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PREPARATION AND STANDARDIZATION STUDIES ON *VEERA MEZHUGU* – A SIDDHA ANTICANCER FORMULATION

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ABSTRACT

Veeramezhugu is a *Siddha* formulation which is often prescribed in cancer theraphy. It is a poly herbo-metalic preparation comprising *Veeram* (Corrosive sublimate), *Rasam* (Mercury), *Pooram* (Calomel), *Lingam* (Cinnaber), *Sudam* (Camphor), *Sambiran*i (Benzoin), Perungayam (Asafoetida), Vediuppu (Potassium nitrate), Navacharam (Ammonium chloride), Vengaram (Borax), *Nervalam* seed (*Croton tiglium*) and honey. In the present work this anticancer Siddha formulation is studied from process and product standardization point of view. Physicochemical parameters are determined for the end product as per Siddha Pharmacopoeia. Such kind of standardization studies will contribute in establishing scientifically the merits of Siddha herbo-metalic preparations.

Keywords: *Veera mezhugu*, Preparation, Purification, Standardization.

INTRODUCTION

In Siddha system of medicine there are 2-types of medicines available. According to the mode of usages, these are classified into 2-types, one is internal medicines and another one is external medicines. Totally there are 32 types of internal medicines and 32-types of external medicines. *Mezhugu* is one of the internal medicines, prepared by way of grinding well the ingredients with certain juices or extracts till a soft waxy consistency is attained. In tamil *mezhugu* means wax, so it is like ointment in its form. The semisolid consistency of these medicines is attained by the addition of mucilageneous or pectinaceous media, oils, fats, waxes or butter. It retains it's potency for five years. These are stored in wide mouthed glass bottles or food container.

Veera mezhugu is a polyherbometallic preparation consisting of 13-drugs used in cancer therapy. Also prescribed for ascitis, skin diseases, severe anemia, sinusitis, tuberculosis and uterine disorders. Ingredients of the selected herbo metallic formulation *Veera mezhugu* (VM) and purification methods used are discussed in sequel. Medicinal uses are specified in the Siddha texts and the formulation is prepared according to the formula given in Anuboga vaithiya navaneetham, written by Hakkim P. M. Abdulla Shagippu, published by Thamarai noolagam, 7, N.G.O.colony,Chennai-26.

Ingredients [1]

1. *Veeram* - Corrosive sublimate (Mercury chloride)

2. Rasam – Mercury (Hydrargyrum)

3. Pooram- Calomel

4. Lingam- Cinnabar (red sulphate of mercury)

5. Sudam - Camphor

6. Sambirani - Benzoin

6. Perungayam- Asafoetida

7. Vediuppu – Potassium nitrate

8. Navacharam –Ammonium chloride

9. Vengaram – Borax (sodium biborate)

10. Nervalam seed- *Croton tiglium*

11. Honey

The drugs enumerated in the recipe are powdered well, ground in an electrical mortar and pestle and made a fine powder form and mixed well. Finally honey is added (mucilageneous media) and ground for 3-hours.

Veera mezhugu

The major two steps involved in the preparation of *veera mezhugu* are 1) purification of the ingredients and 2) preparation of the medicine. Various purification agents like milk, lemon juice, turmeric, ghee, cow's urine and cow dung were used.

MATERIALS AND METHODS

The metal, mineral and plant materials were procured from Raw drug shop of Thanjavur market. The drug was purified as per the methods mentioned in authentic Siddha text books [2,3]. Physico-Chemical analyses were carried out employing modern sophisticated instrumentation techniques such as FTIR, Zeta Sizer analyzer as well as standard textual procedures.

Veeram

It is corrosive sulphate. Highly toxic material. Used in very small doses as stimulant and in skin diseases, used in combination with other drugs after proper processing and purification.

Names in Regional languages [5]

English: Mercury chloride.

Tamil: Veeram

Hindi: Sowweera pasanam

Sanskrit: Sowweera

Method of veeram Purification

Soaked in cow's milk, kept under sunlight for three days. After that washed and dried up.

Corrosive suplimate

Lingam

It is red sulphate of mercury. It occurs in nature and could also be prepared. It is restorative, alterative and tonic. Used in syphilis, diarrhea, fever, consumption and asthma.

Names in Regional languages [5]

English: Cinnabar

Tamil: Elingam

Hindi:Hingool

Sanskrit:Lingam

Telugu:Ingileekam

Malayalam:Chayilyam

Kannada:Ingleeyaka

Method of veeram Purification

Lime juice, milk and *Acalypha indica* juice were taken in equal quantity. Lingam placed in an earthen plate. The plate is heated while adding the mixture little by little for three hours.

