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# IN VITRO FREE RADICAL SCAVENGING ASSAY (DPPH (2, 2-DIPHENYL 1-2 PICRYLHYDRAZYL METHOD) OF SIDDHA HIV HERBAL FORMULATION DEVA CHOORNAM

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## ABSTRACT

**Background:** *Siddha* Herbal powder formulations like *Choornams*, are now well accepted as a supportive therapy for most of the life style diseases and other prevalent ailments in our society owing to its considerable antioxidant property for quenching harmful free radicals formed inside the body. *Deva Choornam*, a poly herbal formulation has been successfully implemented for improving the Quality of life in Immuno compromised conditions especially AIDS. **Objectives:** The study aims to analyze the free radical scavenging activity of *Deva choornam* (DC) against DPPH (2,2-diphenyl 1-2 picrylhydrazyl) Free radical. **Methods:** The *Choornam* were prepared as with reference to *Siddha* texts. Reaction mixture containing 1 ml of 0.3 m M DPPH methanol solution was added to 2.5 ml of sample solution of *Deva choornam* and standard solution of Ascorbic acid of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample DC 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 $37^0$C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank. **Results:**

The effective concentration of test sample DC required to scavenge DPPH radical by 50% ($IC_{50}$ value) was obtained as 73.24 ± 18.47 in compare with Ascorbic acid (13.88 ± 0.93). **Conclusion:** *Deva choornam* exhibited moderate Anti oxidant property as compared with the standard and thereby validating a supportive role in its successive clinical applications in HIV for improving the quality of life.

**KEYWORDS:** *Siddha medicine*, HIV, *Deva Choornam*, DPPH free Radical scavenging Activity, Anti Oxidant.

## I. INTRODUCTION

Oxidative stress is a key factor in the dysregulation of immune system and the body continuously maintains a perfect equilibrium between free radical generation and its innate scavenging components known as Anti oxidants. The deficiency of micronutrients and the innate antioxidant principles brings on the suppression of Immune function of the individual reflected as the defective antibody responses and the innate Tcell mediated responses resulting in Immuno compromised status and further opportunistic infections. Therefore it is the crucial step to correct the effects of malnutrition in Immuno compromised diseased states and also to provide adequate antioxidants supplementation.[1,2]

Herbs are richest source of Anti oxidant phytocomponents along with essential nutrients. ***Deva Choornam*** (DC*)*, a herbal combination is the perfect blend of three vital antioxidant herbs like Alpinia galangal, Cedrus devadaru and Cinnamomum tamala. The up-to-date scientific studies claimes the Anti oxidant activities of all the three herbs.[3] Previous studies on the aqueous extract of DC revealed the presence of Phytochemicals like Alkaloids, Flavanoids, steroids, Terpenoids, Coumarins, phenols, saponins and tannins. Most of these compounds like phenols and Flavanoids have proven Anti oxidant property.[4]

The combination is found to be effective in Immuno compromised conditions especially for the victims of HIV or its associated conditions. On regular basis the formulation were found to improve the overall quality of life, health and well being of the sufferers. With the success in clinical practice along with the supportive scientific validations of its Antioxidant activities it is the further initiation to screen the Free radical scavenging property of this effective formulation.

## II. AIM AND OBJECTIVES

The present study aims to screen the antioxidant potential of *Siddha* formulation Deva *Choornam* (DC)[3] by using DPPH (2, 2-diphenyl 1-2 picrylhydrazyl assay).

## III. MATERIALS AND METHODS

### A. Ingredient Details[5]

There are 3 principle herbal ingredients in this formulation, *Devadaru* (Cedrus devadaru), *Chittarathai* (Alpinia galangal), and *Ilavanga Pathiri* (Cinnamomum tamala) (Fig. A, B, C).

**Fig A. *Cedrus devadaru***

**Fig B. *Alpinia galangal***

**Fig C. *Cinnamomum tamala***

### B. Method of Preparation of DC Sample (Fig. D)[6]

All the ingredients were purchased from reputed herbal suppliers, purified well with reference to *Siddha* texts, powdered nicely and preserved for studies.

