Formulation, standardization and pharmacological studies of Saraswataristam: A polyhedral preparation

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Abstract: Ayurvedic preparations achieved paramount importance in contemporary life owing to the safety and efficacy when compared with those of synthetic drugs. But due to lack of proper standardization at each stage from starting to culmination results in inferior quality and less demand. Saraswataristam, a fermented ayurvedic preparation, has been employed for treating central nervous system disorders and dermatological problems. Saraswataristam, containing *Centella asiatica* (L) urban (Umbelliferae) as the major ingredient is prepared as per the Ayurvedic Pharmacopoeial specifications, characterized and standardized for determining the quality, safety and efficacy of herbs used in it. Saraswataristam was prepared and subjected to phytochemical screening by FTIR analysis and HPTLC fingerprinting, heavy metal determination by AAS, determination of alcohol content, test for *E. coli*, *S. aureus*, aerobic bacteria, yeasts and mould, oral toxicity studies and anti-epileptic activity by MES method. The physico-chemical studies showed total ash content as 1.1%, extractive values and some trace elements such as Lead, Mercury, Cadmium and Arsenic with 3.1, 0.047, 0.17 and 0.46 ppm respectively and all values are found within the acceptable limits specified by WHO. FTIR and HPTLC studies showed the presence of asiaticoside in Saraswataristam, resulting in its chemical standardization. The formulation showed signs of dose dependent significant ($P<0.001$) reduction in various episodes of epileptic seizures in comparison with standard phenytoin, thereby making it biologically standardized. The physico-chemical and pharmacological analysis to standardize Saraswataristam confirmed its use as a safe anti-epileptic ayurvedic formulation.

Keywords: Ayurvedic formulation, HPTLC, asiaticoside, antiepileptic activity.

INTRODUCTION

Ayurveda is a comprehensive natural health care system that originated in India around 5000 BC and is still widely in use. Literature studies revealed that ayurvedic products were highly in demand worldwide in the recent past. These are widely used as herbal products, nutraceuticals and cosmetics (Arun *et al*., 2011). The quality herbal products may be obtained only through maintaining proper care from starting to culmination of its formulation. These include proper identification of plants, season and area of collection, extraction, purification and rationalizing the combination in case of polyherbal formulations.

Arista, an ayurvedic preparation, is effectively used to treat many diseases (Khalsa, 2007, Sekhar and Mariappan, 2008). It is obtained by soaking the crude drugs, either in powdered form or as decoction, in jaggery solution. While doing so, it undergoes fermentation to produce alcohol, which helps to extract the phytoceuticals from the crude drugs (Ayurvedic Formulary of India, 2003). Though the requirement for herbal medicines are increasing, the major drawbacks encountered by the herbal drug companies include lack of proper documentation, validation and determination of biomarkers besides the non-existence of rigid quality control profiles for herbs and their formulations. This creates an urgent need for standardization of herbal drugs, which enhances the quality, safety and efficacy of their use for various ailments.

The present investigation deals with standardization of Saraswataristam which is majorly used as stimulant, soporific, emmenagogue, nervine tonic, cardiotonic, stomachic, carminative and diuretic. It is also used in the treatment of central nervous system disorders and dermatological problems. The main objective of the study includes for*m*ulation of Saraswataristam, subjected to phytochemical, physico-chemical, microbiological, toxicological and pharmacological evaluations using modern analytical tools.

MATERIALS AND METHODS

Plant material

The herbs used in the preparation of Saraswataristam were obtained from the Tirumala hills, Tirupati, India and, authentified by Dr. Jayaraman, PARC, Chennai, India.

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Animals and media

Wistar albino rats (100-120 g) of four groups, including control and standard group, each with six animals were selected. Nutrient broth and culture media were obtained from the SD Fine Chemical Ltd, Mumbai, India. Rats were kept in acrylic cages at standard conditions of temperature at 25±2ºC and, relative humidity of 45-55%, in a well ventilated room keeping 12: 12 h light: dark cycle. Standard rodent diet and water ad libitum were fed to them and animals were acclimatized for a week before the study. The guidelines of the CPCEA (Committee for the Purpose of Control of Experiments on Animals) (Reg. No.930/a/06/CPCEA) were strictly followed and obtained the approval of IAEC, SVCP, Tirupati.

