https://doi.org/10.48047/AFJBS.6.14.2024.1068-1076

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Screening the antioxidant activity of "*Kameshwara Karpa avizhtham*" – Siddha *Karpa Marunthu*

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Volume 6, Issue 14,2024

Received: 10 JULY 2024

Accepted: 31 JULY 2024

Published: 02 AUG 2024

doi:10.48047/AFJBS.6.14.2024.1068-1076

Abstract:

The Siddha System of medicine is one of the ancient medical systems, which delivers 'Holistic Health'. The system provides defensive, primitive, curative, rejuvenation, rehabilitative health care with scientific and holistic approach. *Karpa* Medicine is one form of Siddha medicine which is formulated to prevent ageing, disease and to cure disease. *Karpa Marunthu* (Rejuvenation) has rich antioxidant activity. Antioxidant compounds had defensive mechanism against oxidative damage of cells. The main aim of this study is to screen the antioxidant activity of *Kameshwara Karpa Avizhtham. Kameshwara karpa avizhtham*. It is screened for Antioxidant activity using DPPH (2,2 – Diphenyl -1-picryl hydrazyl) assay, Nitric oxide assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay and H2O2 (Hydrogen peroxide assay). It shows antioxidant activity of 5.029 ± 1.742 to 35.79 ± 8.032 % and IC50 value 170.3 ± 16.9 (µg /ml), 3.685 ± 3.876 to 23.47 ± 5.825 % and IC50 value 232.9 ± 53.66 (µg /ml) and 2.736 ± 1.685 to 23.01 ± 2.816 % and IC50 value 204 ± 18.26 (µg /ml), 2.553 ± 3.288 to 24.59 ± 4.708 and 221.1 ± 47.02 DPPH, Nitric oxide and ABTS assay, H2O2 respectively. Thus, the trial drug has promising antioxidant activity by invitro assay.

Keywords: *Kameshwara karpa avizhtham*, Antioxidant activity, DPPH assay, ABTS say, Nitrous oxide assay, H2O2 assay.

INTRODUCTION

The Siddha System of medicine is one of the oldest medical systems, which delivers 'Holistic Health'. The system provides defensive, primitive, curative, rejuvnative, rehabilitative health care with scientific and holistic approach[1]. Siddha medicine (Marunthu) is classified into two types. They are 32 types of Internal Medicine and 32 types of External medicines. Of these 32 types of Internal Medicine *Karpam Marunthu* is one among them[2]. According to Siddha, A Medicine is a compound which can able to cure disease, treats psychiatric issues and prevents disease[3]. The word *Kaya karpam* means (*Kayam* – body, Karpam – *Kall polakkuthal*) to make our body competent and youthful[4]. There are two ways is available to prevent ageing, disease and death i.e., *Yogam* and *Kaya karpam. Karpa* Medicine is formulated to prevent ageing, disease and to cure disease. *Karpa Marunthu* (Rejuvenation) has rich antioxidant activity[5]. Antioxidant compounds had defensive mechanism against oxidative damage of cells by reducing the number of free radicals. Oxidation is a normal chemical process that takes places in the body every day. When there are disruptions in the natural oxidation process, highly unstable and potentially damaging molecules called Free radicals are developed[6].

Free radical's reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Prolonged oxidative stress can result in permanent damage to vital organs in our body, which could eventually lead to chronic disorders such as heart disease, diabetes, cirrhosis, malaria, neurodegenerative diseases, AIDS, cancer and premature aging[6]. 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[7].

In order to globalize Siddha medicines, first mechanism of action of the drug and their activities is needed to analyze and to standardize the drug[8]. Hence this study is carried to analyze the activity of the drug.

OBJECTIVES:

To screen the antioxidant activity of *Kameshwara Karpa Avizhtham* in DPPH assay, Nitric oxide assay and ABTS assay, H2O2 assay.

Materials and Methods

A. Procurement of Raw Drugs:

The Raw drugs for the preparation of Medicine ***Kameshwara Karpa avizhtham*** was purchased from the reputed indigenous drug store at Parry's Corner, Chennai.

Raw drugs

a. *Kattu Maangai Paruppu* (*Buchanania lanzan*) – 35gm
b. *Murungai Paruppu* (*Moringa oleifera*) – 35gm
c. *Munthirigai Paruppu* (*Anacardium occidentalae)*– 35gm

d. *Kothumai* (*Triticum aestivum*) – 35gm
e. *Vaathumai Paruppu* (*Prunus dulcis)*-35gm
f. *Naruvili* (*Cordia dichotoma)*– 35gm
g. *Atthippaal* (Latex of *Ficus carica*) -1260 gm
h. Ghee – required quantity
i. *Sakkarai* (Country Sugar) – required quantity.

