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Research Article

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Physico chemical analysis and anti oxidant activity of ethanolic extract and HCl extract of *Tetran vithai kudineer chooranam* (A siddha poly herbal formulation)

Dr. R. Keerthana*, Dr.S. Visweswaran MD(S)**

Dr. N.J. Muthukumar MD(S) *, Dr.V. Banumathi MD(S)******

* National Institute of Siddha, Tambaram Sanatoriuom, Chennai-47

** Lecturer, National Institute of Siddha, Tambaram Sanatoriuom, Chennai -47

***Associate Professor, National Institute of Siddha, Tambaram Sanatoriuom, Chennai-47

**** Director, National Institute of Siddha, Tambaram Sanatoriuom, Chennai-47

*Corresponding Author: sugeebsms@gmail.com

Abstract

Siddha Medicine (Tamil *Citta-* or *Tamil-maruttuvam*) is a system of traditional medicine originating in Tamil Nadu in South India .The drugs used by the Siddhars could be classified into three groups: *thavaram* , *thathu* and *jangamam* ***Tetranvithai kudineer chooranam*** is a drug under the category of thaavaram which is mentioned in the text Gunapadam mooligai vaguppu. It is traditionally used for the management of Madhumagam (diabetes mellitus). But scientifically it is not characterized so for. Therefore in the present study, the physic- chemical characters like pH value, Total ash value, Acid soluble ash content and water soluble ash content of Tetranvithai kudineer chooranam were determined. Also the Ethanolic extract and HCl extract of the drug were tested for its anti oxidant activity against nitric oxide scavenging ability.

Keywords: **Tetranvithai kudineer chooranam,** anti oxidant activity, Nitric oxide, Ethanolic extract and HCl extract.

Introduction

World Health Organisation (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and evolved guidance to support the member states in their efforts to formulate national polices on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.

Since very old times, herbal medications have been used for relief of symptoms of disease . Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care.

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects .

Strychnos potatoram (Tetran) seeds are used in the Indian traditional system of medicine for the treatment of hepatopathy, nephropathy, gonorrhea, leucorrhoea, microbial infection chronic diarrhoea, diabetes and eyes disease. The rips seeds are used for clearing muddy water.

For the useful application of the plant parts in modern medicine, physico-chemical and phytochemical standardization is very important, so that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world.

The objective of this study was to prepare Tetranvithai kudineer chooranam(as per protocol given by PLIM) analyze the physicochemical characteristics and antioxidant activity . The Physico-chemical parameters like pH value, Total ash value, Acid soluble ash content and water soluble ash content also were estimated.

Materials and Methods

Collection of the raw material

The raw drugs Strychnospotatoram (tetran), Terminaliya chebula (kadukkai),Cassia auriculata (avarai), Limonia acidissima (vilambpicin) were procured from the local market and authenticated by Dr.D.Aravind Assistant professor (Botany), National Institute of Siddha, Tambaram, Chennai. The drugs were dried, powdered separately and mixed.

Preparation of the extract

The drug mixer (25 g) was boiled with 75 ml of methanol for 15 minutes and filtered through Whatman No.1 fiter paper. The filtrate was collected and the extract was stored at 4 ^{O}C.

Physico - chemical characterisation:

The physico-chemical analysis was done as per the protocol for testing of Ayurvedic, Siddha and Unani Medicines, by PLIM, Ghaziabad, under the Ministry of AYUSH, Ministry of Health and Family Welfare, New Delhi.

1. pH

0.5 gm of the prepared drug was dissolved in ethanol and the pH of the solution was found out using pH meter.

2. Loss on drying

Accurately 1 gm of sample was weighed and taken in the dish. The dish was covered with lid and dried in the drying chamber till two consecutive weights remain within ± 0.5 mg. After drying was completed, the sample was cooled in desiccators and weighed. From the difference of weights the ash content was calculated.

$$\text{Loss on drying (\%w/w)} = \frac{\text{Loss in weight (g)} \times 100}{\text{Mass of the sample (g)}}$$

3. Total ash

3 gm of sample was accurately weighed and incinerated in a silica dish at a temperature of 650º C until free from carbon. Then the residue was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

$$\text{Percentage of total ash (\% w/w)} = \frac{\text{Mass of ash (g)} \times 100}{\text{Mass of the sample (g)}}$$

4. Acid insoluble ash

The ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected and washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated

$$\text{Percentage of acid insoluble ash (\%w/w)} = \frac{\text{Mass of acid insoluble matter (g)} \times 100}{\text{Mass of the sample (g)}}$$

5. Water soluble extractive

5 gm of the coarsely powdered drug was macerated with 100 ml of water in a closed flask for 24 hours and allowed to stand for 18 hours. The content was filtered rapidly, evaporated and dried at 105^{o} C, in an oven, to constant weight. The percentage of water soluble extractive with reference to the air-dried drug was calculated.

$$\text{Percentage of water soluble extractive (\%w/w)} = \frac{\text{Mass of the residue (g)} \times 100 \times 100}{\text{Mass of the sample (g)} \times 25}$$

Evaluation of anti oxidant activity

The antioxidant activity of the above prepared drug was tested by Nitric oxide radical scavenging assay. The procedure is based on the method where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions. This undergo diazoziation with sulphanilic acid. The diazotized product was added with Napthylethylenediminedihydrochloride ((NEDD) gives red dye.

