



EVALUATION OF *IN VITRO* ANTIBACTERIAL ACTIVITY AND MEDIAN LETHAL DOSE ESTIMATION OF A SIDDHA POLYHERBAL FORMULATION: *SINGINATHA CHOORNAM* IN RAT MODEL

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ABSTRACT

Singinatha Choornam, a Siddha polyherbal formulation has been prescribed for Allergic Rhinosinusitis in the literature. The objective was to evaluate the antibacterial activity and safety of *Singinatha Choornam* in a rat model. Initially, Physico chemical characterization studies were done adopting a methodology of Pharmacopoeial Laboratory for Indian Medicine. Antibacterial study was done by Kirby-Bauer disc diffusion method against gram positive and negative strains comparing with Ciprofloxacin. An Acute toxicity study was done on female wistar albino rat under OECD guidelines 423. Physicochemical parameters results can be used as standard in future for maintaining quality of this drug. *Staphylococcus aureus* and *Escherchia coli* were susceptible to 50 µl/disc of aqueous extract of the test drug prepared with 5 g of sample in 50 ml of distilled water. Acute oral toxicity study of *Singinatha Choornam* revealed no mortality even at the dosage of 2000 mg/kg body weight justifies its recommended therapeutic dosage of 1 g.

KEYWORDS: *Singinatha Choornam*, Siddha, Rhinosinusitis, Acute toxicity and Antibacterial study



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INTRODUCTION

In Siddha system of medicine, herbs and herbal formulations are considered as an initial choice of drugs followed by higher order medicines of parpams and chendurams prepared from minerals and metals, if the patient is not responding well to herbal medicines¹. *Singinatha Choornam* (SC) is a powder form poly-herbal formulation cited in the literature *Agasthiyar Attavanai Vagadam* indicated for treating Allergic Rhinosinusitis (*Mokkuneerpaichal*) at one gram dosage with warm water². Robert Saper reported the presence of heavy metals in Ayurvedic herbal medicine products which makes it essential to perform characterization and safety study in Indian system of medicine to validate its usage in community³. As the drug SC has not been evaluated before, this study was carried out to establish the physicochemical characterization data, antibacterial activity and safety profile for this *choornam* to validate its therapeutic usage.

MATERIALS AND METHODS

(i) Preparation of Singinatha Choornam

Singinatha Choornam (SC) was prepared by the method described in *Agasthiyar Attavanai Vagadam*². The herbal ingredients used for the preparation of *Singinatha chooranam* were purchased from local market. An equal proportion of *Chukku* (dried rhizome of *Zingiber officinale* Roscoe), *Milagu* (dried fruit of *Piper nigrum* Linn), *Thippili* (dried fruit of *Piper longum* Linn), *Karkadaga Shingi* (Gall of *Rhus succedanea* Linn), *Katthari ver* (Root of *Solanum melongena* Linn) and *Kandankatthari ver thol* (Root cortex of *Solanum surattense* Burm) were used as ingredients. Before preparation all the ingredients were purified for maintaining quality standard. *Chukku* was purified by removing the outer cortex; *Milagu* and *Thippili* were purified by frying up to dryness in mud pan. *Karkadaga Shingi*, *Katthari ver* and *Kandankatthari ver thol* were washed in water for seven times and dried under sunlight. Each purified ingredients was ground using pulverizer, filtered through the mesh of the sieve

size no. 125 and fine powder made. All the powders were mixed well and preserved in an air tight container.

(ii) Physicochemical analyses

SC was subjected for determination of physicochemical parameters such as total ash, water soluble and acid insoluble content, extractive values, moisture content and Thin layer chromatographic (TLC) analysis according to the standard methods adopted by Pharmacopoeial Laboratory of Indian Medicine⁴. For TLC analysis, the extract was prepared by adding 4g of SC in 25 ml alcohol and refluxed on a water-bath for 30 min for three times using 25ml alcohol each time, filtered and concentrated to 10 ml. 10 µl of concentrated extract was applied to the TLC Aluminium plate precoated with silica gel and the plate was developed to a distance of 8 cm using toluene: ethyl acetate (5: 2) as mobile phase. After development, the plate was air dried and examined under ultraviolet (254 & 366 nm). *UV-VIS Spectroscopic analysis*: A solution of SC in chloroform 10µ/ml was prepared. After baseline correction, the sample was scanned in the range of 800-200 nm, the peak pattern was obtained. The major peak observed was noted.

