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QUALITATIVE PHYTOCHEMICAL ANALYSIS AND PHARMACOLOGICAL ACTION OF SIDDHA FORMULATION SADHAKUPPAI CHOORANAM

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ABSTRACT

Sadhakuppai Chooranam (*SKC*) is a polyherbal formulation indicated in *Siddha* literature for the treatment of Worm infestations in pediatrics. This study was aimed at evaluating the phytochemical and anthelmintic activity of Sadhakuppai Chooranam (*SKC*). Water extract of two concentration of Sadhakuppai chooranam (90 and 180mg) was tested against the nematode Ascaris lumbricoides and the results were expressed in terms of paralysis and mortality of worms. The drug Piperazine citrate 10mg was used as standard reference drug for this study. The results were statistically analysed by'*t* test'. The results showed that the test drug Sadhakuppai Chooranam(180mg) showed early paralysis and mortality of the nematode worms Ascaris lumbricoides when compared to the standard drug Piperazine citrate 10mg. Hence the anthelmintic activity of the Sidha drug Sadhakuppai Chooranam has been demonstrated for the first time.

KEYWORDS: *Siddha*, *Sadhakuppai Chooranam* (*SKC*), Worm infestation, herbal medicine, Anthelmintic activity.

INTRODUCTION

The Siddha system of medicine has been traditionally used among the people of South India for the treatment of common ailments such as *Kudal Kirumi* (worm infestations). Since time immemorial the ancient saints known as Siddhars, had a vast knowledge about the medicinal uses of flora and fauna of this universe. As these herbs are in use over centuries, a wealth of literature is available in manuscripts indicating the medicinal use of these herbs.

Intestinal parasitic infections have been reported to be the most common public health problem and an important cause of malnutrition and cognitive behavioral impairment in Children.[2] According to World health organization (WHO) it has been estimated that over a billion of world's population is infested with soil transmitted diseases and there are 3 billion worm infections in today"s world population.[1] The poorest global estimates of urban and rural areas of India have nearly 450 million Children affected by worm infestation.[2-5]

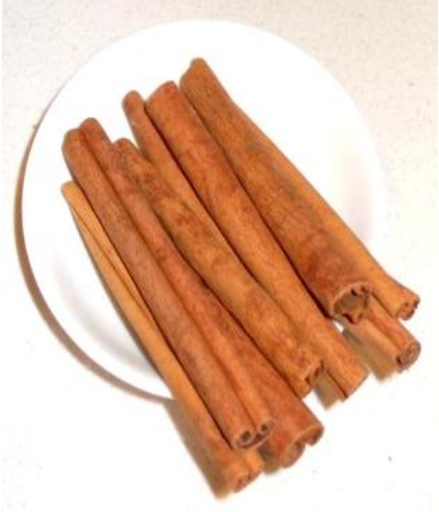
Sadhakuppai Chooranam is one among such classical formulation indicated for the treatment of Intestinal worm infestations. It is a polyherbal formulation containing nine herbal ingredients. Although these natural drugs are safe, there exists an unquestionable need to evaluate the safety and toxicity parameters of these herbal drugs. This study was performed to evaluate the Physicochemical, biochemical and pharmacological aspects of Sadhakuppai Chooranam (*SKC*). The results of pharmacological study were analysed using 't' test.

MATERIALS AND METHODS

Sadhakuppai Chooranam

The ingredients are *Sadhakuppai Chooranam (Anethum graveolens), Seeragam(Cuminum cyminum), Peruncheeragam (Pimpinella anisum), Karuncheeragam(Nigella sativa), Elam (Elettaria cardamomum), Lavangapattai (Cinnamomum verum) Athimadhuram(Glycyrrhiza glabra), Kothumalli(Coriandrum sativum), Lavangam (Syzygium aromaticum) and Cheenakarkandu (White sugar candy).*

Sadhakuppai (Anethum graveolens)	***Seeragam (Cuminum cyminum)***	***Peruncheeragam (Pimpinella anisum)***
		

Karuncheeragam (Nigella sativa)	*Elam (Elettaria cardamomum)*	*Lavangapattai (Cinnamomum verum)*
		

Athimadhuram (Glycyrrhiza glabra)	*Kothumalli (Coriandrum sativum)*	*Lavangam (Syzygiumaromaticum)*	*Cheena karkandu (White sugar candy)*
			

Fig 1: Herbal ingredients of Sadhakuppai Chooranam.