CinnabarPurifying solution

Purified Cinnabar

Pooram

This is mercurous chloride. It is insoluble in water. In Siddha System of Medicine, it is always processed with other drugs and administered to reduce the unwanted massive tissue growth.

Names in Regional languages [5]

English: Calomel

Tamil: Pooram

Hindi:Ras kaapoor

Sanskrit: Rasakarpoora

Telugu: Rasakarpoora

Malayalam:Rasakarpoora

Kannada: Rasakarpoora

Calomel

A paste is made of black pepper and betel leaves weighing about 8.5 gm. Mixed this paste with 1.3 liters of water and taken in an earthen pot. Pooram is taken in a cotton cloth placed in the pot filled with the above mixer and boiled till the liquid was fully evaporated. Finally pooram was taken out washed and dried.

Rasam

Mercury widely used in the siddha system in several medicines in various forms. It is therapeutically used as an alternative, tonic, laxative, diuretic and in venereal diseases.

Names in Regional languages [5]

English: Mercury
Tamil: Paadarasam, rasam
Hindi:Paara
Sanskrit:Paarada, rasa
Telugu:Paadarasam
Malayalam:Paadarasam
Kannada:Paadarasa

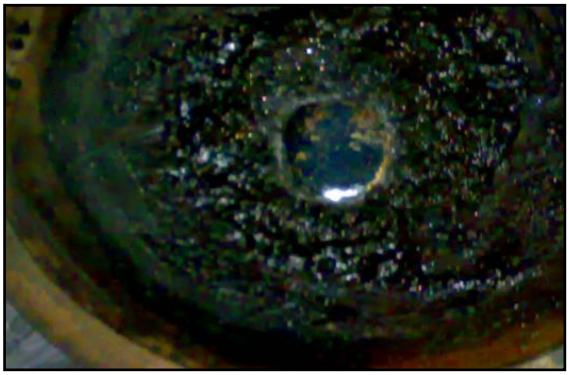
Method of rasam Purification

Raw material is ground well with brick powder till it's color turn's to white and removed. Again ground with turmeric powder till it's color changes to black and the turmeric powder will remain separately and it is removed. Finally semi purified rasam is boiled with the *Acalypha* plant juice. Washed, dried and used.

Mercury ground with turmeric powder

After grinding the turmeric powder remains isolated

Washing with water



Boiling with *Acalypha* juice

Purified mercury

Vediuppu

This is potassium nitrate. It has diuretic, refrigerant and diaphoretic properties. It reduces the frequency of pulse. In large doses, it is irritant. Useful in dropsy, small pox, measles, influenza, catarrh, gonorrhea, acute rheumatism and in bleeding from lungs and other internal organs.

Names in Regional languages [5]

English: Pottassium nitrate

Tamil: Vediuppu

Hindi:Sarakalmi

Sanskrit:Surakshara

Telugu:Surekaramu

Malayalam:Vediuppu

Kannada:Patluppu

Method of Purification

Dissolved with cow's urine and kept under sunlight for drying.

Potassium nitrate

Navacharam

This is ammonium chloride. It is expectorant, cholagogue and alterative in small doses. In large doses, it is purgative. It stimulates mucosa, relieves hepatic congestion and urinary secretion. Useful in dropsy, urinary disorders and when urine is full of lithates.

Names in Regional languages [5]

English: Sal ammoniac

Tamil: Navachaaram

Hindi:Nowshadar

Sanskrit:Navasagara

Telugu:Navasaramu

Malayalam:Navasaram

Kannada:Navasagara

Method of Purification:

Dissolved with cow's urine and boiled till it acquires a solid stage. Then it is kept under sunlight for drying.

Sal ammoniac

Boiled with cow's urine

Sambirani

This is the resinous gum obtained from Styrax benzoin and native of Malaya. It is antiseptic, disinfectant, stimulant and expectorant. Diuretic used in Respiratory disorders, jaundice and incontinence of urine among children.

Names in Regional languages [5]

English: Gum Benzoin

Tamil: Saambirani

Hindi:Oodh

Sanskrit:Devadhupika

Telugu:Saambsaani

Gum Benzoin

Sudam

The drug is sedative, antiseptic, diaphoretic and anthelmintic. It is stimulant in small doses. In moderate doses binds the motions. Used also in cough and cold to clear the congestion and causes expectorant action.

Names in Regional languages [5]

English: Camphor

Tamil: Karpooram

Hindi:Kaapoor

Sanskrit:Karpoora

Telugu: Karpoora

Malayalam:Karpooram

Kannada: Karpoora

Camphor

Perungayam

Consists of the dry resinous exudates of *Ferula foetida* Regel of the family Umbelliferae. The drug is carminative, stimulant and antiseptic. It contains essential oil, ferulic acid, umbelliferone, resinous matter and organic sulphur compounds. It is used as an abortifacient and as emmenagogue in substantial doses.

