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IN-VITRO ANTIOXIDANT PROPERTY OF SIDDHA POLYHERBAL FORMULATION *THETRAN ILAGAM*

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ABSTRACT

Siddha system of medicine is a renowned holistic system of traditional medicine emphasizing curative and preventive measures. The medicines used in siddha are of plant origin, metals, minerals and animal products. Kaya-karpam (Elixir science) is a treasure for the siddha system as they improvise the longevity of life through their anti-oxidant activities**.** *Thetran Ilagam* is a Siddha polyherbal formulation medicine mainly useful in treating Male infertility. Antioxidant activity of *Thetran Ilagam* is being tested scientifically by using the samples of prepared *Thetran Ilagam*. The qualitative and quantitative analysis of antioxidant activity of *Thetran Ilagam* extract was determined.

KEYWORDS: Antioxidant, *Thetran Ilagam*, Siddha system, Tamil medicine, Male infertility.

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules, viz., nucleic acids, proteins, lipids, and DNA and can initiate different

degenerative diseases such as neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, and arthritis.[11] Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders. Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as superoxide dismutase, catalase, and antioxidant compounds, viz., ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione. Prior and Cao (1999) reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Plants have a longest history of use as a medicine, food source, and for a variety of daily needs. Of the 250,000 known plant species on the Earth, more than 80,000 are utilized for medicinal purposes. India is one of the world's 12 biodiversity centres with the presence of over 45,000 different plant species. Of these, about 15,000-20,000 plants have a potent medicinal value. However, only 7000-7500 species are utilized in routine by traditional communities for their medicinal value. In India, drugs of herbal origin have been used by Ayurveda, Siddha, and Unani systems of medicines since ancient times. Siddha system of medicine is one of the oldest one from Dravidian culture. This system is mainly focused on food as medicine. Kayakarpam is also called as elixir science is unique and treasure of the siddha system. Kayam means body karpam means stone alsoknown as life span of Brahma according to Hindu mythology. Hence, this medicine is one which makes human body as stone and not affected by any diseases or aging. These kinds of medicines are available from herbal preparation, metals and from animal products also. Many of siddhars such as sage Agathiyar and Bohar are written about in various literatures. These medicines are preventive as well as cure the disease. The kayakarpam prevent the aging process as one of the actions is antioxidant property. The *Thetran Ilagam* (TI) composition of 20 different herbs viz., (In Tamil) *Thetrankottai, Narseeragam, Kasakasaa, Lavangapattai, Vaalmelagu, Sapjavithai, Saathikkaai, Saathipathri, Melagu, Esappukool vithai, Poonaikan kungiliyam, Madhulai vithai, Neermulli vithai, Seemai thanneervittankizhangu, Nilappanai kizhangu, Omam, Thiratchai pazham, Perichchan pazham, Saarap paruppu, Padham paruppu* the Ilagam form of this medicine used for Male infertility. The aim of the present study is to evaluate the in vitro free radical scavenging activity of TI and for this 1, 1-diphenyl phenyl hydrazyl (DPPH) radical scavenging activity, ABTS radical scavenging activity, nitric oxide (NO) radical scavenging activity, and Hydrogen peroxide radical scavenging activity was determined as per standard procedures.

MATERIALS AND METHODS

Collection of plant materials

The plant materials required for the formulation of Siddha medicines were collected from the commercial Siddha raw drug stores. All the ingredients were shade dried, powdered and sieved was formulated into medicines and stored in porcelain pots for further use. The Siddha formulation was prepared as prescribed in the written siddha text.

Table 1: Composition of *Thetran Ilagam* (TI).

S.No	Siddha Name	Botanical Name
1	Thetran Kottai	*Strychnos potatorum*
2	Narseeragam	*Cuminum cyminum*
3	Kasakasaa	*Papaver sominiferum*
4	Lavangapattai	*Cinnamomum verum*
5	Vaalmelagu	*Piper cubeba*
6	Saathikkaai	*Myristica fragnans*
7	Sapjavithai	*Ocimum basilicum*
8	Saathipathri	*Myristica fragnans*
9	Melagu	*Piper nigrum*
10	Esappukool vithai	*Plantago ovate*
11	Poonaikan kungiliyam	*Pistacia lentiscus*
12	Madhulai vithai	*Punica granatum*
13	Neermulli vithai	*Hygrophila auriculata*
14	Seemai thanneervittankizhangu	*Asparagus racemosus*
15	Nilappanai kizhangu	*Curculigo orchiodes*
16	Omam	*Trachyspermum ammi*
17	Thiratchai pazham	*Vitis vinifera*
18	Perichcham pazham	*Phoenix dactylifera*
19	Saarap paruppu	*Buchanania lanzan*
20	Padham paruppu	*Prunus dulcis*

Sample Extraction

Sample Extraction were carried out with chloroform and the resulting extract was utilized for the Anti-oxidant assay.

RESULT AND DISCUSSION

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay of *Thetran Ilagam*

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

10 ml whose concentration was then10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml 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 at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100 µg/ml) was noted after 15 min incubation period at 37^0C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

The effective concentration of test sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations.