(iii) Phytochemical analyses

Aqueous extract of SC was prepared by boiling 5g of medicine in 50 ml of distilled water for 10 min and cooled and filtered through Whatman filter paper 41. The phytochemical tests were done on the extract as the method illustrated in Prashant Tiwari⁵ and Harborne⁶.

(iv) Antimicrobial activity

In-vitro antimicrobial activity of *Singinatha Choornam* was screened against bacteria strains such as *Streptococcus mutans*, *Staphylococcus aureus*, *Escherchia.coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by Kirby-Bauer disc diffusion method⁷. The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18 h

and stocked at 40°C in Mueller-Hinton Agar. The culture was inoculated in sterile Nutrient broth and incubated at 37°C for 3h until the culture attained a turbidity of 0.5 McFarland units. The density of the organisms was adjusted to 10⁸ cfu/ml by comparing its turbidity with that of 0.5 McFarland opacity standards. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. The sterile blotting paper discs (10 mm) were soaked in different concentrations (10µl, 25 µl, 50 µl) of Aqueous extracts (5 g/100 ml distilled water) and in Ciprofloxacin (50 µg) for standard and in sterile distilled water (50 µl) for control and dried well. The prepared discs were placed on the surface of agar using sterile forceps and pressed slightly to provide uniform contact and the plates were incubated at 37°C for 18 h. By using transparent plastic ruler, the zone of complete growth inhibition around each discs were measured including discs diameter. The interpretation of zone size was classified as susceptible (≥ 21 mm), moderately susceptible (16 – 20 mm) and resistant (≤ 15 mm).

(v) Acute oral toxicity study

Wistar strain Albino rat of female sex, weighing 120-130 g was purchased from King Institute of Preventive Medicine, Guindy, Chennai, India and they were acclimatized in the animal house of Sairam Advanced Centre for Research, West Tambaram, Chennai, India at 21-23 °C. Animal ethical guidelines of CPCSEA, Ministry of Animal Husbandry and Welfare, Govt. of India were strictly followed of the care and maintenance of procured animals. The animals were fed on standard rodent pellet and RO water was provided *ad libitum*. The animals were kept for overnight fasting before experimentation. Acute oral toxicity in the animals has been conducted as per the OECD guidelines 423 (Acute Toxic Class Method)⁸.

The acute toxic class method is a stepwise procedure with 3 animals of a single sex per step⁹. Since the formulation is relatively non toxic in clinical practice the highest dose of 2000 mg/kg/p.o (as per OECD guidelines "Unclassified") was used in the acute toxicity study. The animals were observed closely for behavioral toxicity, if any, by using FOB (Functional observation battery).

RESULTS AND DISCUSSIONS

1. Physiochemical analysis

The organoleptic character revealed the *choornam* was brownish grey in colour having mild irritant odour and hot with an astringent taste. The evaluation of organoleptic characters makes simplest and quickest route to establish the identity and quality of an herbal formulations¹⁰. Quality test performed for moisture content, ash content, water soluble and alcohol soluble extract values, pH and their results (Table 1) revealed that the ingredients used for the preparation were in better quality and purity. Ashing involves an oxidization process of the crude drugs in the preparation and gives inorganic material residual. Less ash value for SC (8.43%) is an indicative for reduced contamination and free from adulteration implies better quality. The moisture content at 105°C is 0.25% revealed stability of the drug is more as due to dryness. Dryness makes the formulation free from molds from reduced microbial content¹¹. The pH of the formulation shows slightly acidic nature (6.3) due to the presence of acidic salts such as Sodium, Zinc and Sulphate (Table 1). The alcohol soluble and water soluble extractive values of SC shows 79.80% and 86.23% respectively as depicted in table 1 implies both water and alcohol are better solvents for extraction of this formulation

