Research Article

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# EVALUATION OF IN VITRO ANTI-OXIDANT ACTIVITY OF SIDDHA DRUG EACHURAMOOLI CHOORANAM

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## ABSTRACT

The Siddha system of medicine profounded by siddhars is an ancient documented medical system in the world. This study,aims to investigate the Anti-oxidant activity of siddha drug Eachuramooli chooranam( EC) made from the plant Eachuramooli (Aristalochia indica Linn) using DPPH, NO, Hydrogen peroxide and ABTS radical scavenging assay.The results of the study indicates that the DPPH radical scavenging activity of the formulation EC the percentage inhibition ranges from 12.8 to 61.7 %. NO radical scavenging activity the percentage inhibition of the test drug ranges from 9.11 to 44.5 %. Similarly in ABTS assay the percentage inhibition ranges from 12.05 to 68.9 % for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 6.59 to 52.7 %. In conclusion the siddha drug Eachuramooli chooranam (EC) possesses promising antioxidant activity against all four tested assay and hence it may be effective in the clinical management of the several oxidative stress related disorders.

**KEY WORDS:** ***Eachuramooli chooranam, Anti-oxidant activity, Siddha, DPPH, NO, Hydrogen peroxide, ABTS, Radical scavenging assay***

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## 1. Introduction

The Siddha system of medicine profounded by siddhars is an ancient documented medical system in the world [1].It is based on 96 concepts of philosophy (96 thathuvam)[2,3].There are several plants, as raw minerals, metals, arsenics, salts & animal origin used for medicinal preparations, the value of which the Siddha medicine proclaims should be scientifically studied & recorded for future use[4]. Antioxidants are widely used in the food industry as potential inhibitors of lipid peroxidation [5]. Many synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may accumulate in the body, resulting in liver damage and carcinogenesis [6]. Research activities focusing on medicinal plants have been encouraging because of their high content of potent antioxidants, accessibility,economic viability and next-to-no side effects [7].This study, aims to investigate the Anti-oxidant activity of siddha drug Eachuramooli chooranam made from the plant Eachuramooli (Aristalochia indica Linn).

Aristalochia indica Linn belonging to the family Aristalochiaceae is a perennial shrubby glabrous twiner with a long woody root stock; leaves simple, alternate, short petioled, entire with somewhat undulate margins; flowers greenish white or light purplish in axillary cymes or fascicles with swollen or inflated basal part, contracted middle part and narrow funnel shaped distal part; fruits rounded or oblong or hexagonal, septicidal 6 – valved capsules opening from below upwards; seeds flat, winged found growing throughout India at low elevations , on hedges and bushes[8].

## 2.Materials and Methods

### 2.1. Botanical Authentication

The plant material Aristalochia indica Linn (Eachuramooli) was identified and authenticated by the Director, Plant Anatomical Research Centre, Chennai.

### 2.2. Method of Preparation

The plant material was collected and authenticated by the botanist. Then it was washed well in water, shade dried, finely powdered and made into Eachuramooli chooranam (EC) for the study purpose.

### 2.3. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay [9]

The antioxidant activity of test drug sample EC was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample EC 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 EC 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 370C. 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 EC required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations

### 2.4. Nitric Oxide Radical Scavenging Assay [10]

The concentrations of test sample EC 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 EC 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 drugEC and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug EC and gallic acid were calculated using the following formula:

Percentage nitrite radical scavenging activity:

nitrite scavenging% = [AControl - ATest / AControl] X 100

Where AControl = absorbance of control sample and ATest = absorbance in the presence of the samples extracts of standards.

### 2.5. ABTS Assay [11]

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

Radical scavenging% = [AControl - ATest / AControl] X 100

### 2.6. Hydrogen Peroxide Radical Scavenging Assay [12]

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

Radical scavenging% = [AControl - ATest / AControl] X 100

## 3.Results

### 3.1. Effect of EC on DPPH radical scavenging activity

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 12.8 to 61.7 % when compared with standard ascorbic acid with percentage inhibition ranges from 40.5to 95.5 %. The IC50 value of the trial drug was found to be 78.6 (µg /ml) when compared with standard ascorbic acid with (IC50 value 11.5µg/ml)

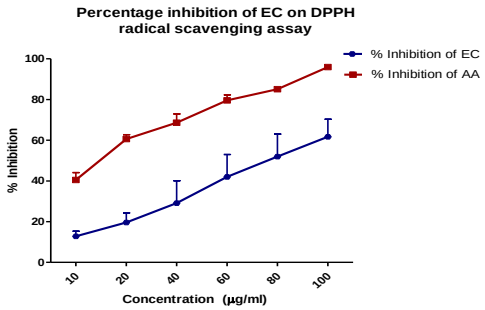


**Figure 1: Effect of EC on DPPH radical scavenging activity**

### 3.2. NO radical scavenging activity

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 9.11 to 44.5 % when compared with standard gallic acid with percentage inhibition ranges from 44.6 to 92.8 % .The corresponding IC50 value of the trial drug was found to be 112.3 (µg /ml) when compared with standard gallic acid with (IC50 value 19.5µg/ml)