### C. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay[7]

The antioxidant activity of test drug sample DC was determined using the 2, 2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample DC 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 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 DC 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 $37^0$C. 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 DC 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.

## IV. RESULTS AND DISCUSSION

DPPH Free radical assay of *Deva Choornam* has reported the following results.

- ☆ The Percentage inhibition of DC and standard at 6 different concentrations were monitored. For DC at final 100 µg/ml concentration %inhibition was 59.67% ± 5.94 as when compared with the standard Ascorbic acid (96.1% ± 1.68) (Table. 1, Fig graph E).
- ☆ The IC50 Values of DC was 73.24 µg /ml ± 18.47 as compared with standard ascorbic acid (13.88 ± 0.93) (Table. 2).

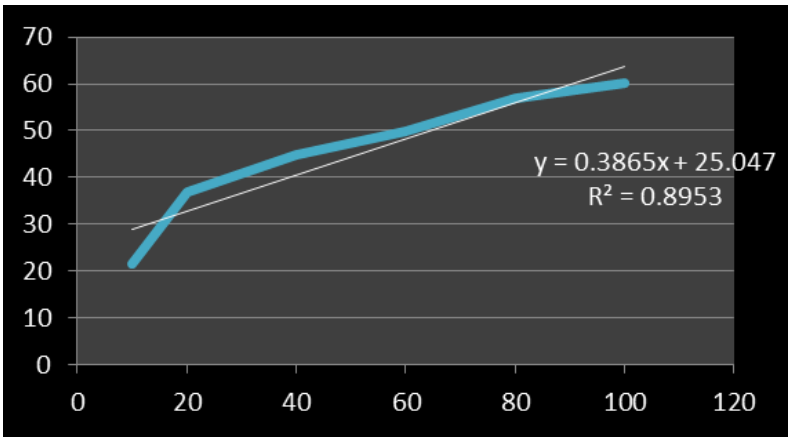
The IC 50 Value was calculated to find out the effective concentration to scavenge or decline half of the concentration of DPPH free radical. More the Value lesser will be the antioxidant potential. According to many research works IC50 Value of extracts ranging from 50-100 µg /ml is grouped as intermediate Anti oxidants (Having Average radical scavenging property) and from 10-50 µg /ml as strong radical scavengers. As per the present study here the Herbal drug *Deva Choornam* can be considered as an Antioxidant with intermediate potency.[8] The presence of Anti oxidant principles like phenols and Flavanoids in DC Formulation may have a significant role in this property.

**Table 1: Percentage inhibition of test drug DC on DPPH radical scavenging assay.**

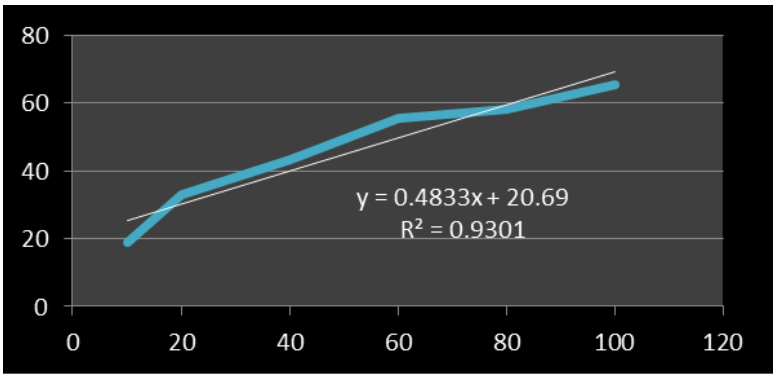
| Concentration (µg/ml) | % Inhibition of DC | % Inhibition of Ascorbic Acid |
|---|---|---|
| 10 µg/ml | 19.09 ± 2.31 | 43.1 ± 4.10 |
| 20 µg/ml | 30.58 ± 7.66 | 53.26 ± 2.60 |
| 40 µg/ml | 39.03 ± 8.62 | 71.52 ± 3.79 |
| 60 µg/ml | 46.81 ± 10.38 | 76.93 ± 3.14 |
| 80 µg/ml | 52.9 ± 8.28 | 84.37 ± 2.30 |
| 100 µg/ml | 59.67 ± 5.94 | 96.1 ± 1.68 |

Data are given as Mean ± SD (n=3).