Names in Regional languages [5]

English: Asafoetida

Tamil: Perungayam

Hindi:Hing

Sanskrit:Hingu

Telugu:Inguva

Malayalam:Kaayam

Kannada:Hingu

Method of Purification

Fried and used.

Asafoetida

Vengaram

This is sodium biborate. It acts as an emmenagogue, astringent, sedative, diuretic and antiseptic. Used in skin diseases, puerperal convulsions, amenorrhoea and cystitis.

Names in Regional languages [5]

English: Borax

Tamil: Vengaram

Hindi:Tincal

Sanskrit:Tankana

Telugu:Veligaram

Malayalam:Pongaaram

Kannada:Biligaara

Method of Purification

Fried till the water of crystallization gets evaporated

Borax

Fried borax

Nervaalam

This is seeds of *Croton tiglium* Linn. of the family Euphorbiaceae. The seed and roots are emetic and are exceedingly purgative. Seeds contain 55% of oil. The oil contains toxic resin, purgative and vesicant principles, toxic proteins, sucrose, and a glycoside crotonoside. Wild in the dry deciduous forest and scrub jungles.

Names in Regional languages [5]

English: Croton

Tamil: Nervaalam

Hindi:Jamal gotta

Sanskrit:Danti

Telugu:Nepala

Malayalam: Nervalam

Kannada:Japala

Method of Purification

Collected the seed in cotton cloth and dipped in the pot containing cow dung solution. Boiled for 3-hours. (This method is called thulayenthra murai). After cooling the seeds were taken out and washed. Afterwards removed the seed coat and inner cotyledons. Finally boiled the seeds with lemon juice and fried with ghee.

Croton seed

Boiled with lemon juice

Fried with ghee

RESULT AND DISCUSSION

Table 1: Physico chemical Data

Parameters	Data
Description	Grey coloured semisolid mass
Loss on Drying	23.0939 %
Total Ash	12.0035 %
Acid Insoluble ash	0.2647 %
Water soluble extractive	51.2821 %
Alcohol soluble extractive	15.4589 %
pH (1% w/v solution)	7.29

Broad peaks at ca.1600cm^{-1} and ca.3500 are due to OH stretching vibrations. Peaks at ca.830 cm^{-1} and 770 cm^{-1} are due to bending vibrations of nitrate. Peak at 1380 cm^{-1}, 1015 cm^{-1}and ca.590 cm^{-1} may be due to the stretching and bending vibrations from inorganic borates. Broad peaks at 3180 cm^{-1} and 1430 cm^{-1} signifies the presence of ammonium in the sample. Presence or absence of chlorides and sulfides of mercury could not be evaluated from the FTIR spectra. Presence of hydrocarbon peaks at ca. 2980 cm^{-1} and ca.1650 cm^{-1} signifies the presence of organic materials in the sample.

***In vitro* studies on EAC cell lines**

MTT assay procedure

In vitro cytotoxicity study employing MTT assay was performed as per standard textual procedures [12]. Ehrlich ascites carcinoma (EAC) cells were cultured in RPMI-1640 medium supplemented with 2nM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin, maintained at 37^{0}C in an atmosphere of 95% O_2 and 5% CO_2. In each assay, 0.1X10^6 EAC cells were harvested in culture medium and plated in 96-well flat bottom culture plates and incubated at 37^{0}C for 24 h in humidified 5% CO_2. After 24 hours, 10 µL aliquots of serial dilutions of plant extract (1000-1.95 µg/ml) in DMSO were added to EAC cells and incubated for 48 h. Cell viability was assessed through the MTT assay. Briefly, 25 µL of MTT (5 mg/mL) was added and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilized with 100 µL of a solution containing 50% N, N-dimethylformamide and 20% Sodium dodecyl sulfate. The optical density of each well was measured using Epoch microplate spectrophotometer (BioTek, USA) set at 590 nm filter. Cells viability was calculated and tabulated.