Preparation of study drug

The above said drugs were purified separately and were allowed to dry in the proper sunlight. All the dried drugs (except White sugar candy) were powdered and then finally it was mixed with sugar candy.Then it was filtered with a piece of cloth and then stored in a air tight bottles. The powdered drug was placed on the cloth tied on top of a vessel containing milk for purification of Chooranam and was steamed until the milk evaporated. The purified powder was taken and then dried.[6]

Dosage for Children

3 – 7 years 500 mg twice daily

8 – 12 years 1 gm twice daily

Adjuvant and therapeutic duration: Hot water for a period of 20 days

MATERIALS AND METHODS

Qualitative phytochemical analysis of acidic / basic radicals of test drug – Sadhakuppai Choornam[7,8]

Preparation of Extract

5 gm of Sadhakuppai Choornam was weighed accurately and placed in a 250 ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes and was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

ICP-OES analysis of Sadhakuppai Chooranam

Heavy metal (lead, cadmium, mercury and arsenic) content in Sadhakuppai Chooranam was determined by using (ICP-OES).The samples are usually made up in 2% w/v Nitric Acid (Trace Metal Grade from Fisher Sci, Cat#A509-500) prepared with 18 M Ohm D.I Water. If 1 or 2 ml of sample are to be diluted then the stock solution of 2% w/v Nitric Acid may be used. If a larger volume of sample is to be prepared then the quantity of concentrated Nitric Acid required to provide a 2% w/v Nitric Acid concentration in the final solution needs to be calculated.

Anthelmintic activity

Sadhakuppai choornam (SKC) was prepared by the method prescribed in the text book of Siddha medicine-Agasthiyar attavanai vagadam. All drugs used for the study was suspended each time with 1% (w/v) solution of sodium carboxy methyl cellulose before administration.

Drugs and chemicals

Standard Drugs and fine chemicals used in these experiments were obtained from Sigma Chemicals Company, U.S.A. Other analytical grade chemicals were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Experimental animals

Colony inbred animals strains of Wistar rats of either sex weighing 200 - 250 g were used for the pharmacological study. The animals were kept under standard conditions 12:12 (day/night cycles) at 22^0C room temperature, in polypropylene cages and were fed on standard pelleted diet (Hindustan Lever Pvt Ltd., Bangalore) and tap water *ad libitum*. They were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory

conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) at National Institute of Siddha, Tambaram, Chennai.

In vitro Anthelmintic activity in experimental Wistar rats

Adult Ascaris worms were collected from the abdomen of sheep slaughtered at the Chennai Corporation Ambattur. Immediately after slaughter, the abdomen were collected and transported to the laboratory. The parasites were then collected, washed and kept in phosphate buffered saline (PBS). The experiment was conducted according to Eguale *et al.*[9] Ten actively moving worms were placed in *Petri dishes* containing 90 and 180mg (each ml contains 9 and 18mg, respectively) aqueous solution of the test drug in PBS and PBS alone for the control group, in a total volume of 10 ml. Piperazine citrate dissolved in DMSO and diluted in PBS at concentration of 10mg/ ml (each ml contains 1mg of piperazine citrate) was used as the positive control. After 24 hours, the test drug and piperazine citrate were washed away and the parasites suspended in PBS for 30 minutes for possible recovery of parasite motility. The number of motile (alive) and immotile (dead) worms were counted under the dissecting microscope, and recorded for each concentration. Death of worms was ascertained by the absence of motility for an observation period of 5-6 seconds. A mortality index was calculated as the number of dead worms divided by the total number of worms per Petri dish.

RESULTS

Table 1: Test for Acidic Radicals.

Procedure	Observation	Inference
Test for Calcium: 2 ml of extract is taken in a clean test tube. To this add 2 ml of 4% ammonium oxide solution.	White precipitate is formed	Presence of calcium
Test for Sulphate: 2 ml of the extract is added to 5 % barium chloride solution.	White precipitate is formed	Presence of Sulphate
Test for Chloride : The extract is treated with Silver nitrate solution	No white precipitate is formed	Absence of Chloride
Test for carbonate : The substance is treated with Conc. HCl.	No effervescence is formed	Absence of carbonate
Test for Starch : The extract is added with weak iodine solution	No blue colour is formed	Presence of starch
Test for Iron (Ferric) : The extract is treated with glacial acetic acid and potassium ferrocyanide	No blue colour is formed	Absence of Ferric iron
Test for Iron (Ferrous) : The extract is treated	Brick red colour is	Presence of

