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Antioxidant potential of novel Siddha Polyherbal distillate *Sanjeevi theeneer* investigated through DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) method

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Abstract
Sanjeevi theeneer (ST) is a novel *siddha* distillate formulation used mainly in conditions of Jaundice, and Cardiac diseases. Previous preliminary phytochemical studies on the drug had shown the presence of phenolic compounds that are natural radical scavengers. The present study aimed to investigate the antioxidant activity of *Sanjeevi theeneer* (ST) against DPPH (2, 2-diphenyl 1-2 picrylhydrazyl) free radical. 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 was read out at 517 nm using double-beam U.V Spectrophotometer. The effective concentration of test sample ST required to scavenge DPPH radical by 50% (IC_{50} value) was obtained as 90.19 ± 8.57 µg /ml as compared with Ascorbic acid (46.91± 9.93 µg /ml) showing its Intermediate anti-oxidant activity. The report of the present assay supports the Anti-oxidant potential of *Sanjeevi theeneer* for its wide clinical applications.

Keywords: siddha medicine, *Sanjeevi theeneer*, DPPH free radical scavenging activity, antioxidant

1. Introduction
Free radical generation within the body is the most possible triggering factor for accelerated ageing process and at least hundreds of diseases most prevalent being liver diseases and cardio vascular ailments [1]. Human body has its own defense mechanisms to nullify or balance the radical effects and in certainly there are conditions in which this natural detoxification processes are impaired or insufficient to provide the equilibrium [2]. The importance elixir therapies as mentioned as *Kalpa* treatments in *siddha* medicine is an approach were the Physical, and psychological aspects of the human body is vitalized through several therapies including drug, diet or supplementations [3]. The formulations mentioned under this class shall include or possess the properties of Tonics, rejuvenators, Anti-oxidants simultaneously correcting the imbalanced Trihumors and moreover as a fact all these formulations act in a broad spectrum way.
Herbal sources are the first criteria for rejuvenation therapies either in the form of single drug or compound formulations as they are the chief source of antioxidants [4]. Out of this aqueous distillates, defined as *Theeneer* are the vital extracts of the herbs that stand unique in promoting wellness for the individual [5]. Numerous herbs especially spices, fruits are individually or collectively distilled and used in siddha medical practice for this purpose. *Sanjeevi theeneer* is a premium distillate product which has a wide role in curative, palliative and rejuvenation therapies in conditions of liver, spleen, blood and cardiac disorders. The formulation is a blend of eleven herbs and one mineral [6]. Most of the herbs in this formulation is considered as very important *kalpa Mooligaigal* (Herbal Elixirs) and so many studies are justifying its Anti-oxidant activity [7- 15].
The objective of this study is to investigate the free radical scavenging property of ST using DPPH assay with standard drug Ascorbic acid.

2. Materials and methods
2.1 Collection of Raw material and Test sample preparation
All the raw drugs were purchased from reputed country merchants, purified as referred in the texts. The ingredients were pounded nicely and soaked in water for a period of 7 days. On the 8th day the distillate was prepared as per the standard procedures [6] [Table. 1]

Table 1: Ingredients of *Sanjeevi theeneer*

S. No	Ingredient	Botanical Name	Part Used	Quantity
1	*Chukku*	*Zingiber officinale*	Dry Rhizome (Outer skin removed)	60 g
2	*Milagu*	*Piper nigrum*	Dry fruit	60 g
3	*Thippili*	*Piper longum*	Dry Berry	10g
4	*Kadukkai*	*Terminalia chebula*	Dry fruit (seed removed)	25g
5	*Nellikkai*	*Phyllanthus emblica*	Dry fruit (seed removed)	50g

6	*Tantrikkai*	*Terminalia belerica*	Dry fruit (seed removed)	25g
7	*Omam*	*Trachyspermum ammi*	Dry fruit	25g
8	*Vaividangam*	*Embelia ribes*	Dry fruit	25g
9	*Chithramoolam*	*Plumbago zeylanica*	Dry Root Bark	30g
10	*Korai kizhangu*	*Cyperus rotundus*	Dry Tuber	25g
11	*Panam karkandu*	*Borassus flabellifer*	Palm Candy	20g
12	*Irumbu Podi*	Purified Ferrum powder	--	60 g
13	Water			6 Litres

2.2 Dpph (2, 2-Diphenyl 1-2 picrylhydrazyl) Free radical scavenging Assay [16]

The assay was carried out to screen the Anti oxidant property of the test drug. Stock solution was prepared with ST and standard Ascorbic acid by using solvent, methanol (90%). Different concentrations of 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of this solution were prepared. 1 ml of 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. The mixture was kept in the dark for 30 mins time period. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer. The effective concentration of test sample ST required to scavenge DPPH radical by 50% (IC_{50} value (µg/ml) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

$$\% \ Scavenging = \frac{[Absorbance \ of \ Control \ - \ Absorbance \ of \ Test \ sample}{\text{Absorbance of Control] X 100}}$$

3. Results & discussion

Usually a herbal distillate primarily yields different classes of compounds belonging to Phenols, organic acids, volatile compounds and so many intermediate constituents [17-20]. In previous studies the Qualitative analysis of phytochemical variables in *Sanjeevi theeneer* reported the presence of Phenolic compounds. Phenolic compounds are considered as the most active anti oxidant group of metabolites derived from the herbal resources (Ali *et al.*, 2008 and Singh 2007).