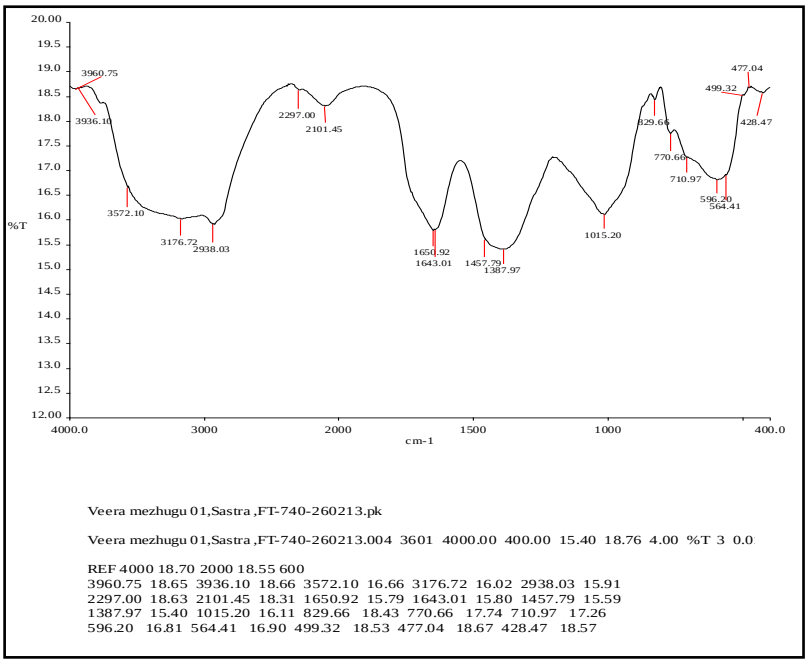


Fig. 1: FTIR Spectrum of Veera Mezhugu

Table 2: Cytotoxicity effect of Veera Mezhugu (VM) on EAC

Drug Concentrations (µg/ml)	Percentage inhibition	IC_{50}
1000	74.05±1.41	93.59 µg/ml
500	72.06±0.87	
250	62.95±1.30	
125	60.19±0.43	
62.5	42.20±0.76	
31.2	25.43±0.97	
15.6	20.49±5.03	
7.81	5.68±6.93	

The cytotoxicity of Veera Mezhugu was evaluated using *in vitro* MTT assay on EAC cell lines. Various concentrations of test drug (VM) ranging from 7.8 to 1000 µg/ml were reacted with EAC and the cytotoxicity was assessed. In the present study, 74.05 % cytotoxicity was observed at 1000µg/ml concentration of the test drug Veera Mezhugu.

In vitro antioxidant studies

In vitro antioxidant studies employing chemical methods such as DPPH and reducing capacity assays were carried out as per Blois (1958) and Oyaizu (1986) [13, 14].

Table 3: Antioxidant efficacy of Veera Mezhugu (VM)

Drug Concentrations (µg/ml)	DPPH Assay	Reducing Capacity Assay
	Percentage inhibition	Absorbance
500	28.27±1.86	0.53±0.01
250	26.19±1.03	0.43±0.01
125	20.83±1.36	0.39±0.02
62.5	16.67±2.06	0.33±0.02
31.25	15.77±2.25	0.26±0.01
15.62	11.90±1.86	0.13±0.02
7.81	5.06±2.25	0.05±0.01

The data on the antioxidant efficacy studies employing chemical methods depicted that the test drug (VM) possesses moderate antioxidant potential.

DISCUSSION

In the present study the test drug Veeram was prepared as per standard siddha texts, using various purification steps as mentioned in the literature. Physico chemical standards to decide the quality of the finished product was also determined. The FTIR spectrum of the end product revealed incorporation of inorganic and organic groups from the purifying agents and ingredients used. When VM was subjected to invitro cytotoxicity studies, 74.05 % cytotoxicity was observed at 1000µg/ml concentration of the test drug Veera Mezhugu.

In living system, reactive oxygen species are constantly generated. When the level of ROS levels exceed, oxidative damage is caused to tissues and bio-molecules leading to various pathological conditions [15].

The ability of the test drug (VM), to donate hydrogen or to scavenge free radicals can be assessed by DPPH assay. DPPH radical (deep violet) is a stable free radical which when react with an antioxidant substance reduces to diphenylhydrazine (light yellow). The change in colour can be measured spectrophotometrically [16]. In the present study, different concentrations of Veera Mezhugu were made to react with DPPH radical and the change in colour was observed. The sample at the concentration of 500 µg/ml showed 28.27 % inhibition.

Total reducing capacity of test drug (VM) was presented in Table no. 3. Depending upon the concentration of the test drug (VM) a change in colour was noticed which is due to the conversion of ferric to ferrous form. The colour changes from yellow to different shades of green and blue [16]. Amongst various concentration of drug (VM) tested 500 µg/ml showed maximum absorbance of 0.53±0.01. The data obtained in the present study revealed that the test drug Veera Mezhugu has good reducing capacity.

To sum up from the data generated in the present study, It is concluded that Siddha formulation, Veera Mezhugu possess both antioxidant and anticancer potentials justifying scientifically its administration in cancer patients by Siddha practioners.

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