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Research article

Determination of serum antioxidant activity of *Sinna Sivappu Maathirai* for respiratory symptoms

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Abstract

Siddha medicine is presently practiced by Tamil speaking community in South part of India and North East part of Sri Lanka. Siddha medicine believes herbo-mineral drug to be potent than herbal alone and gradual unavailability of herbals make attention towards utilization of mineral based drugs. But heavy metal toxicity is a major safety issue. *Sinna Sivappu Maathirai (SSM)* is a herbo mineral drug which is prepared and prescribed in Siddha hospitals of Northern Province for respiratory symptoms with the reference literature .Most of Individual ingredients of SSM has the antioxidant property in the previous research works. So far, the antioxidant activity of *SSM* has not been published previously. *SSM* may be safety by antioxidant capacity. The study objected to determine serum antioxidant activity of *SSM* in patients with respiratory symptoms. 50 patients were selected with inclusion and exclusion criteria. All subjects treated for one week as the dose of 260mg bd/pc with 5ml of betel extract as an *Anupanam* (vehicle) and followed to find the serum antioxidant activity. Mean difference between base line and after treatment such as completion of 1st week analyzed by the method of "Ferric Reducing Antioxidant Power assay (FRAP)". Antioxidant power of *SSM* is higher in male (0.45±0.09 to 0.61±0.09) patients than the female (o.43±0.06 to 0.56±0.07). Antioxidant power is higher in the age group of 49years to 58years (0.38±0.10 t0 0.60±0.06) than other age groups. The mean difference of base line and after treatment was the p value of 0.0000. It indicated that the *SSM* has the serum antioxidant capacity significantly (0.44±0.08 to 0.58±0.08). Antioxidant capacity can be checked through the individual materials of *SSM* in the future research prospect. All three oils showed activity against all tested *Candida* sp ZOI from 8.3 ± 0.5 - 30.0 ± 0.0mm. MIC of three oils is similar for all tested *Candida* sp in both methods. The extremely low MIC (0.0045µg/mL) of the oil of *S. aromaticum* for all the tested *Candida* strains is note worthy. However, all the tested oils were active against *Candida* with MICs ranging from 0.0045 –2.5 µg/mL. MBC was the same or differed by only one dilution as the MIC for tested *Candida sp.* suggesting that the oils are fungicidal. Three oils have ability to inhibit *Candida* sp with low MIC.

Introduction

Indigenous medical system appeared in the company of ancient Dravidian. Siddha medical system is the one of the well-liked system surrounded by all other indigenous medical systems such as Ayurveda, Unani, Accupuncture, Homiopathy and traditional medicine. It is practiced by Tamil speaking community in South part of India and North East part of Sri Lanka. Siddha medicine believes herbo-mineral drugs to be potent than herbal alone and gradual unavailability of herbals make attention towards utilization of mineral based drugs [1]. As per the WHO, Herb-mineral products are natural and people believe it as a safe medicine but heavy metal toxicity is a major safety issue [6]. Toxicity may be prevented and safety by antioxidant capacity in Siddha formulations [8]. So far, limited studies have been published on herbo-mineral preparations with the antioxidant in Sri Lanka but individual plants has been published previously [3-4]. Hence researcher is trying to search the solution to dissolve the above problem by the scientific study of herbo-mineral medicine. The preliminary study was conducted in five Siddha drug preparing centers in Northern Province. All these centers are preparing and providing herbo-mineral based drugs. Among the mineral based drugs, all these units are preparing *Sinna Sivappu Maathirai* (*SSM)* which is a compound herbo-mineral Siddha drug. Sathilingam is the one mineral. SSM is currently prescribed for cough, chest pain, breathing difficulties cough with fever. It is prepared from nine raw materials of herbs such as *Saussurea lappa*, *Aconitum ferox*, *Piper nigrum*, *Alpinia officinarum*, *Eugenia caryophyllata*, *Carum copticum*, *Cuminum cyminum*, *Acorus calamus*, *Zingiber officinalis*, salt as Borax/

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Sodium Bi Borate (Porikaram) and a mineral as Red sulphide of mercury (Sathilingam) [5-6].

Suthesa oudatha Seimurai (1985) mentions the preparation and indication of *Sinna Sivappu Maathirai* in details as follows:

1) *Saussurea lappa* (Koddam) 5g
2) *Aconitum ferox* (Nabi) 5g
3) Red sulphide of mercury (Sathilingam) 5g
4) *Piper nigrum* (Milaku) 5g
5) *Alpinia officinarum* (Sitarathai) 5g
6) *Eugenia caryophyllata* (Karambu) 5g
7) *Carum copticum* (Omam) 5g
8) *Cuminum cyminum* (Seerakam) 5g
9) *Acorus calamus* (Vasampu) 5g
10) Borax/ Sodium Bi Borate (Porikaram) 5g
11) *Zingiber officinalis* (Ginger extract) required quantity

