WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 808-816. Research Article ISSN 2277– 7105

EVALUATION OF ANTI-OXIDANT PROPERTY OF SIDDHA HERBAL FORMULATION "THOOTHUVELAI KARPAM"

Rahini Nivedhidha C.*[1], Ranjitham K.[1], Sivakkumar S.[2], Visweswaran S.[3], Maariappan A.[4] and Banumathi V.[5]

[1]P.G Scholar, Department of Gunapadam, National Instiute of Siddha.

[2]Lecturer, Department of Gunapadam, National Institute of Siddha.

[3]Head of the Department, Department of Gunapadam, National Institute of Siddha.

[4]Lecturer, Department of Gunapadam, National Institute of Siddha.

[5]The Director, National Institute of Siddha.

Article Received on
26 July 2018,

Revised on 16 August 2018,
Accepted on 06 Sept. 2018

DOI: 10.20959/wjpr201817-13241

***Corresponding Author**
Rahini Nivedhidha C.
P.G Scholar, Department of Gunapadam, National Instiute of Siddha.

ABSTRACT

Siddha system of Medicine is one of the ancient systems of medicine in India. Siddha medicine serves both therapeutic and prophylactic concepts and hence Holistic in nature. Kaya Karpam, a special branch of this medical system is a science of rejuvenation, longevity, and spiritual wellbeing. Concept of Kaya Karpam was briefly explained by saint Thirumoolar in his text Thirumanthiram. The word Kaya and Karpam denotes the meaning of Prevention of body from diseases. Nowadays, this can be correlated with the Anti-Oxidant concept. It deals with the Prevention of *Narai* (Whitening of hairs), *Thirai* (Shrinking of Skin), *Moopu* (Aging), *Saakadu* (Death). Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. A plant-based drug protects against chronic oxidative stress-related diseases. Kaya Kalpa drugs have proven scientific evidences to prevent and manage the Non communicable diseases like Diabetes mellitus, Obesity and also Chronic deliberating diseases like cancer etc. This study is aimed to screen the antioxidant effect of *Thoothuvelai Karpam* by using DPPH free radical scavenging assay, Nitric Oxide radical scavenging, ABTS radicals, Hydrogen Peroxide Radical Scavenging Assay. The results of this study shows that the percentages of inhibition in DPPH, Nitric Oxide and ABTS, Hydrogen Peroxide radical scavenging studies are 50.73% (standard ascorbic acid 86.07%), 69.38% (standard Gallic acid 91.38%) and 67.1% (standard Gallic acid 94.48%), 70.74% (standard BHA 89.98%) respectively and thus, our findings

provide evidence that *Thoothuvelai Karpam* could be a potential source of natural antioxidant and it may be used as rejuvenating medicine for vast therapeutic effects, gives a powerful body, mind and soul with long-lasting life.

KEYWORDS: Thoothuvelai karpam, Kayakalpam, Siddha medicine, Antioxidant.

INTRODUCTION

Oxidation in living organisms is essential for the generation of energy during catabolism but these metabolic processes result in the continuous production of free radicals and reactive oxygen species (ROS) *in vivo*. Free radicals or more generally ROS are highly reactive species that are generated by cells during respiration and cell-mediated immune functions. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, and pesticide. The instability and reactivity of free radicals due to the lone electron in the outer shell can cause them to attack specific bio-molecules in the body such as protein and lipids. Normally, there is a balance between the quantity of free radicals generated in the body and the antioxidant mechanisms which scavenge/quench these free radicals preventing them from causing deleterious effects in the body. The antioxidant mechanisms include endogenous and exogenous systems such as catalase and vitamin antioxidants, respectively. When the generation of free radicals exceeds the scavenging capacity of the cell's endogenous systems, the excess free radicals seek stability through electron pairing with biological macromolecules of healthy cells such as proteins, lipids, and DNA. The pairing of the free radicals with bio-molecules can eventually result in the induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases. Prolonged oxidative stress can result in permanent damage to vital body organs, which could eventually lead to chronic disorders such as heart diseases, diabetes, cirrhosis, malaria, neurodegenerative diseases, AIDS, cancer, and premature aging. It has been noted that about 95% of the pathologies observed in people above 35 years of age are associated with production and accumulation of free radicals. Natural antioxidants are considered to be safe and bioactive. The antioxidants from natural sources are the only alternative to synthetic antioxidants in counteracting the free radicals associated disease. The antioxidant activities of phenolic compounds are mainly due to the redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers, in addition to their metal chelating potential. The antioxidant activity of phenolics plays an important role in the adsorption or neutralization of free radicals. In recent