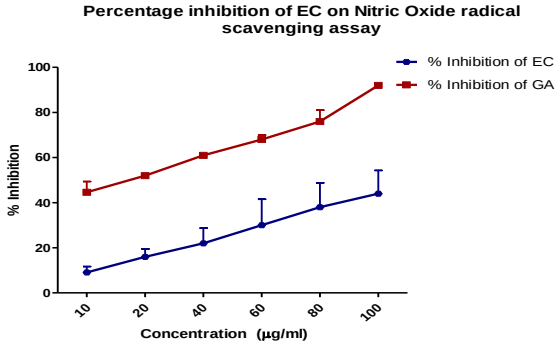


**Figure 2: Effect of EC on NO radical scavenging activity**

### 3.3. ABTS radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 12.05 to 68.9 % when compared with standard gallic acid with percentage inhibition ranges from 27.3 to 94.01 % .The corresponding IC50 value of the trial drug was found to be 66.26 (µg /ml) when compared with standard gallic acid with (IC50value 29.55µg/ml).

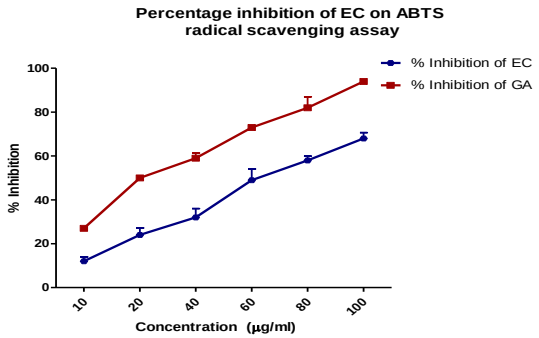


**Figure 2: Effect of EC on ABTS radical scavenging activity**

### 3.4. Hydrogen peroxide radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 6.59 to 52.7 % when compared with standard BHA with percentage inhibition ranges from 29.5 to 85.6 % .The corresponding IC50 value of the trial drug was found to be 98.28 (µg /ml) when compared with standard BHA with (IC50 value 38.03µg/ml)

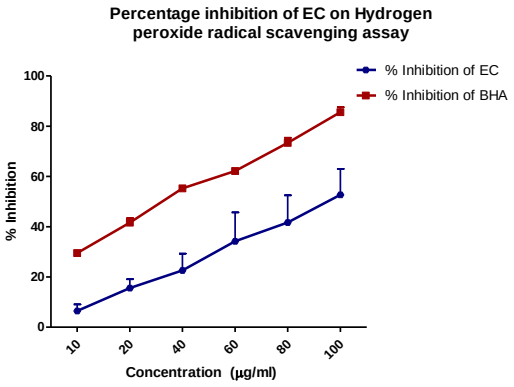


**Figure 2: Effect of EC on H2O2 radical scavenging activity**

## 4.Discussion

Free radicals derived from oxygen, nitrogen and sulphur molecules in the biological system are highly active to react with other molecules due to their unpaired electrons. These radicals are important part of groups of molecules called reactive oxygen/ nitrogen species (ROS/RNS), which are produced during cellular metabolism and functional activities and have important roles in cell signaling, apoptosis, gene expression and ion transportation. However, excessive ROS attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, and cause oxidative stress, which can damage DNA, RNA, proteins and lipids resulting in an increased risk for cardiovascular disease, cancer, autism and other diseases. Intracellular antioxidant enzymes and intake of dietary antioxidants may help to maintain an adequate antioxidant status in the body. In the past decades, new molecular techniques, cell cultures and animal models have been established to study the effects and mechanisms of antioxidants on ROS. The chemical and molecular approaches have been used to study the mechanism and kinetics of antioxidants and to identify new potent antioxidants.

Antioxidants can decrease the oxidative damage directly via reacting with free radicals or indirectly by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes [13]. The results of the study indicates that the DPPH radical scavenging activity of the formulation EC the percentage inhibition ranges from 12.8 to 61.7 %. when compared with standard ascorbic acid with percentage inhibition ranges from 40.5to 95.5 %. NO radical scavenging activity the percentage inhibition of the test drug ranges from 9.11 to 44.5 % when compared with standard gallic acid with percentage inhibition ranges from 44.6 to 92.8 %.Similarly in ABTS assay the percentage inhibition ranges from 12.05 to 68.9 % when compared with standard gallic acid with percentage inhibition ranges from 27.3 to 94.01 % for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 6.59 to 52.7 % when compared with standard BHA with percentage inhibition ranges from 29.5 to 85.6 %. Intracellular antioxidant enzymes and intake of dietary antioxidants may help to maintain an adequate antioxidant status in the body.

## 5.Conclusion

Based on the results obtained from the In-vitro anti-oxidant assay for the sample EC it was concluded that the siddha drug EC has promising anti-oxidant activity in the estimated assays.

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## Conflict of interest – Nil -

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