The antioxidant activity was measured in a reaction mixture containing 2 ml of sodium nitroprusside, 0.5 ml of phosphate buffer saline and varying volumes of Ethanolic extract of the drug (10,20,30,40,50 µg) . The reaction mixture was incubated at 25^{0}C for 15 mins. After incubation 0.5 ml of reaction mixture was added

with 1 ml of sulphanic acid and allowed to stand for complete diazotization. To this 1 ml of Napthylethylenediminedihydrochloride was added, the absorbance of which was read at 540 nm, and compared with standard ascorbic acid. IC_{50} value is the concentration of sample required to inhibit 50% of $NO^{\bullet}$ .The percentage of $NO^{\bullet}$ radical scavenging was calculated as given below:

% of scavenging [$NO^{\bullet}$] =

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

$$= \frac{\text{Optical density of control} - \text{optical density of test}}{\text{optical density of control}} \times 100$$

$$= \frac{A_0 - A_1}{A_0}$$

A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample of drug or ascorbic acid.

Results and Discussion

Physico - chemical characterisation:

The result obtained in the physic -chemical characterisation is listed below;

S No	Test parameter	Unit of measurement	Result	Method of testing
1	Ash content	% by wt	3.12	IS.1448 PART4- 1984
2	Acid insoluble ash	%by mass	1.10	FSSAI lab manual 4
3	Water soluble ash	%by mass	0.75	FSSAI lab manual 4
4	Loss on drying	%by mass	10.20	FSSAI lab manual 4

The nitric oxide scavenging assay of Thetranvithai kudineer solution was studied and the results obtained are tabulated below

Table: 1 Nitric oxide radical scavenging assay of extract

The concentration of drug **solution** used and its optical density are listed below

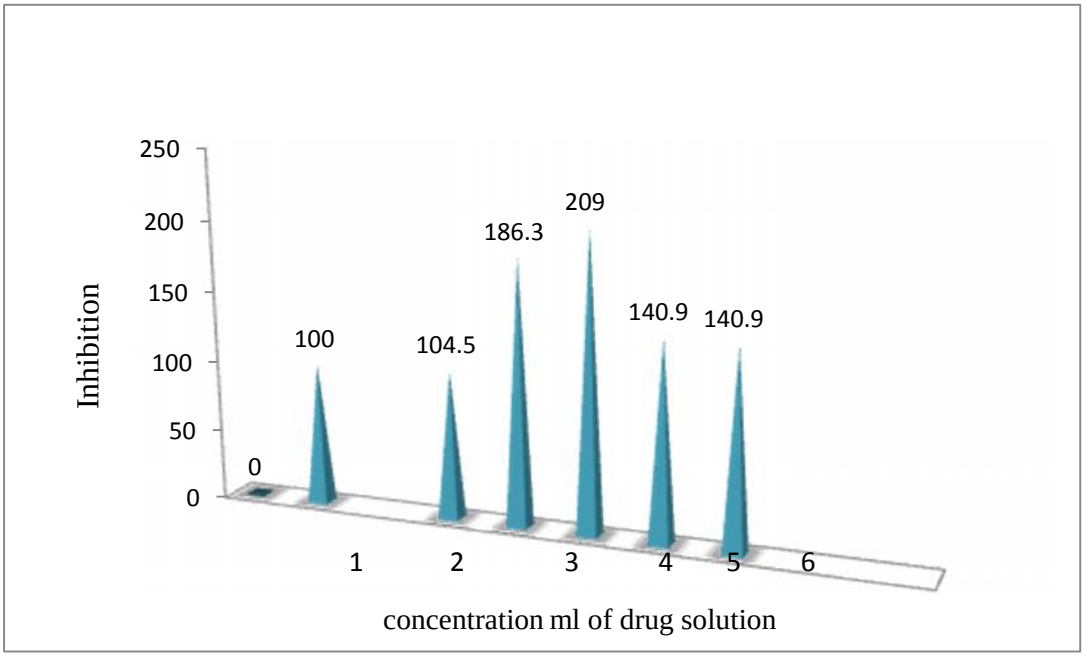
Calculation of percentage inhibition:

