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Research Article

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Evaluation of anti-oxidant activity of *Kukkilathi choornam* (A Siddha herbo mineral preparation)

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Abstract

Siddha system is a renowned holistic system of traditional medicine that emphasis on healthy long life by preventing ageing and degenerative diseases with medicines having anti-oxidant property. Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract and cancer. Anti-oxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them or by promoting their decomposition. *Kukkilathi Choornam* is a Siddha herbo mineral formulation which is indicated for *Alkulputru* (cervical cancer), *Moolam* (haemorrhoids) , *Vipuruthi* (cancer) , *Kranthi* (glandular swelling) and *Pouthiram* (fistula). Even though this drug has been used by traditional siddha physicians,there is no scientific validation has been carried out to evaluate its anti-oxidant property. The aim of this study is to evaluate the anti-oxidant property of *Kukkilathi Choornam* through DPPH, Nitric Oxide, Hydrogen Peroxide and ABTS radical scavenging assays. The study results reveal that the drug *Kukkilathi Choornam* shows the percentage of inhibition on DPPH 59% (Ascorbic acid 93%) , Nitric oxide 43% (Gallic acid 93%) , Hydrogen peroxide 43% (BHA 92%) and ABTS 71% (Gallic Acid 93%) respectively. This result concludes that the drug *Kukkilathi Choornam* has a potent anti-oxidant activity and it can be used for the management of Cervical Cancer.

Keywords: : Kukkilathi Choornam, Free radicals, Herbo mineral formulation, Anti-oxidant activity, Siddha medicine

Introduction

Siddha system of medicine is an ancient system of medicine. It was enriched by the super special souls called siddhars. Herbs, Metals, Minerals and Animal products has been used for medicine preparations and treating the illness. Anti-oxidant compounds are exogenous or endogenous in nature which either prevents the generation of toxic oxidants, intercept any that are generated and inactivate them and thereby block the chain propagation reaction produced by these oxidants. These anti-oxidants neutralise the activity of free radicals. Hence, they are also known as “Free Radical Scavengers”.

Free radicals are highly reactive that they have the potential to harm cells. They are formed naturally in the body and play an important role in many normal cellular processes. At high concentrations, however free radicals can be hazardous to the body and damage all major components of cell, including DNA , may play a role in the development of cancer and other health conditions.

Cancer is a global problem and serious in nature. It is the second leading cause of death next to Cardiac diseases throughout the world. The ancient text of Siddha Medicine, reported that the Siddhars had given details about “*Puttru Noi*” and its treatments. Scientific documentation of traditional system of medicine is increasing and need for preparing it fir siddha formulation has become the need of the hour.

Materials and Methods

The herbo mineral siddha formulation *Kukkilathi Choornam* (KC) is studied for its Anti-oxidant activity. It is a combination of one mineral and six herbal drugs.

Ingredients:

Tamil name	Botanical name
Purified Kukkil	*Shorea robusta*
Purified Parangi sakai	*Smilax china*
Purified Gandhagam	Sulphur
Kandanthippili	Root of *Piper longum*
Arisithippili	*Piper longum*
Vetpalai arisi	*Wrightia tinctoria*
Vaaividangam	*Embelia ribes*

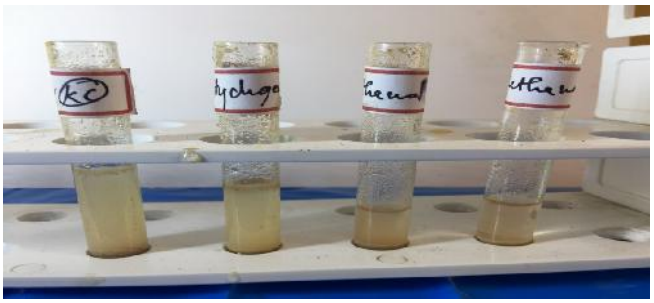
Method of preparation:

The reference of this drug was found in the text ANUBOGA VAIDHYA NAVANEETHAM by Hakkeem Abdullah Sahib. As per the text, the raw drugs were selected and purified. Finally the dried product was powdered and filtered with cloth (Vasthirakaayam). Then the powder is weighed and stored in an air tight container.

Sample description:-

State	Solid
Appearance	Pale Milky Brown
Nature	Dry Fine powder
Odor	Mild Pungent

Solubility assay:-



S.No	Solvent Used	Solubility
1.	Water	Soluble
2.	Methanol	Soluble
3.	Ethanol	Soluble
4.	Hydrogen Peroxide	Soluble

Anti-oxidant activity:

Anti-oxidant activity of the drug Kukkilathi Choornam drug (KC) was evaluated through *in-vitro* anti-oxidant assays namely.

- DPPH Assay
- Nitric oxide radical scavenging Assay
- ABTS Assay
- Hydrogen peroxide radical scavenging Assay

DPPH Assay:-

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions.

Nitric oxide radical scavenging Assay:-

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases.