Formulation of Saraswataristam (SA)

Saraswataristam (SA) was formulated as per the specifications of Ayurvedic Pharmacopoea following good management and laboratory practices. The quantity of ingredients used for the formulation is presented in table 1. The ingredients 1 to 7 were crushed well and boiled in water. The volume of the decoction was reduced to half the initial volume followed by the addition of the ingredients 8 to 10. Later, coarse powders of the remaining ingredients (11 to 22) were added to the cold filtered decoction. The vessel was kept tightly packed. The ingredients were fermented for one month (Nadakarni, 1993) to obtain the final product. It is then filtered, kept in tightly stoppered glass bottle and stored at 10ºC.

Characterization of Saraswataristam

The prepared Saraswataristam was evaluated for its physical characters like colour, odour, taste and pH. Physicochemical evaluation like total ash, water soluble ash, acid insoluble ash, sulphated ash, water, ether and alcohol soluble extractive value, loss on drying, and determination of foreign matter were carried out as per the WHO guidelines (Parihar *et al*., 2010; WHO, 1998). Preliminary phytochemical studies were carried out to determine the type of chemical constituents present in it (Harborne, 1973). SA was subjected to Fourier Transform Infra Red (FTIR) analysis to study the nature of functional groups. FTIR spectrum of formulation and powder of *Centella asiatica* were derived and compared for the presence of identical groups. In the study, tests for heavy metal content was carried out, which involved determination of lead, cadmium, mercury and arsenic using atomic absorption spectroscopy (AAS).

HPTLC fingerprinting

High Performance Thin Layer Chromatography (HPLTC) is employed in the identification and estimation of phytoconstituents present in the plant extract. The details pertaining to the class of active constituents present can also be identified (Wagner and Bladt, 1996). In this study, Saraswataristam was subjected to HPTLC analysis for confirming the presence of biomarker compound and its quantification. The major ingredient of SA is *Centella asiatica* belonging to the family Umbelliferae. The plant contains Vallarine, Asiaticoside, Hydrocotylin, Pectic acids, Phytosterols, Hersaponin, Bacogenin, Monnierin, Triterpenes and Tannins (Kokate *et al*., 1996). Therefore, asiaticoside is selected as biomarker in the present study. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS- 4 software was used. The solvents used were of HPLC grade obtained from MERCK Ltd, India and weighing was carried out on Precisa XB 12A digital balance.

Preparation of extract

The formulation (10 ml) was extracted using methanol (10 ml) at 50-55ºC temperatures for 10 min. The filtered extract was used for HPTLC fingerprinting. Weigh accurately about 0.05g of asiaticoside, dissolve in distilled water HPLC grade, make up to 10 ml so as to obtain a concentration of 5 mg/ml and, used for spotting.

30µl of methanol extract solution was applied as 11.6 mm band on a precoated aluminium TLC plate (20 x 10 cm) with silica gel 60 $F_{254,}$ using Linomat V applicator and a Hamilton syringe. Applied plate was developed in an overnight saturated twin trough chamber containing chloroform:glacial acetic acid: methanol: water (6:3.2: 1.2:8) as mobile phase. The plate was developed for a migration distance of 76.5 mm and further scanned under wavelengths, 220 nm using Deuterium lamp.

Determination of alcohol content in Saraswataristam

The preparation (25ml) was accurately measured, transferred to a distillation flask and, diluted with 150ml of distilled water. From this, more than 90 ml of distillate was taken into a 100ml volumetric flask. The relative density was determined by maintaining the temperature at 24-25ºC. The percentage of ethanol contained in the preparation was determined (Indian Pharmacopoeia, 1996).

Determination of microbial contaminants

The standard procedures followed for microbial growth determination (Kalaiselvan *et al*., 2010) are explained as follows.