Materials and method

The research proposals with questionnaire and consent forms were submitted and obtained Ethical clearance dated on 28th of February 2017 (J/ERC/16/76/DR/0034) to the Ethical Review committee, Faculty of Medicine, University of Jaffna. Written permission was obtained from the commissioner, Dept. of Ayurveda, Colombo, SriLanka for the study placeas Siddha teaching hospital Kaithady. All patients were informed about the aim of the study and were included in the study after written consent. Informed consent form with information sheet (Tamil translation) was given and adequate time was given to take decision and consent was obtained from the subjects. All the necessary steps were taken to maintain confidentiality of the information and data which were collected during the research. All chemicals and reagents used were of Analytical grade and purchased from *Diagnosticm Zrt* (Pvt) Ltd, Colombo. Patients included that patients prescribed with SSM for the first time and excluded that patients were Children below 12years, pregnant women, mentally incompetent patients and patients with hepatic and renal impairment. The observation was conducted at Siddha teaching hospital Kaithady. At the enrollment, each subject was requested to support the interviewer interviewed questionnaire for data collection. A medical history, details of their food habits and life style of each subjectswere collected. After that, 5ml of blood sample was drawn from the anticubital vein using single use disposable painless blood collecting sterile needle and syringe (BD Emerald™). The blood was transferred into the evacuated tube. The blood specimens were transported to Dept. of Biochemistry, Faculty of Medicine, University of Jaffna with appropriate cold storage precautions. The maximum prescribed dose of *ssm* was 260 mg twice a day after meals with 5ml of betel extract for a week. The samples were centrifuged at 4500 rpm for 5 minutes and serum was pipette into sterilized clean polypropylene tubes and observed for the consistency and colour. If there any hemolytic (bright red) appearance, those samples were discarded. Serum was separated and used for the analysis. The FRAP (Ferric reducing antioxidant power assay) procedure described by Benzie and Strain (1999). The principal of this method based on the reduction of a ferric- tripyridyl triazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5ml of a (10mmol/L) TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40mmol/L HCl plus 5ml of $FeCl_3$ (20 mmolL) and 50 ml of acetate buffer, (0.3mol/L pH=3.6) and was prepared.1mmolar $Feso_4$ used as the standard. 31.2mg TPTZ weighed in analytical balance dissolved in 10 ml of 40 mol/l HCl and placed in a magnetic rotator for 30 minutes to get the clear solution. Ferric chloride 108mg dissolved in 20ml of distilled water. Acetic acid 3.42 ml and Sodium acetate 4.92 g measured and mixed together in a beaker added 180 ml of distil water stared well by glass rod and adjusted PH in PH meter by adding acetic acid until get the PH 3.6. The solution made up to 200 ml by adding distilled water. 5ml of TPTZ solution, 5 ml of $fecl_3$ and 50 ml of acetate buffer mixed together in conical flask and kept in water bath at the temperature of 37^{o}C for 10 minutes. 1.5 ml of FRAB solution mixed with 0.5ml of serum. The triplicate in each sample and blank kept for 10 minutes in 37 ^{o} C water bath. Absorbance read in UV-VIS Spectrophotometer.

Results and discussion

All data were analyzed using the SPSS 21.0. Data was expressed as percentage of age and sex variables, means and standard deviation of ferric reducing power for baseline and end of one week treatment of *SSM*. The significance between mean of baseline and end of one week treatment was tested using the paired 'T' test. A probability value of < 0.05 was considered statistically significant.

According to the table 1, during the study period, 72 % of the male subjects treated for respiratory symptoms with *SSM*. Antioxidant power of *SSM* is higher in male subject than the female subject for comparing between base line and end of one week treatment (0.16).

According to the table 2, antioxidant power is higher in the age group of 49years to 58years than among above age groups (Mean difference 0.22). *SSM* has the serum antioxidant activity among the fifty subjects. The mean value and standard deviation of baseline and end of one week treatment showed in the table 2.

Table: 1. Male and female prevalence on Antioxidant capacity

Sex	Number of patients	Base line	End of one week	Mean difference
Male	36	0.45±0.09	0.61±0.09	0.16
Female	14	0.43±0.06	0.56±0.07	0.13

Table: 2. Antioxidant capacity with Age and Serum antioxidant activity of SSM

Age group (Years)	Number of patients	Mean and standard deviation of base line	Mean and standard deviation of end of one week	Mean difference
18 – 28	17	0.44±0.07	0.58±0.08	0.14
29 – 38	14	0.43±0.09	0.58±0.08	0.15
39 – 48	08	0.43±0.07	0.56±0.07	0.13
49 – 58	06	0.38±0.10	0.60±0.06	0.22
Above 58	05	0.49±0.08	0.62±0.07	0.13
Mean ± SD for SSM	50	0.44±0.08	0.58±0.08	0.14

Statically, the significant level of serum antioxidant activity between base line and end of one week treatment is the value of 0.0000. It indicated that the *SSM* has the serum antioxidant capacity significantly. But the SSM that could be safety due to the antioxidant capacity should be tested in the future research prospect.

Conclusion

The Siddha drug "SSM" for its Antioxidant action through a trial of one week, From the observations of the present study, it is clear that the Ferric Reducing Antioxidant Power (FRAP) of the serum in the study participants significantly increased after one weeks of intervention. Hence *SSM* has the antioxidant activity significantly and SSM that may be safety due to the antioxidant property should be tested for the conclusion of antioxidant positive correlation with the safety.

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