ABTS Assay:-

ABTS Antioxidant *Assay* can be used to determine the total antioxidant capacity of biological fluids, cells, and tissue. It can also be used to *assay* the *antioxidant activity* of naturally occurring or synthetic compounds for use as dietary supplements, topical protection and therapeutics.

Hydrogen peroxide radical scavenging Assay:-

Hydrogen peroxide (H_2O_2) is a biologically important, non-radical reactive oxygen species (ROS) that can influence several cellular processes. Ability of anti-oxidants to scavenge H_2O_2 can be measured by several methods.

Inferences and objectives:-

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

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

% scavenging =

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

The effective concentration of test sample KC 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 KC 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 KC 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 KC and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug KC 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 KC 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 KC was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample KC 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 KC (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 vortexed 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 KC and standard was calculated and recorded. The percentage radical scavenging activity of the test drug KC 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 and Discussion

I. DPPH radical scavenging activity

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 9.97 to 59.21 % when compared with standard ascorbic acid with percentage inhibition ranges from 46.13 to 93.72 %. The IC50 value of the trial drug was found to be 82.98 (µg /ml) when compared with standard ascorbic acid with (IC_{50} value 14.98 µg/ml)

Percentage inhibition of test drug KC on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of KC	% Inhibition of Ascorbic Acid
10 µg/ml	9.97 ± 3.47	46.13 ± 2.81
20 µg/ml	20.58 ± 5.83	56.06 ± 5.85
40 µg/ml	28.53 ± 5.60	62.09 ± 6.64
60 µg/ml	36.48 ± 2.36	69.18 ± 8.0
80 µg/ml	48.23 ± 2.27	76.27 ± 9.53
100 µg/ml	59.21 ± 0.65	93.72 ± 0.22

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

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	14.98 ± 9.956
KC	82.98 ± 2.316

II. NO radical scavenging activity

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 5.18 to 43.7 % when compared with standard gallic acid with percentage inhibition ranges from 31.23 to 93.61 % .The corresponding IC50 value of the trial drug was found to be 118.2 (µg /ml) when compared with standard gallic acid with (IC_{50} value 32.12 µg/ml)

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

Concentration (µg/ml)	% Inhibition of KC	% Inhibition of Gallic Acid
10 µg/ml	5.185 ± 1.69	31.23 ± 1.06
20 µg/ml	11.85 ± 0.64	47.55 ± 2.67
40 µg/ml	20 ± 2.94	57.12 ± 2.77
60 µg/ml	31.11 ± 7.77	68.82 ± 2.15
80 µg/ml	35.93 ± 10.02	72.72 ± 4.63
100 µg/ml	43.7 ± 8.48	93.61 ± 0.22

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

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
KC	118.2 ± 33.87
GALLIC ACID	32.12 ± 1.806

III. ABTS radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 13.35 to 71.93 % when compared with standard gallic acid with percentage inhibition ranges from 49.2 to 93.41 % .The corresponding IC50 value of the trial drug was found to be 63.74 (µg /ml) when compared with standard gallic acid with (IC_{50} value 6.04 µg/ml)

Percentage inhibition of test drug KC on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of KC	% Inhibition of Gallic Acid
10 µg/ml	13.35 ± 3.34	49.2 ± 3.40
20 µg/ml	27.94 ± 7.89	58.03 ± 2.20
40 µg/ml	39.61 ± 5.50	67.56 ± 4.77
60 µg/ml	49.46 ± 8.35	74.63 ± 5.33
80 µg/ml	58.58 ± 4.76	81.34 ± 4.01
100 µg/ml	71.93 ± 15.5	93.41 ± 1.00

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

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
KC	63.74 ± 13.02
GALLIC ACID	6.042 ± 3.124

IV. Hydrogen peroxide radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 10.75 to 43.33 % when compared with standard BHA with percentage inhibition ranges from 47.08 to 92.63 % .The corresponding IC50 value of the trial drug was found to be 119.1 (µg /ml) when compared with standard BHA with (IC_{50} value 5.38 µg/ml)

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

Concentration (µg/ml)	% Inhibition of KC	% Inhibition of BHA
10 µg/ml	10.75 ± 2.23	47.08 ± 2.20
20 µg/ml	18.27 ± 2.70	58.74 ± 4.01
40 µg/ml	24.71 ± 3.45	70.39 ± 2.25
60 µg/ml	31.16 ± 3.33	77.45 ± 5.65
80 µg/ml	36.53 ± 5.46	84.37 ± 7.22
100 µg/ml	43.33 ± 5.40	92.63 ± 1.54

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

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
KC	119.1 ± 17.95
BHA	5.386 ± 3.213

Conclusion

The Siddha formulation Kukkilathi Choornam exhibits potent antioxidant property in various methods when compare to standards. It is a potent antioxidant candidate which can be used for the treatment of various diseases, which involve oxidative stress in its pathogenesis. This result is for the world at large from Siddha system of medicine.

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