Test for E. coli

The accurately measured formulation (10 ml) was made up to 100ml using lactose broth and incubated at 35-37ºC for 4 hrs. From this, 1ml of sample was taken and serial dilutions were performed with 9 ml of MacConkey broth of concentrations 100, 10 and 0.01 mg/ml. These were incubated at 37ºC for 18 h. 1ml of each was inoculated on

MacConkey agar media, and further incubated at 35-37ºC for 24 h. The plates were then observed for growth of *E. coli*.

Biochemical test

The colonies obtained on the MacConkey agar media were inoculated on 5 ml of peptone water and the culture was incubated at 35-37ºC for 24 h. From this, 3 ml of peptone culture was pipetted out into a test tube and equal volume of Kovac's reagent was added. Appearance of a pink ring is the indication of presence of *E. coli*. Parallel tests were conducted with pure stain.

Test for Staphylococcus aureus

Sample (10 ml) of was taken, made up to 100 ml with Nutrient Agar (NA) broth and incubated at 35-37ºC for 4h. 1ml of this sample was placed on Baird-Parker agar media F, and incubated at 35-37ºC for 48 h.

Biochemical test

DNAse test or coagulase were carried on the colonies obtained on Baird-parker agar media. In this test, *Staphylococcus aureus* culture and plasma (5-10 times diluted sample) were added and incubated for 2-4 h. The formation of coagulam indicates the presence of *Staphylococcus aureus.*

Test for aerobic bacteria, yeasts and mould

Accurately weighed sample (10 ml) made up to 100 ml with NA broth was incubated separately at two different temperatures i.e. at 35-37ºC for 4 h and 40-42ºC for 4 h. 1 ml of sample taken from the former was placed on casein-soyabean digest agar and further incubated at 35-37ºC for 4 h while, Streptomycin was added to the latter and incubated at 40-42ºC. From this, 1 ml each was placed on corn meal agar media and Sabouraud's dextrose agar (SDA) media and both were incubated at 35-37ºC for 4 days.

Acute oral toxicity studies

The formulation (3 ml) was administered to overnight fasted albino rats (100-120 g). Rats were not given food after administration for 3-4 h and, observed for signs for toxicity. The body weight of rats before and after administration was noted and changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and salivation, diarrhea, lethargy, sleep and coma were noted. Histopathological studies were performed on the tissues of stomach, kidney and liver to examine the toxicity of the formulation.

Anti-Epileptic activity of Saraswataristam

Maximal electro shock (MES) method in rats – Healthy albino wistar rats of either sex weighing 100-120 g were housed in acrylic cages and, provided with standard diet and water *ad libitum*. The animals were divided into four groups (n=6). Group I served as control receiving 1 ml of 5% CMC p.o. and group II served as standard group receiving phenytoin 20 mg/kg p.o. Group III and group IV animals were administered with two different doses of Saraswataristam (0.5 ml and 1.0 ml respectively). All groups were subjected to gastric incubation for 14 days and, seizures were induced to the groups by an Electro convulsiometer on the 15th day. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to its application so as to increase contact and reduce fatalities. The duration of various phases of epilepsy were noted. The percentage protection was estimated by observing the number of animals showing abolition of Hind Limb Tonic Extension (HLTE) or extension not greater than 90º (Ghosh, 2005).

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was carried out to understand the significance of differences among the treated group. This was followed by followed by Dunnet's *t*-test

RESULTS

Saraswataristam was formulated as per the Ayurvedic Pharmacopoeial specifications, characterized and subjected to standardization procedures. The physical characterization showed that it is highly soluble in ethanol, methanol and water and insoluble in pet ether, chloroform and benzene. It has sweet taste and have odour sane as that of alcohol. Saraswataristam was dark brown in colour and is acidic in nature with a pH of 3.88.

The results of physical characterization of Saraswatarishtam are presented in table 2 and, all values are observed within the limits. The alcohol content is obtained as 9.08% v/v. Preliminary phytochemical screening revealed the presence of secondary metabolites like alkaloids, flavonoids, steroids, flavonoids, tannins and saponins.