S.No	Concentration ml of drug solution (ml)	A_0	A_1	A_1-A_0	inhibition = $\frac{A1-A0}{A_0} \times 100$
	Blank	**0.22**			
1	1	0.22	0.45	23	104.5
2	2	0.22	0.63	41	186.3
3	3	0.22	0.68	46	209.0
4	4	0.22	0.53	31	140.9
5	5	0.22	0.53	31	140.9

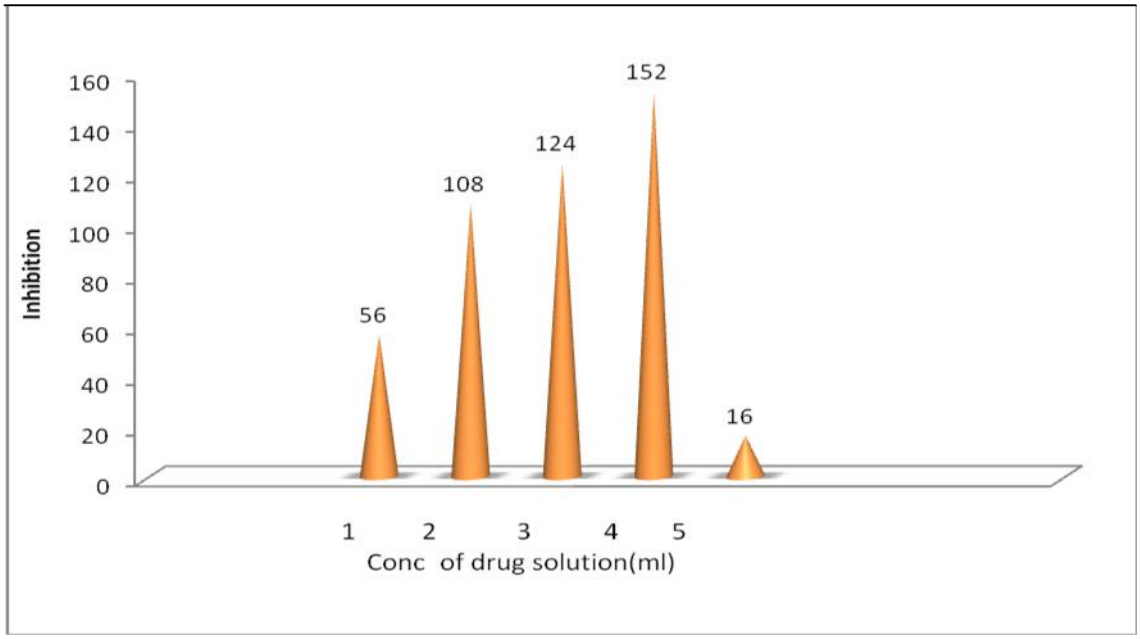
Table 2: The concentration of HCl extract of drug solution used and its optical density are listed below:
Calculation of percentage inhibition:

S.No	Concentration of drug solution (ml)	A_0	A_1	A_0-A_1	Inhibition $\frac{A1-A0}{A_0} \times 100$
	Blank	**0.25**			
1	1	0.25	0.39	14	56
2	2	0.25	0.52	27	108
3	3	0.25	0.56	31	124
4	4	0.25	0.63	38	152
5	5	0.25	0.65	4	16

Graph: 1 Nitric oxide scavenging assay of ethanolic extract thetran vithai kudineer



Nitric oxide scavenging assay of HCl extract of thetran vithai kudineer



Summary and Conclusion

The present study was focused on the preparation, physico- chemical characteristics and antioxidant characteristics of ethanolic extract and HCl extract of Thetranvithai kudineer. The drug was prepared as per the PLIM protocol. Its physico chemical characteristics like pH value, Total ash value, Acid soluble ash content and water soluble ash content were also estimated.

The low ash content of the prepared drug (3.12) reveals the fact that the unwanted material in the drug is very low which makes the drug as a better one than any other drug. Low value of loss on drying (10.2) helps to store the drug for a long period. The anti oxidant study of the drug was carried out by in vitro trials towards the scavenging of nitric oxide free radical. Its inhibition towards the free radical produced were determined using optical density measurements. The percentage inhibition value for ethanolic extract was found to be 209 when the concentration of the extract was 3 ml. and that of HCl extract was 152 at 5 ml. These values were compared with the standard natural anti oxidant ascorbic acid (256 at 3 ml).

The above result showed that the drug **Thetranvithai kudineer chooranam** has more free radical scavenging tendency in alcoholic medium than acid medium. Concentration versus inhibition graph for both extract were drawn. The graph obtained reveals the fact that the anti oxidant property is concentration dependent. Further research is required to evaluate the active principle of the extract of the drug.

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