Nitric Oxide Radical Scavenging Assay *Thetran Ilagam*

The concentrations of test sample are made into serial dilution from 10−100 µg/mL and the standard gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10−100 µg/mL) and incubated at 25°C for 180 mins. The test drug was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm using a Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged } (\%) = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

ABTS Assay of *Thetran Ilagam*

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug against 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals.

The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1 : 44, v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of test sample (10-100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

INFERENCE AND OBSERVATION

DPPH radical scavenging activity of *Thetran Ilagan*

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 4.14 to 29.34% when compared with standard ascorbic acid with percentage inhibition ranges from 37.95 to 88.99%. The IC50 value of the trial drug was found to be 181.4 (µg /ml) when compared with standard ascorbic acid with (IC_{50} value 22.44 µg/ml).

NO radical scavenging activity of *Thetran Ilagan*

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 7.96 to 41.59% when compared with standard gallic acid with percentage inhibition ranges from 17.13 to 85.44%. The corresponding IC50 value of the trial drug was found to be 127.7(µg /ml) when compared with standard gallic acid with (IC_{50} value 44.4 µg/ml).

ABTS radical scavenging activity of *Thetran Ilagan*

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 5.58 to 43.59% when compared with standard gallic acid

with percentage inhibition ranges from 40.54 to 97.52%. The corresponding IC50 value of the trial drug was found to be 117.4 (µg /ml) when compared with standard gallic acid with (IC_{50} value 15.22 µg/ml).

Hydrogen peroxide radical scavenging activity of *Thetran Ilagan*

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 1.47 to 25.17% when compared with standard BHA with percentage inhibition ranges from 37.65 to 87.65%. The corresponding IC50 value of the trial drug was found to be 196 (µg /ml) when compared with standard BHA with (IC_{50} value 23.21 µg/ml).

RESULTS

Percentage inhibition of test drug TI on

DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of TI	% Inhibition of Ascorbic Acid
10 µg/ml	16.05 ± 0.2762	46.85 ± 2.166
20 µg/ml	33.73 ± 0.99	61.76 ± 2.081
40 µg/ml	38.45 ± 1.844	69.74 ± 2.619
60 µg/ml	49.5 ± 0.5808	78.41 ± 2.753
80 µg/ml	59.63 ± 1.127	86.01 ± 8.025
100 µg/ml	77.23 ± 0.6707	95.75 ± 0.2166

Data are given as Mean ± SD (n=3).

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

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	4.838 ± 1.31
TI	58.73 ± 0.5627

Data are given as Mean ± SD (n=3).

Percentage inhibition of test drug TI on

Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of TI	% Inhibition of Gallic Acid
10 µg/ml	9.97 ± 5.606	34.79 ± 4.732
20 µg/ml	20.95 ± 6.013	51.23 ± 2.099
40 µg/ml	30.8 ± 10.31	61.72 ± 2.776
60 µg/ml	42.92 ± 4.731	70.82 ± 3.206
80 µg/ml	56.18 ± 8.194	78.16 ± 1.603
100 µg/ml	66.79 ± 5.72	88.65 ± 1.603

Data are given as Mean ± SD (n=3).

IC50 Values for Nitric Oxide radical scavenging assay by TI and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
TI	105.1 ± 57.92
GALLIC ACID	25.16 ± 4.351

Data are given as Mean ± SD (n=3).

Percentage inhibition of test drug TI on

ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of TI	% Inhibition of Gallic Acid
10 µg/ml	16.61 ± 1.923	41.8 ± 2.616
20 µg/ml	29.22 ± 2.922	63.98 ± 1.04
40 µg/ml	39.79 ± 1.041	70.56 ± 1.2
60 µg/ml	50.67 ± 2.605	77.84 ± 2.616
80 µg/ml	58.92 ± 3.828	82.34 ± 3.176
100 µg/ml	73.4 ± 0.5128	95.26 ± 1.002

Data are given as Mean ± SD (n=3).

IC50 Values for ABTS radical scavenging assay by TI and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
TI	60.65 ± 2.284
GALLIC ACID	6.957 ± 2.123

Data are given as Mean ± SD (n=3).

Percentage inhibition of test drug TI on

Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of TI	% Inhibition of BHA
10 µg/ml	11.34 ± 1.775	35.48 ± 3.529
20 µg/ml	24.72 ± 0.628	49.75 ± 4.95
40 µg/ml	34.79 ± 1.026	59.27 ± 8.573
60 µg/ml	35.44 ± 4.423	70.61 ± 5.07
80 µg/ml	56.61 ± 10.12	78.84 ± 3.736
100 µg/ml	63.15 ± 8.971	87.18 ± 1.775

Data are given as Mean ± SD (n=3).

IC50 Values for Hydrogen peroxide radical scavenging assay by TI and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
TI	75.83 ± 10.49
BHA	26.35 ± 8.377

Data are given as Mean ± SD (n=3).

CONCLUSION

Thetran Ilagam has proved its antioxidant activity. 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 TI possess significant antioxidant property and may act therapeutically in treating several oxidative stress related disorders. Further present investigation had generated an evidence based data with respect to purity, standards and antioxidant potential of the formulation TI.

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