Previous Analytical Gas Chromatography – Mass Spectrometry (GC-MS) studies on the same drug revealed the presence of major compound Oleic acid, Dasycarpidan, Monocyclic phenolic compounds like Thymol and Isothymol, 1,6-Octadien-3-ol, 3,7-dimethyl-(Linalool) and other secondary metabolites which may have significant biological properties contributing to the Therapeutic potential of *Sanjeevi theeneer* as an Anti-oxidant [21-24]. So based on this outcome we decided to assess the radical scavenging effect of the drug sample on the stable DPPH free radical.

DPPH assay is a reliable, rapid and effective procedure to analyze the radical scavenging property of the drug solution and selecting the proper solvent for the *In vitro* assays is very crucial as each of them responds with the bioactive compounds in a different manner. Here polar solvent like methanol was used in the mixture solution preparation as it can effectively extract volatile or phenolic compounds from a distillate drug. Dpph has the capacity to accept an electron or hydrogen radical thereby changing to a stable diamagnetic molecule and when the drug solution is allowed to interact with the DPPH radical the reduction in the absorbance is indicated by the changes in color from purple to yellow. This clearly depicts the presence of anti oxidant principles in the drug solution [25].

Here In the present study % inhibition of test drug *Sanjeevi Theeeneer* (ST) and the standard Ascorbic acid on DPPH radical was measured and assessed on the basis of this principle.(Table 1) Here 6 different concentrations (10, 20, 40, 60, 80, 100 µg/ml) demonstrated the different percentage of inhibition of the test drug and the standard. The scavenging activity of both was increased in a concentration dependent manner. Concentration at 100 µg/ml showed the best % inhibition of the test drug (55.4 ± 3.58) [Table. 2 & Graph 1]

The Inhibition Concentration value (IC_{50}) was measured to find out the effective concentration (in µg/ml) of the drug sample to inhibit half of the free radical. The IC_{50} Values of ST was 90.19 ± 8.57 µg/ml as compared with standard ascorbic acid (46.91 ± 9.93 µg/ml) [Table. 2]. With reference to studies of Phongpaichit [25] were he grouped the anti oxidant activity in accordance with the IC_{50} (µg/ml) ranging from 10-50 µg/mL as strong anti-oxidants, 50-100 µg/mL as Intermediate antioxidants and >100 µg/mL as weak Anti-oxidants [Table 3]. The test drug and the standard in this study fall under anti-oxidants with intermediate activity. However the radical scavenging activity of the drug ST was lesser but considerable as compared with the standard.

Table 2: Percentage Inhibition of test drug *Sanjeevi Theeeneer* (ST) on DPPH radical scavenging assay

Concentration (µl)	% Inhibition of Ascorbic acid	% Inhibition of ST
10 µg/ml	23.7 ± 8.34	6.881 ± 1.79
20 µg/ml	35.93 ± 4.57	15.77 ± 2.42
40 µg/ml	45.56 ± 9.71	24.29 ± 2.49
60 µg/ml	57.41 ± 3.32	33.55 ± 6.20
80 µg/ml	68.89 ± 7.74	45.4 ± 5.04
100 µg/ml	85.19 ± 2.37	55.4 ± 3.58

Data are given as Mean ± SD (n=3)

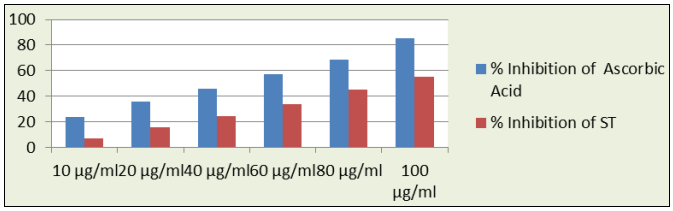

Fig 1

Table 3: IC_{50} Values of *Sanjeevi theeneer* (ST) and Standard.