FT-IR spectrum of sample showed a number of well defined peaks in 400-4000 cm^{-1} region along with peaks in higher region. The broad peaks at 3446 cm^{-1} and 3442 cm^{-1} is due to OH in formulation and centella powder revealing the presence of similar functional groups in both samples. The broadening of peak in formulation indicates the presence of multiple functional groups related to other ingredients in formulation along with those found in centella alone. The spectra were shown in fig. 1.

AAS results revealed the presence of heavy metals in the formulation and their concentration is presented in table 3. HPTLC analysis showed a peak of asiaticoside in the

Table 1: Composition of Saraswataristam for 500 ml

Ingredients	Biological source	Parts used	Quantity used (SA) g*
Brahmi	*Centella asiatica*	Whole plant	1000
Shatavari	*Asparagus recemosus*	Roots	225
Vidari	*Ipomea digitata*	Tubers	225
Haritaki	*Terminalia chebula*	Whole plant	225
Usira	*Vetiver zizinaloides*	Roots	225
Ardraka	*Gingiber zerumbet*	Rhizomes	225
Sata puspa	*Anithum graveolens*	Seeds	225
Madhu	Honey	---	450
Sakara	Sugar	---	1100
Dhataki	*Woodfordia floribunda*	Flowers	300
Arenuka	*Piper aurentiacum*	Seeds	30
Trivrt	*Turpitha*	Roots	30
Lavanga	*Eugenia caryophyllus*	Flower buds	12
Pippali	*Piper longum*	Seeds	13
Vacha	Sweet flag	Plant	12
Kustha	*Costus*	Roots	14
Asvagandha	*Withania somnifera*	Root	15
Guduchi	*Tinospora cardifolia*	Stem and root	12
Ela	*Elletteria cardamom*	Fruits	14
Lavanga tvak	*Cinnamomum zeylanicum*	Bark	12
Vidanga	*Emblica officinallis*	Fruits	10
Jala	Water	---	12.5

*****All quantities taken in grams and some of them in milliliters whichever relevant.

chromatogram as shown in fig. 2. It also demonstrated some other peaks that indicate the presence of other active principles apart from asiaticoside.

The results of microbiological studies are shown in fig. 3. The studies showed negative results indicating SA free from growth of *E. coli*, *Staphylococcus aureu*s, yeast and mould. This is one of the most important and primary requirement for any ayurvedic formulation.

The results of toxicological studies are shown in table 4. The rats showed no signs of toxicity as their body weights remained constant before and after administration of SA. In the study, sleep was noticed in all the rats for sometime soon after the administration of SA indicating the formation of alcohol in the product due to which sleep occurred. The findings of histopathological studies are shown fig. 4. The study revealed that the structure of cells in each tissue was normal before and after administration of formulation proving that SA can be used safely. Toxicity studies showed no renal, hepatic and gastro intestinal toxicity of SA.

Saraswataristam showed signs of dose dependent significant ($P<0.001$) reduction in various episodes of epileptic seizures in comparison with standard phenytoin as given in table 5. There was also a significant reduction in the time required for the writhing reflex (recovery) in the aristam treated groups.

Table 2: Physical characterization of Saraswataristam

Tests	SA (% w/w)
a) Foreign matter	-
b) Sand and Silica	-
Ash values	
a) Total ash content	1.1
b) Acid insoluble ash	0.54
c) Water insoluble ash	0.4
d) Sulphated ash value	0.35
Extractive values	
a) Alcohol soluble extractive	30.2
b) Water soluble extractive	28.5
c) Ether soluble extractive	15.8

Table 3: Concentration of trace elements

Element	Concentration in SA (ppm)
Lead (Pb)	3.1
Mercury (Hg)	0.047
Cadmium (Cd)	0.17
Arsenic (As)	0.46

