



## Evaluation of Antioxidant potential of the Siddha Formulation *Sambirani Poo Kuligai* by *in-vitro* DPPH radical scavenging assay

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### Abstract

Oxidative stress has become a primary factor in the progression of many degenerative diseases, such as cancer, hyperlipidemia, gastric ulcer, and diabetes. Antioxidants are vital substances which possess the ability to protect body from damage caused by free radical-induced oxidative stress. Radicals and other reactive oxygen species (ROS) are formed constantly in the human body and are removed by the enzymatic and non-enzyme antioxidant defense systems. The usage of Siddha medicine has considerably increased over the past two decades and it is steadily crossing the various geographical boundaries owing to its rich antioxidant, therapeutic potential and inexpensiveness compared to conventional modern medicines and has fairly high acceptance rates because of its herbal origin and therefore its nontoxic nature. The main aim of the present investigation is to evaluate the *In-vitro* antioxidant potential of the novel siddha preparation *Sambirani Poo Kuligai* (SPK) by DPPH radical scavenging assay. The antioxidant activity of test drug sample SPK was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay at the concentration of 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml and 20 µg/ml using ascorbic acid as standard. Results of the DPPH free radical scavenging assay of SPK shows the highest percentage inhibition of about 82.52% at 20 µg/ml when compared to standard ascorbic acid with 89.62%. In conclusion the trial drug SPK possess significant antioxidant property which contributes to the beneficial effect oxidative stress associated disorders.

**Keywords:** Oxidative stress, Siddha medicine, *Sambirani Poo Kuligai*, DPPH, Antioxidant.

## 1. Introduction

Oxidative stress has been identified as the root cause of the development and progression of several diseases. Supplementation of exogenous antioxidants or boosting endogenous antioxidant defenses of the body is a promising way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage. Herbs have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS- induced oxidative damage. Several *in vitro* methods have been used to screen plants for their antioxidant potential, and in most of these assays they revealed potent antioxidant activity.

Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate [1]. Antioxidants can be synthesized *in vivo* (e.g., reduced glutathione (GSH), superoxide dismutase (SOD), etc.) or taken as dietary antioxidants [2]. Plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential [3]. The interest in the exogenous plant antioxidants was first evoked by the discovery and subsequent isolation of ascorbic acid from plants [4]. Since then, the antioxidant potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress [5].

Siddha system of medicine pioneering in emphasize the biological activity of the various phytocomponents with respect to the etiology and pathophysiology of various dread full disease emerging in humans and animals. It is evident that there are some medicinal plants used in siddha has potency of acting as an anesthetics, analgesics, anti-microbial, immune modulators, Hepato , neuro and nephron protectant. But the most pathetic scenario is most of these potential herbs are extinct and not be used currently. Siddha formulations offers tremendous advantage in clinical practice against metabolic and lifestyle disorders including neuro degenerative diseases. Often investigation on siddha preparations attempted on

reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically and now several investigation are being made on its preclinical aspect. Hence the main aim of the present investigation is to evaluate the antioxidant potential of the novel siddha preparation *Sambirani Poo Kuligaiby* DPPH radical scavenging assay and to document the evidence based data for the purview of the researcher in future.

## 2. Materials and Methods

### 2.1. Source of raw drugs:

The Required raw materials were procured from a well reputed indigenous drug shop from Parys corner, Kanda Samy Temple, Chennai, Tamil Nadu, India. All raw drugs were authenticated by the Botanist and faculties of Gunapadam department, Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India. The test drug *Sambirani poo kuligai* was prepared as per Agasthiar Paripuranam 400.

### 2.2. Ingredients

The siddha formulation *Sambirani poo kuligai* Comprises of the following ingredients

<i>Sambirani [Styrax benzoin]</i>	-
250 g	
<i>Korosani [Felbovinum purifactum]</i>	-
6 g	
<i>Kirambu [Syzygium aromaticum]</i>	-
20 g	
<i>Vetrilai [Piper betel]</i>	-
50 ml	

### 2.3.Purification of Raw Drug

***Styrax Benzoin***[6]: The gums were purified by removing the sand, dust and odd particles.

***Fel bovinum purifactum***: The unwanted debris substances were removed.

***Syzygium aromaticum*** [7]: The flower buds were removed and fried slightly.

***Piper betle*** :The stalk and the middle vein were removed.

### 2.4.Method of preparing Sambirani poo kuligai [8]

The purified *Styrax benzoin* was powdered well and was placed in a small pot. Then a paper was pasted on the inner surface of the big pot. The big pot was

placed over the small pot and their mouths oppose each other. The gap between their mouths were covered by a seven layered muddy wet cloth and they allowed to dry. Then it was subjected to sublimation process for 12 hours (4 samam). After finishing sublimation process let the pot undisturbed to give away heat. Followed by this the seal were opened and the sublimed product was scrapped and collected.

### 2.4.1. Kuligai Process

*Syzygium aromaticum* and *Felbovinum* are powdered well and sieved through a white cloth. Finely powdered *Syzygium aromaticum* powder and *Felbovinum* powder are added along with the sublimate. Then all these substances are grounded well with *Piper betle* leaf juice for 48 minutes [2 *Nazhigai*]. The paste was made into pills in the size of seeds of *Abrus precatorius* [*Kundri size*] which was equivalent to 130 mg, dried in the shade and bottled up.