Table 1
Physico chemical analyses of Singinatha Choornam

Parameters	Result
Total ash value	8.43 %
Acid insoluble ash value	8.01 %
Water soluble ash value	5.38 %
Alcohol soluble extractive value	79.80 %
Water soluble extractive value	86.23 %
Loss on drying at 105°C	0.250 %
pH	6.3
Test For Sodium	+ ve
Test For Zinc	+ ve
Test for Sulphate	+ ve

2. Phytochemical analyses

The results of phytochemical analyses of the formulation revealed the presence of high contents of Flavonoids, Phenols and Phytosterols. Phytosterol has been reported as an anti cancer and immunomodulatory compound¹². FDA reported 2 g/day of phytosterol consumption in diet reduce high cholesterol level and lowering Cardio Vascular Disease (CVD) risk¹³. Phenolic compounds present in the SC suggest the drug may act as a Chemo preventive drug¹⁴. The presence of flavonoids leads to further studies on SC for the anti bacterial, anti aging, Vitamin C sparing and mild vasodilator activity¹⁵.

3. Thin layer Chromatography and Ultraviolet visible spectroscopic analysis of Singinatha Choornam

TLC finger print of SC seen under UV 254 nm shows major spots at Rf 0.29, 0.33, 0.57, 0.69 and 0.85 revealed presence of five major compounds. Under UV 366 nm, it shows major spots at Rf 0.35, 0.54, 0.67, 0.72, 0.82 implies the presence of five major compounds. Table 2 shows the absorbance value of SC at ten different wave length and revealed the presence of ten major compounds.

Table 2
UV-VIS Spectroscopic analyses of Singinatha Choornam

Wave Length (nm)	Absorbance
400	3.000
420	3.000
425	2.673
430	2.190
450	1.223
460	0.964
480	0.608
500	0.444
540	0.289
560	0.240

4. Antimicrobial activity

According to the antibacterial profile of SC (Table 3), it was observed that gram positive bacterial strain *Staphylococcus aureus* and gram negative bacterial strain *Escherchia coli* were moderately susceptible with SC at 50

µl/disc concentration whereas resistant against *Streptococcus mutans*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in comparison of standard Ciproflaxion(50 µg/disc). Antimicrobial activity of SC is reported might be due to presence of flavanoids and phytosterols¹⁶

Table 3
Antimicrobial activity of Singinatha Choornam

Organism	Standard drug Ciprofloxacin 50 mcg/disc	Test drug (µl/disc)		
		Zone of inhibition in mm		
		10µl	25µl	50µl
<i>S. mutans</i>	32	11	13	14
<i>S. aureus</i>	31	14	16	19
<i>E. coli</i>	31	12	15	17
<i>K. pneumoniae</i>	31	9	11	12
<i>P. aeruginosa</i>	29	7	8	8

5. Acute oral toxicity study

The result of acute oral toxicity study show SC at the dose of 2000mg/kg/po did not exhibit mortality and did not show any signs of acute toxicity and behaviour changes. As per OECD 423 guidelines, the dose is said to be "Unclassified" under the toxicity scale⁸. Hence, further study with higher doses was not executed. So, the drug SC falls under class 4 where LD50 value lies more than 2000mg/kg).

CONCLUSION

It can be concluded that the *Singinatha Choornam* has significant anti bacterial activity against some bacterial strains causing upper respiratory and gastrointestinal infections which lead to evidence for its traditional usage against Rhino-Sinusitis. Moreover, it is a safer drug in its therapeutic dosage indicated in the literature since its median lethal dose is evaluated more than 2000 mg/kg. Further, chronic toxicity and pharmacological studies have to be carried out in future for further establishing its safety and potency.

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