B. Identification and Authentication

The raw drugs for the preparation of ***Kameshwara Karpa avizhtham*** was identified and authenticated by the experts of Medicinal Botany and Gunapadam, National Institute of Siddha, Chennai – 47.

C. Procedure

Purification –The raw drug was weighed and purified by getting rid of sand, dust particles, small wooden sticks and other adulterants.

Preparation[9]:

Kattu Maangai Paruppu, *Murungai Paruppu*, *Munthirigai Paruppu Kothumai*, *Vathumai Paruppu*, *Naruvili* were taken 35gm (each drug) and placed in mud pot.1.260 Kilogram of Atthippaal was collected and pour into the mud pot. Allowed to dry it, at sunlight for 6 days. After that external layer of ***Vaathumai Paruppu***, ***Kattu Maangai Paruppu*** was removed. Again, the raw drugs were allowed to dry until it completely dried. After that powder it and weighed. Equal quantity i.e., 200gm of Sugar (Brown Sugar) and 200gm of ghee is added to that mixture. After that the drug is stored in airtight container.

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

Introduction[10]:

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in colour (from deep violet to light yellow) was measured. The intensity of the yellow colour depends on the amount and nature of radical scavenger present in the sample or standard compounds.

Method[11]:

The antioxidant activity of test drug sample KKA was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay.

The effective concentration of test sample KKA 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[12]:

The concentrations of test sample KKA 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 KKA 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 micro plate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug KKA and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug KKA and gallic acid were calculated using the following formula:

Radical Scavenging activity of Nitrous oxide = [A (Control) – A (Test)]/ A (Control) ×100

Note: A (control) – Absorbance of control sample

A (test) – Absorbance in the presence of the samples extracts or standards

ABTS Assay[13]:

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug KKA 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 per sulfate. 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 at the concentration of 10-100µg/ml in chloroform 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 KKA was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample KKA was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

Radical Scavenging = [A (Control) – A (Sample)]/ A (Control) ×100

Hydrogen Peroxide Radical Scavenging Assay[14]:

A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample KKA (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 KKA and standard was calculated and recorded. The percentage radical scavenging activity of the test drug KKA and BHA were calculated using the following formula:

Radical Scavenging = [A (Control) – A (Sample)]/ A (Control) ×100

Results:

Percentage inhibition of test drug KKA on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of KKA	% Inhibition of Ascorbic Acid
10 µg/ml	5.029 ± 1.742	12.7 ± 0.2785
20 µg/ml	11.68 ± 6.923	17.27 ± 1.48

40 µg/ml	16.95 ± 9.431	41.55 ± 2.196
60 µg/ml	21.28 ± 10.68	51.64 ± 4.479
80 µg/ml	29.66 ± 8.55	64.59 ± 4.502
100 µg/ml	35.79 ± 8.032	82.07 ± 0.782

Data are given as Mean ± SD (n=3)

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

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
KKA	170.3 ± 16.92
ASCORBIC ACID	58.26 ± 1.671

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug KKA on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	**% Inhibition of KKA**	**% Inhibition of Gallic Acid**
10 µg/ml	3.685 ± 3.876	17.6 ± 2.539
20 µg/ml	8.966 ± 4.567	32.84 ± 4.746
40 µg/ml	14.26 ± 3.991	44.68 ± 2.002
60 µg/ml	17.02 ± 4.478	52.43 ± 1.982
80 µg/ml	19.73 ± 5.749	77.5 ± 1.758
100 µg/ml	23.47 ± 5.825	88.71 ± 2.102

Data are given as Mean ± SD (n=3)

IC50 Values for Nitric Oxide radical scavenging assay

by KKA and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
KKA	232.9 ± 53.66
GALLIC ACID	48.6 ± 3.205

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug KKA on ABTS radical scavenging assay

Concentration (µg/ml)	**% Inhibition of KKA**	**% Inhibition of Gallic Acid**
10 µg/ml	2.736 ± 1.685	15.85 ± 5.077
20 µg/ml	5.786 ± 2.485	38.43 ± 2.743
40 µg/ml	13.13 ± 3.28	49.75 ± 3.94
60 µg/ml	18.51 ± 2.748	67.49 ± 2.198
80 µg/ml	21.89 ± 3.335	77.07 ± 9.804

100 µg/ml	23.01 ± 2.816	87.73 ± 0.9786

Data are given as Mean ± SD (n=3)

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

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
KKA	204 ± 18.26
GALLIC ACID	43.51 ± 4.975

Data are given as Mean ± SD (n=3)

Percentage inhibition of test drug KKA on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of KKA	% Inhibition of BHA
10 µg/ml	2.553 ± 3.288	17.43 ± 5.152
20 µg/ml	10.48 ± 2.022	28.62 ± 4.794
40 µg/ml	13.52 ± 3.328	43.51 ± 4.318
60 µg/ml	16.21 ± 3.704	46.87 ± 4.211
80 µg/ml	20.59 ± 4.612	64.77 ± 3.638
100 µg/ml	24.59 ± 4.708	85.02 ± 0.2877

Data are given as Mean ± SD (n=3)

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

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
KKA	221.1 ± 47.02
BHA	54.77 ± 5.109

Data are given as Mean ± SD (n=3)

Discussion

The trial drug ***Kameshwara karpa avizhtham*** was selected from the text ***Theraiyar yemaha venba*** for screening the antioxidant activity of the drug (Invitro Assay).