### 2.5. Organoleptic Investigation

The macroscopical evaluation of Sambirani poo kuligai was performed as per the methods of Khandelwal [9]. Organoleptic characters such as color and texture were studied.

### 2.6. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay [10]

The antioxidant activity of test drug sample SPK was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample SPK was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution the serial dilution the concentration of 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml and 20 µg/ml was made respectively. Ascorbic acid were used as

standard was prepared in same concentration as that of the sample extract by using methanol as solvent. 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 sample SPK at different concentration of (1.25 µg, 2.5µg, 5 µg, 10 µg and 20µg/ml) was noted after 15 min incubation period at 37°C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

$$\% \text{ scavenging} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100$$

The effective concentration of test sample SPK required to scavenge DPPH radical by 50% (IC<sub>50</sub> value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations

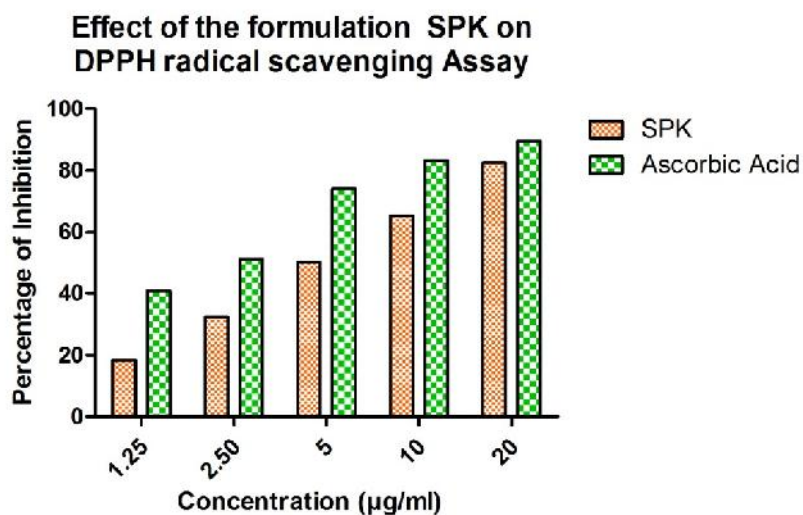
## 3. Results

### 3.1. Result Analysis of DPPH radical scavenging Assay of SPK

The results of DPPH radical scavenging assay of the sample SPK shows that the test drug possesses concentration dependents scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the SPK was given in (Table 1 and Figure 1). The extract of SPK showed the highest DPPH scavenging activity (82.52%) at 20µg/ml and the lowest percentage of inhibition (18.36%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (89.62%) at 20µg/ml and the lowest percentage of inhibition (40.89%) at 1.25µg/ml.

**Table No 1: Results of DPPH radical scavenging Assay of SPK**

Sample concentration (µg/ml)	Percentage of Inhibition	
	SPK	Standard
Control	-	-
1.25	18.36	40.89
2.50	32.40	51.25
5	50.08	74.07
10	65.28	83.33
20	82.52	89.62

**Figure 1: DPPH radical scavenging Assay of SPK**

#### 4. Discussion

Free radicals and other oxidants have gained importance in the field of biology due to their central role in various physiological conditions as well as their implication in a diverse range of diseases. The free radicals, both the reactive oxygen species (ROS) and reactive nitrogen species (RNS), are derived from both endogenous sources (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells etc.) and exogenous sources (pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs like halothane, paracetamol, and radiation). Free radicals can adversely affect various important classes of biological molecules such as nucleic acids, lipids, and proteins, thereby altering the normal redox status leading to increased oxidative stress [11].

Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [12]. Substantial evidence has accumulated and indicated key roles for reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health [13]. Human body has an inherent antioxidative mechanism and many of the biological functions such as the anti-mutagenic, anti-carcinogenic, and anti-aging responses originate from

this property [14,15]. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells [16]. Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance [17,18]. The results of DPPH radical scavenging assay of the sample SPK shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals with the highest percentage inhibition of about 82.52%.

#### 5. Conclusion

Imbalance between the antioxidants and oxidant leads to increased generation of free radicals which in turn causes vigorous damage to macromolecules such as nucleic acids, proteins and lipids. This leads to tissue damage in various disease conditions such as diabetes mellitus, neurodegenerative diseases, cancer, cardiovascular diseases, cataracts, rheumatoid arthritis, asthma etc. and thus severely hastening the disease progression. From the result obtained from the present investigation it was concluded that the formulation SPK possess significant antioxidant property and may act therapeutically in treating several oxidative stress related disorder's. Further present investigation had generated an evidence based data with respect to purity, standards and antioxidant potential of the formulation SPK.

## Acknowledgments

I wish to acknowledge my thanks to The Noble research solutions, Chennai for their technical support.

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### How to cite this article:

M. Vijibala, R. Karolin Daisy Rani, V.Velpandian, M.Pitchiah Kumar. (2017). Evaluation of Antioxidant potential of the Siddha Formulation *Sambirani Poo Kuligai* by *in-vitro* DPPH radical scavenging assay . *Int. J. Adv. Res. Biol. Sci.* 4(12): 257-261.

DOI: <http://dx.doi.org/10.22192/ijarbs.2017.04.12.027>