**Triplicate 1**




**Triplicate 2**

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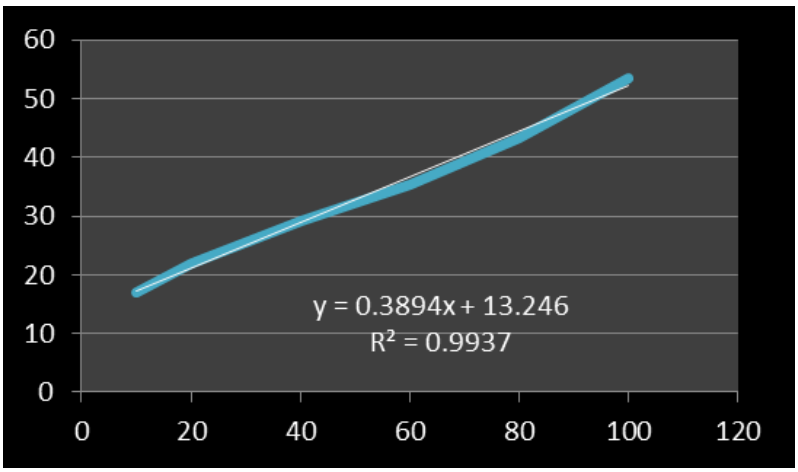


**Fig. E. Percentage inhibition of DC on DPPH radical scavenging assay.**

**Table 2: IC50 Values for DPPH radical scavenging Assay by DC and standard.**

| Test Drug / Standard | IC50 Value DPPH Assay ± SD (µg /ml) |
|---|---|
| ASCORBIC ACID | 13.88 ± 0.93 |
| DC | 73.24 ± 18.47 |

Data are given as Mean ± SD (n=3).

## V. CONCLUSION

*Deva choornam* exhibited moderate Anti oxidant property as compared with the standard and thereby validating a supporting role in its successive clinical applications in HIV for improving the quality of life.

## VI. REFERENCE

1. Iniaghe OM, Malomo SO, Adebayo JO. Hepatoprotective effect of the aqueous extract of leaves of Acalypha racemosa in carbon tetrachloride treated rats. J Med Plants Res., 2008; 2: 301-305.
2. Djacbou D. Sylvie, Pieme constant Anatole, Biaspa Prosper Cabrel, Penlap Beng Veronique. Comparison of In Vitro Antioxidant properties of extracts from three plants used for medical purpose in Cameroon: Acalypha racemosa, Garcinia lucida and Hymenocardia lyrata. Asian Pac J Trop Biomed., 2014; 4(2): S625-S632.
3. Thangadurai K.1*, Suresh K.2, Niranjana N.3, Thirunavukkarasu Dharmalingam4, Banumathi V. A Review on Siddha Herbal Formulation Deva Choornam for improving the QOL in Acquired Immuno Deficiency Syndrome (AIDS). World Journal of Pharmaceutical Research, 2017; 6(5): 319-332.
4. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Complement Altern Med., 2008; 8: 53.
5. K. S. Murugesa mudhaliyar, Gunapadam-mooligai vaguppu, 2nd edition, 2008, Directorate of Indian Medicine and Homeopathy, 547.
6. Siddha Formulary of India, Part 2. Tamil Version, 1st Edition, 2001, Ministry of Health & Family Welfare. Page no. 173-182.
7. Badami, Omprakash, Dongr SH, Suresh B. *In-vitro* Antioxidant property of Solanum *Pseudocapsicum* leaf extract. *Indian J Pharmacol*, 2005; 37: 251-252.
8. S. Phongpaichit, J. Nikom, N. Rungjindamai, J. Sakayaroj, N. Hutadilok-Towatana, V. Rukachaisirikul, K. Kirtikara, FEMS Immunol. Med. Microbiol, 2007; 51(3): 517-525.