation [(1)].

The human body is in a constant battle to postpone aging. Research suggests that free radical damage to cells induces pathological changes associated with aging [(2)]. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property [(3)]. Antioxidant activity will be studied using DPPH assay in In-vitro. DPPH assay is a reliable method to determine the antioxidant capacity of biological substrates [(4)].

Inflammation is a normal protective response to tissue injury. It is a complex process, which is frequently associated with pain and involves occurrences such as the increase in vascular permeability, increase of protein denaturation, and membrane alterations [(5)]. Most human diseases are associated with inflammation. And currently, there is a high demand for herbal anti-inflammatory drugs. In-vitro anti-inflammatory activity test will be studied using the Albumin denaturation technique.

In the present study, *Panchadeepakini Ilagam* was prepared as per the Siddha classical literature, *Siddha vaidhiya thirattu*, and the drug has been evaluated for Antioxidant activity by DPPH assay and Anti-inflammatory activity by Albumin Denaturation assay.

MATERIALS AND METHODS

Collection of raw drugs

The required raw drugs were purchased from an Indigenous raw drug store, in Tambaram, Chennai, Tamil Nadu, India.

Authentication

Raw drugs were authenticated by the Medicinal Botanist of the National Institute of Siddha, Chennai. The test drug *Panchadeepakini Ilagam* was prepared at Gunapadam lab, National

Institute of Siddha, Chennai-47.

Ingredients of the *Panchadeepakini Ilagam* [6]

1) Sukku *(Zingiber officinale)*

2) Milagu *(Piper nigrum)*

3) Thippili *(Piper longum)*

4) Elam *(Elettaria cardamomum)*

5) Seeragam *(Cuminum cyminum)*

6) Cow's milk

7) Pannaivaellam (Palm jaggery)

8) Nei (Ghee)

9) Thaen (Honey)

Preparation of *Panchadeepakini Ilagam*

The above-mentioned five spices are roasted and made as a fine powder. Then palm jaggery is dissolved in cow's milk and filtered to remove any impurities and then boiled until *paagu patham*. In this stage the fine powder is added gradually, and mixed with the ghee. After the mixture cools down, it is mixed with honey and made it into *mezhugu patham*. Then it is stored in an air-tight container.

Dosage

5-6 grams twice a day, 48 days

Indication

Vatham, Pithavayvu, Vali, Erichal, Porumal

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay [7]

The antioxidant activity of PDI was determined using a 2,2-diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging assay. The sample was mixed with 95% methanol to prepare the stock solution in the required concentration (10mg/100 ml or 100µg/ml). From this stock solution, 1ml, 2ml, 4ml, 6ml, 8ml, and 10ml were taken in six test tubes, and by serial dilution with the same solvent, the final volume of each test tube was up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml respectively.

Ascorbic acid was used as a standard which was prepared in the same concentration as that of the sample extract by using methanol as solvent. The final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test samples at different concentrations (10 µg, 20 µg, 40 µg, 60 µg, 80 µg, and 100µg/ml) was noted after 15 mins incubation period at 370C. Absorbance was read out at 517 nm using a double-beam U.V Spectrophotometer by using methanol as blank. The effective concentration of the test sample required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of the dose-response curve plotting between % inhibition and concentrations.

$$\text{Radical scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 10$$

ANTI-INFLAMMATORY ACTIVITY

Albumin denaturation assay [8], [9]

In-vitro anti-inflammatory activity of PDI as studied using protein (albumin) denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample PDI at varying concentration ranges from 100 to 500 µg/ml and standard Diclofenac sodium at the concentration of100 µg /ml of the final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After 22 cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of the test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate. The Percentage protection from denaturation is calculated by using the formulae.

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 10$$

RESULT

Percentage inhibition of test drug PDI on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of PDI	% Inhibition of Ascorbic Acid

10 µg/ml	3.822 ± 1.072	21.16 ± 2.653
20 µg/ml	7.624 ± 2.103	34.15 ± 2.281
40 µg/ml	15.24 ± 3.055	55.83 ± 2.099
60 µg/ml	19.16 ± 2.427	64.87 ± 2.757
80 µg/ml	25.43 ± 5.716	76.4 ± 4.284
100 µg/ml	28.56 ± 6.73	92 ± 0.4547

Data are given as Mean ± SD (n=3)

IC50 Values for DPPH radical scavenging Assay by PDI and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
PDI	198.2± 40.57
ASCORBIC ACID	39.39± 16.73

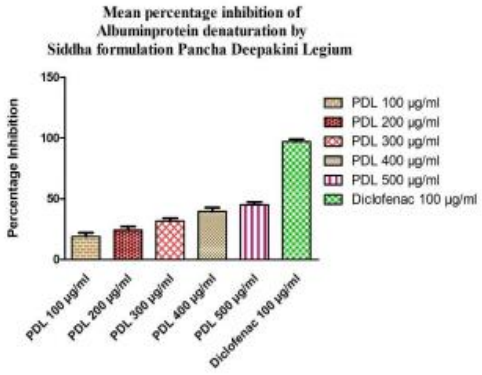
Data are given as Mean ± SD (n=3)

Albumin denaturation assay

Concentration in µg/ml	**Percentage Inhibition of Protein Denaturation**
PDI 100	18.95 ± 3.14
PDI 200	24.36 ± 2.895
PDI 300	31.78 ± 2.336
PDI 400	39.67 ± 3.25
PDI 500	45.15 ± 2.306
Diclofenac sodium (100 µg)	97 ± 1.883

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by PDI and Standard



DISSCUSION

Anti-oxidant activity – DPPH assay

The trial drug was screened for DPPH radical scavenging assay and the percentage inhibition ranges from 3.822 ± 1.072 to 28.56 ± 6.73 % when compared with standard ascorbic acid with percentage inhibition ranges from 21.16 ± 2.653 to 92± 0.4547 %. The IC50 value of the trial drug was found to be 198.2± 40.57 (µg /ml) when compared with standard ascorbic acid with (IC50 value 39.39± 16.73µg/ml).

Anti-inflammatory activity – Albumin denaturation assay

The concentration range of PDI at 100, 200, 300, 400, and 500 µg /ml produces significant inhibition of protein denaturation in a concentration-dependent manner. Maximum percentage of inhibition of about 45.15 ± 2.306 % was observed at 500 µg/ml when compared to Diclofenac sodium, a standard anti–inflammatory agent with the maximum inhibition of about 45.15 ± 2.306 % was observed at 500 µg/ml, when compared to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 97±1.88 % at the concentration of 100 µg/ml. The result obtained from the study indicates that the test drug PDI was effective in inhibiting heat-induced albumin denaturation.

CONCLUSION

In the current study, we used the DPPH assay and the albumin denaturation assay to assess the anti-oxidant and anti-inflammatory properties of the Siddha polyherbal formulation Panchadeepakini Ilagam, respectively. The outcome suggests that the Siddha formulation PDI has strong anti-inflammatory activity, is an efficient DPPH scavenger, and reduced oxidative stress due to its antioxidant qualities. In-Vivo screening and additional research on this topic

will be beneficial for the drug's clinical application. Consequently, it may be said that Panchadeepakini Ilagam exhibits encouraging anti-oxidant and anti-inflammatory properties.

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