The drug was prepared as per the procedure and subjected to various studies to reveal potency and effectiveness against the disease. Literary review about the ingredients of *Kameshwara Karpa Avizhtham* from various text book

gave hope about its activity. Literary review, which consists Botanical Aspect, Gunapadam Aspect and Pharmacological review which support this study.

DPPH radical scavenging activity

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 5.029 ± 1.742 to 35.79 ± 8.032 % when compared with standard ascorbic acid with percentage inhibition ranges from 12.7 ± 0.2785 to 82.07 ± 0.782 93 %. The IC50 value of the trial drug was found to be 170.3 ± 16.9 (µg /ml) when compared with standard ascorbic acid with (IC50 value 58.2 ± 1.67 µg/ml).

NO radical scavenging activity

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 3.685 ± 3.876 to 23.47 ± 5.825 % when compared with standard gallic acid with percentage inhibition ranges from 17.6 ± 2.539 to 88.71 ± 2.102 %. The corresponding IC50 value of the trial drug was found to be 232.9 ± 53.66 (µg /ml) when compared with standard gallic acid with (IC50 value 48.6 ± 3.205 µg/ml).

ABTS radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 2.736 ± 1.685 to 23.01 ± 2.816 % when compared with standard gallic acid with percentage inhibition ranges from 15.85 ± 5.077 to 87.73 ± 0.9786 % .The corresponding IC50 value of the trial drug was found to be 204 ± 18.26 (µg /ml) when compared with standard Gallic acid with (IC50 value 43.51 ± 4.975µg/ml).

Hydrogen peroxide radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 2.553 ± 3.288to 24.59 ± 4.708% when compared with standard BHA with percentage inhibition ranges from 17.43 ± 5.152to 85.02 ± 0.2877%.The corresponding IC50 value of the trial drug was found to be 221.1 ± 47.02 (µg /ml) when compared with standard BHA with (IC50 value 54.77 ± 5.109µg/ml).

Antioxidant activity of trial drug *Kameshwara karpa avizhtham* was found to be 6% to 45%, 6% to 28%, 3% to 25%, 2.5% to 29% for DPPH assay, Nitrous oxide assay and ABTS assay, H2O2 Assay respectively When compared to Standard drug Ascorbic acid and gallic acid they had more Antioxidant activity i.e., 12% to 82%, 19% to 90% and 20% to 87%, 22% to 85% respectively. Thus, trial drug had low antioxidant activity when compared to standard synthetic compound but it sure the trial drug also had antioxidant activity.

IC50 value for the trial drug *Kameshwara karpa avizhtham* for DPPH assay, Nitrous oxide , ABTS assay and H2O2 Assay is 170.3 ± 16.9 (µg /ml), 232.9 ± 53.66 (µg /ml), 204 ± 18.26 (µg /ml), 221.1 ± 47.02 respectively. IC50 value for the standard compound Ascorbic acid, gallic acid for DPPH assay, Nitrous oxide and ABTS assay is 58.2 ± 1.67 µg/ml, 48.6 ± 3.205 µg/ml, 43.51 ± 4.975µg/ml, 54.77 ± 5.109µg/ml respectively. Lowest the IC50 value had highest antioxidant activity[15].

Thus, the drug had low antioxidant activity when compared to standard drug. Standard drug is single compound while trial drug is mixture of raw drugs. And dosage of the trial drug is approximately 5 – 10gm but here 10mg – 100mg of trial drug is tested but these amounts also had enough amount of antioxidant activity.

Conclusion

Based on the results obtained from the In-vitro anti-oxidant assay for the sample *Kameshwara Karpa avizhtham* it was concluded that the Siddha formulation *Kameshwara Karpa avizhtham* has promising anti-oxidant activity in the estimated DPPH, Nitrous oxide, ABTS assays and H2O2 Assay. Antioxidant substance prevent ageing and cell death which is similar to *Karpa marunthu* action as mentioned classical Siddha text. From the above results Siddha text are correct based on Scientific parameters. In future Pre-clinical and Clinical studies are done to evaluate their effectiveness and make strong evidence of Siddhars science.

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