years, various species of plants have been used in preparation of drugs and are consumed as food due to their antioxidant activities. The extracts of medicinal plants and natural products have become a great source of antioxidant and antiaging properties. Recently, much attention has been directed towards the development of ethnomedicines with strong antioxidant properties but with low cytotoxicity. Therefore, antioxidants with free radical scavenging activities of medicinal plants may have great relevance in the prevention of diseases and in therapeutic properties. Plants, rich in their phytochemical compounds, are good sources of antioxidants and radical scavengers. In Indian traditional system of medicine like Siddha system many more herbs are used in antioxidant medicinal formulations which are called as *Kaaya Kalpa* medicines. These formulations have been used to treat the illness and help to regenerate the degenerative conditions and also help to prevent the aging. *Thoothuvelai Karpam* is a herbal Siddha preparation mentioned in ancient Siddha literature. Modern pharmaceuticals and nutraceuticals are currently out of reach of a large proportion of the human population in developing countries. This necessitates the use of other sources of human knowledge to provide common health benefits. Thus, herbal medicines are now regarded as important but underutilized tool against the disease. The main objective of the study was to determine the antioxidant activity of *Thoothuvelai Karpam* by DPPH, Nitric Oxide and ABTS, Hydrogen peroxide radical scavenging assay.

MATERIALS AND METHODS

Thoothuvelai Karpam

The test drug Thoothuvelai Karpam is a herbal Siddha preparation prepared from Thothuvelai samoolam which has been used for Paandu (Anaemia), Athithoolam(obesity), Karappan (Eczema), Gunmam(Ulcer), Erumal(Cough), Increase sperm count, Skin diseases and helps to enhance the immune system. It is a rejuvenating medicine in siddha system and used as a Kaayakalpam.

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of test drug sample *Thoothuvelai Karpam* was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample *Thoothuvelai Karpam* was mixed with 95% methanol to prepare the stock solution in required concentration. 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 test drug 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 *Thoothuvelai Karpam* at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/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.

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

The effective concentration of test sample *Thoothuvelai Karpam* 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

The concentrations of test sample *Thoothuvelai Karpam* 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 *Thoothuvelai Karpam* 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 *Thoothuvelai Karpam* and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug *Thoothuvelai Karpam* 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

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug *Thoothuvelai Karpam* 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 *Thoothuvelai Karpam* was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample *Thoothuvelai Karpam* was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

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

Hydrogen Peroxide Radical Scavenging Assay

A hydrogen peroxide solution (2 mm) was prepared in 50 mm phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample *Thoothuvelai Karpam* (different concentration ranging from 10-100µg/ml) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mm phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexes and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. BHA was used as the positive control. The percentage inhibition of the test drug *Thoothuvelai Karpam* and standard was calculated and recorded. The percentage radical scavenging activity of the test drug *Thoothuvelai Karpam* and BHA were calculated using the following formula:

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

RESULTS

Percentage inhibition of test drug *Thoothuvelai Karpam* on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of *Thoothuvelai Karpam*	% Inhibition of Ascorbic Acid
10 µg/ml	5.767 ± 1.16	48.15 ± 1.69
20 µg/ml	15.07 ± 4.19	59.26 ± 4.20
40 µg/ml	20.5 ± 2.42	64.81 ± 4.27
60 µg/ml	30.96 ± 2.92	72.22 ± 4.06
80 µg/ml	39.1 ± 2.96	82.44 ± 10.81
100 µg/ml	50.73 ± 5.73	86.07 ± 6.22

Data are given as Mean ± SD (n=3)

IC50 Values for DPPH radical scavenging Assay by *Thoothuvelai Karpam* and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	76.53 ± 32.56
Thoothuvelai Karpam	102.8 ± 12.23

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug *Thoothuvelai Karpam* on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of	% Inhibition of Gallic Acid
10 µg/ml	10.37 ± 2.39	31.86 ± 5.13
20 µg/ml	23.78 ± 1.14	45.62 ± 1.25
40 µg/ml	36.04 ± 4.64	52.87 ± 1.88
60 µg/ml	51.75 ± 7.02	65.91 ± 2.17
80 µg/ml	62.86 ± 4.14	76.42 ± 3.32
100 µg/ml	69.38 ± 5.78	91.38 ± 0.41

Data are given as Mean ± SD (n=3)

IC50 Values for Nitric Oxide radical scavenging assay by *Thoothuvelai Karpam* and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
Thoothuvelai Karpam	**63.79 ± 5.546**
GALLIC ACID	34.16 ± 3.453

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug *Thoothuvelai Karpam* on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of *Thoothuvelai Karpam*	% Inhibition of Gallic Acid
10 µg/ml	18.31 ± 1.575	46.78 ± 2.677
20 µg/ml	28.62 ± 3.621	58.13 ± 2.677
40 µg/ml	36.18 ± 5.74	69.12 ± 4.429
60 µg/ml	46.83 ± 6.628	75.5 ± 4.429
80 µg/ml	60.57 ± 7.806	82.6 ± 3.736
100 µg/ml	67.1 ± 8.439	94.48 ± 1.431

Data are given as Mean ± SD (n=3)