with Conc. HNO_3 and ammonium thiocynate	formed	Ferrous iron
Test for phosphate : The extract is treated with ammonium molybdate and conc. HNO_3	Yellow precipitate is formed	Presence of phosphate
Test for Tannic acid : The extract is treated with Ferric chloride	No blue black precipitate is formed	Presence of Tannic acid
Test for Unsaturation: 1 ml of Potassium permanganate solution is added to the extract.	Does not get decolourised	Absence of unsaturated compound
Test for saponins: Dilute extract+ 1ml of distilled water shake well.	No Froth formation	Absence of saponins
Test for sugars : **Benedict method** ; 5ml of Benedict solution heated gently then add 8 drops of diluted extract then heated in a boiling water bath. **Molisch test**; Dilute extract+2 drops of Molisch+3ml conc.H_2SO_4.	No colour change No Reddish violet zones appeared	Indicates the Absence of sugar Absence of carbohydrate
Test for steroids: Liberman Burchard test ; Dilute extract +2 ml aceticanhydride+conc.H_2SO_4	No formation of red colour	Absence of steroids
Test for amino acids: Dilute extract +2ml of Ninhydrin's soln .	Formation of violet colour	Presence of amino acids
Test for proteins: Biuret method ; 1ml of dilute extract+1mlof5%$CuSO_4$+ 1%NaOH.	Formation of blue colour	Presence of proteins
Test for Flavanoids: Dilute extract+ mg bits+2drops of conc.HCl and gently heated.	No formation of pink colour	Absence of Flavanoids
Test for phenol: Dilute extract+2drops of $FeCl_3$ soln.	No formation of Deep green colour	Absence of phenols
Test for Tannins: dilute extract +2ml of 10%lead acetate add.	White precipitate formed	Presence of tannins
Test for alkaloids: Mayer's method: 1ml of dilute extract + 1ml reagent. Dragendroff's method: 1ml of dilute extract+ 1ml of reagent	No cream colour precipitate is formed Appearance of orange colour precipitate	Absence of alkaloids Trace amount of alkaloids present
Test for basic radicals		
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid	Formation of Yellow colour precipitate	Presence of Potassium
Test For Sodium: 2 pinches of the substance is made into paste by using Hcl and introduced into the blue flame of Bunsen burner	Appearance of intense Yellow colour	Presence of Sodium
Test For Lead: 2 ml of extract is added with	No formation of	Absence of Lead

2ml of potassium iodide solution.	yellow colour precipitate	
Test For Mercury: 2m1 of the extract is treated with 2ml of sodium hydroxide Solution.	No Formation of Yellow precipitate	Absence of Mercury

ICP-OES analysis of Sadhakuppai Chooranam

Table 2: ICP-OES analysis of Sadhakuppai Chooranam.

S.NO	Analyte	Mean
1	As	BDL
2	Ca	18.059 mg/L
3	Cd	BDL
4	Hg	BDL
5	Fe	1.286 mg/L
6	K	32.532 mg/L
7	Na	48.985 mg/L
8	P	18.295 mg/L
9	Pb	BDL
10	S	1.009 mg/L

Anthelmintic activity

SKC showed a dose dependent anthelmintic activity against adult Ascaris worms by in vitro method. The therapeutic activity of SKC can be compared to that of Piperazine citrate, the standard anthelmintic drug used in modern medicine.

Table 3: Anthelmintic activity of SKC on Ascaris adult worms.

Concentration of test drug/standard drug	Time taken for paralysis(min)	Time taken for death (min)
SKC-90mg	14.32±2.2	63.57±12.6
SKC-180mg	8.05±1.6	50.33±3.1
Piperazine citrate 10mg	12.05±1.2	57.4±2.1

DISCUSSION

The study was aimed at evaluating the qualitative phytochemical and anthelmintic activity of Sadhakuppai Chooranam. The qualitative phytochemical test of water extract revealed that formulation contains calcium, sulphate, starch, ferrous iron, phosphate, tannin amino acids, proteins and trace amount of alkaloids. It also has potassium and sodium. ICP-OES analysis of Sadhakuppai Chooranam showed the quantitative analysis of all the heavy metals Mercury, Arsenic, Cadmium and Zinc were below the detectable limits. Other trace lements

such as Calcium, Iron, Potassium, Sodium, Phosphorous and Sulphur were under the permissible limits. Hence this Siddha formulation is considered to be safe without any heavy metals. The invitro anthelmintic activity showed a dose dependent activity (90mg, 180 mg) against the adult nematode Ascaris lumbricoids. In this study the control drug Piperazine citrate 10mg showed paralysis of worms at 12.05±1.2min and mortality at 57.4±2.1.min. The present study showed that the exposure of test drug Sadhakuppai Chooranam 180mg showed early paralysis than the standard drug Piperazine citrate at 8.05±1.6 followed by mortality of parasites at 50.33±3.1.

CONCLUSION

The study showed that the Siddha formulation Sadhakuppai Chooranam is a pharmacologically effective drug with anthelmintic property. Further in-vitro and in-vivo studies on safety parameters and clinical trials would confirm its usefulness among pediatric patients with worm infestations.

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