Test Drug / Standard	IC_{50} Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	46.91 ± 9.93
ST	90.19 ± 8.57

Data are given as Mean ± SD (n=3)

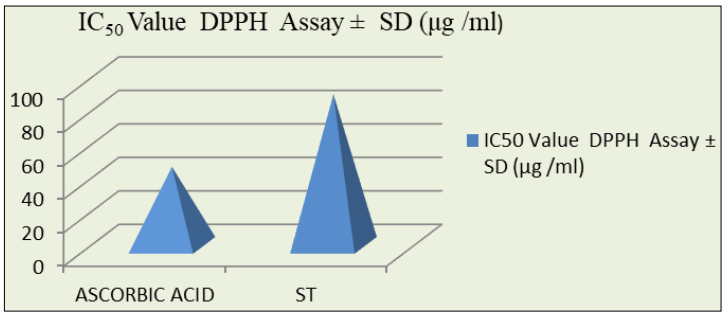

Fig 2

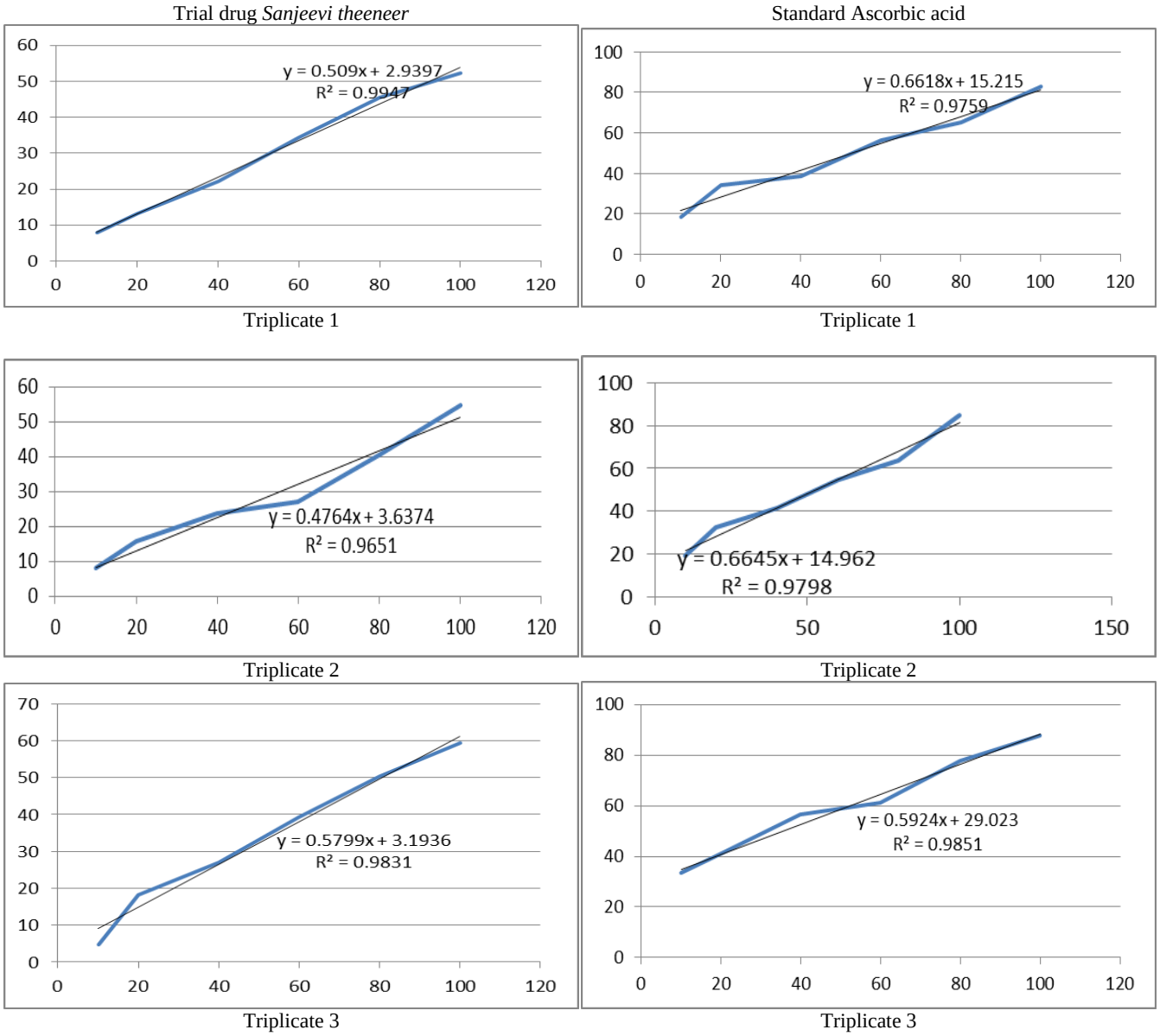

Fig 3: Percentage inhibition of ST and Standard DPPH radical scavenging assay

Table 4: Anti-oxidant Grading with reference to IC_{50} Values (*Phongpaichit, 2007*) [25]

Anti-Oxidant Category	IC_{50} (µg /ml)
Strong Anti-Oxidants	10-50 µg/mL
Intermediate Anti-Oxidants	50-100 µg/mL
Weak Anti-Oxidants	>100 µg/mL

4. Conclusion

Thus the *Sanjeevi theeneer* merits further analytical procedures and in vivo assessments for its further successful clinical validations and moreover with the growing necessity of natural anti-oxidant health products, the introduction of such *siddha* distillates has numerous opportunities in the field

of neutraceutical. Not only it could the synthetic antioxidant products and also it can be used as safe and therapeutically acclaimed health promoters on regular basis or for special medical purposes.

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