IC50 Values for ABTS radical scavenging assay by *Thoothuvelai Karpam* and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
Thoothuvelai Karpam	66.03 ± 13.01
GALLIC ACID	7.927 ± 5.193

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug *Thoothuvelai Karpam* on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of *Thoothuvelai Karpam*	% Inhibition of BHA
10 µg/ml	13.89 ± 2.11	41.42 ± 2.21
20 µg/ml	26.25 ± 2.20	56.57 ± 6.68
40 µg/ml	38.61 ± 0.61	65.44 ± 4.99
60 µg/ml	46.73 ± 3.81	75.05 ± 4.19
80 µg/ml	58.38 ± 3.89	85.65 ± 2.37
100 µg/ml	70.74 ± 3.40	89.98 ± 0.11

Data are given as Mean ± SD (n=3)

IC50 Values for Hydrogen peroxide radical scavenging assay by *Thoothuvelai Karpam* and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
Thoothuvelai Karpam	64.51 ± 2.04
BHA	14.05 ± 8.633

Data are given as Mean ± SD (n=3)

SUMMARY AND DISCUSSION

I. DPPH radical scavenging activity

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 5.76 to 50.73% when compared with standard ascorbic acid with percentage inhibition ranges from 48.15 to 86.07%. The IC50 value of the ***Thoothuvelai Karpam*** was found to be 102.8 (µg /ml) when compared with standard ascorbic acid with (IC_{50} value 76.53 µg/ml).

II. NO radical scavenging activity

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 10.37 to 69.38% when compared with standard gallic acid with percentage inhibition ranges from 31.86 to 91.38%. The corresponding IC50 value of the ***Thoothuvelai Karpam*** was found to be 63.79 (µg /ml) when compared with standard gallic acid with (IC_{50} value 34.16 µg/ml).

III. ABTS radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 18.31 to 67.1% when compared with standard gallic acid with percentage inhibition ranges from 46.78 to 94.48%. The corresponding IC50 value of the ***Thoothuvelai Karpam*** was found to be 66.03 (µg /ml) when compared with standard gallic acid with (IC_{50} value 7.92 µg/ml).

IV. Hydrogen peroxide radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 13.89 to 70.74% when compared with standard BHA with percentage inhibition ranges from 41.42 to 89.98%. The corresponding IC50 value of the ***Thoothuvelai Karpam*** was found to be 64.51 (µg /ml) when compared with standard BHA with (IC_{50} value 14.05 µg/ml)

CONCLUSION

Based on the results obtained from the In-vitro anti-oxidant assay for the sample *Thoothuvelai Karpam* it was concluded that the siddha formulation *Thoothuvelai Karpam* has promising anti-oxidant activity in the estimated assays.In vitro study indicates that the test drug has a significant source of natural antioxidants, which might be helpful in promoting the

Siddha concept of 'Kaaya karpam' thereby preventing the progress of Narai (Whitening of hairs), Thirai (shrinkening of Skin), Moopu (aging), Pini (disease) and Saakadu (Death).

REFERENCES

1. The Siddha text book "*ANUBOGA VAITHIYA NAVANITHAM –PART 8"* 53,54.
2. Badami, Omprakash, Dongr SH,Suresh B. *In-vitro* Antioxidant property of *Solanum Pseudocapsicum* leaf extract. *Indian J Pharmacol*, 2005; 37: 251-252.
3. B. N. Panda, A. B. Raj, N. R. Shrivastava, and A. R. Prathani, "The evaluation of nitric oxide scavenging activity of Acalypha indica Linn Root," Asian Journal Research Chemistry, 2009; 2(2): 148–150.
4. N. Pellegrini, M. Ying, and C. Rice-Evans, "Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2′-azobis (3-ethylbenzothine-6-surfonic acid) radical cation decolorization assay," Methods in Enzymology, 1999; 299: 384–389.
5. Ruch R. J., Cheng S.-J., Klaunig J. E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10(6): 1003–1008.
6. Okuda T, Kimura Y, Yoshida T, Hatano T, Okuda H, Arichi S. Studies on the activities of tannins and related compounds from medicinal plants and drugs. I. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. Chem Pharm Bull (Tokyo), 1983; 31(5): 1625-31.
7. Lata H, Ahuja G. Role of free radicals in health and disease. Indian J Physiol Allied Sci., 2003; 57: 124.
8. Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacogn, 1993; 110: 701.
9. Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant activities and phenolic contents of the methanol extracts of the stems of Acokanthera oppositifolia and Adenia gummifera. BMC Complement Altern Med., 2008; 8: 54-60.
10. 10.Bhaumik UK, Kumar AD, Selvan VT, Saha P, Gupta M, Mazumder UK. Antioxidant and free radical scavenging property of methanol extract of Blumea lanceolaria leaf in different in vitro models. Pharmacol Online, 2008; 2: 74-89. 23.
11. Olayinka AA, Anthony IO. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complement Altern Med., 2010; 10: 21.