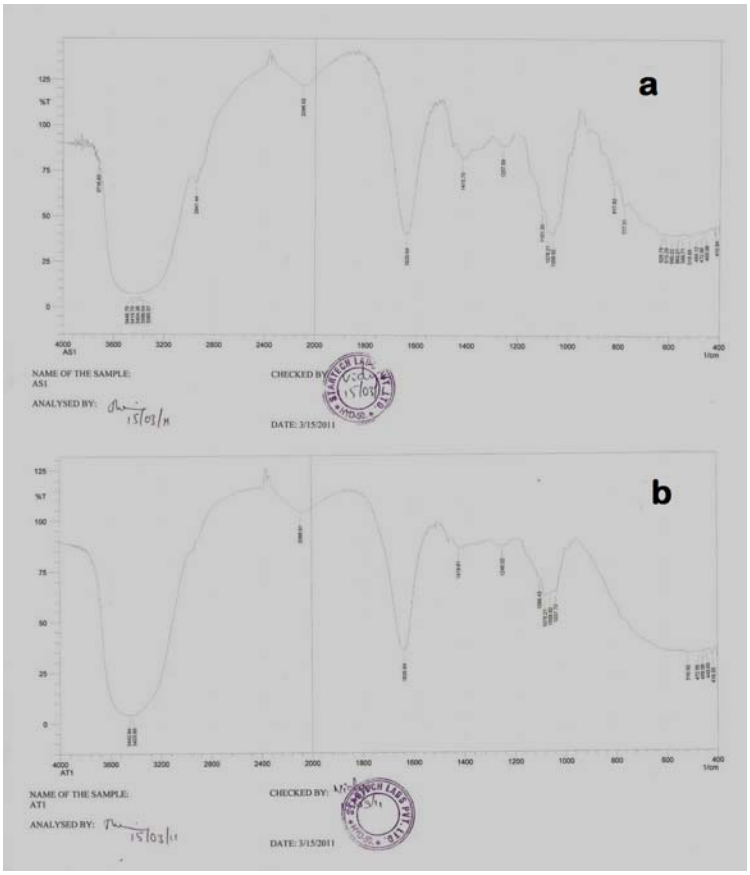

Fig. 1: FTIR Spectra of (a) Saraswataristam and (b) Centella powder

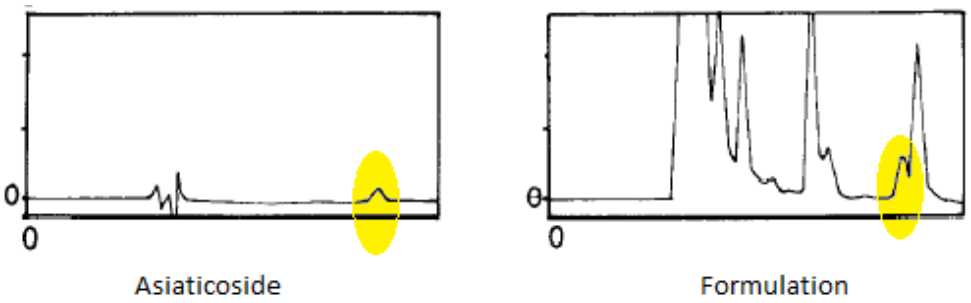

Fig. 2: HPTLC of asiaticoside and saraswataristam

Fig. 3: Antimicrobial activity of Saraswataristam on (a) *E. coli*, (b) *Staphylococcus aureu*s and (c) yeast and mould

Table 4: Results of Acute oral toxicity studies

Animal No.	Dose/kg b.w	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of Study
		Before Test	After Test			
1	3 ml	100 g	102 g	No signs of Toxicity	Nil	14 days
2	3 ml	110 g	110 g	No signs of Toxicity	Nil	14 days
3	3 ml	115 g	114 g	No signs of Toxicity	Nil	14 days
4	3 ml	106 g	105 g	No signs of Toxicity	Nil	14 days
5	3 ml	120 g	120 g	No signs of Toxicity	Nil	14 days
6	3 ml	118 g	119 g	No signs of Toxicity	Nil	14 days

Table 5: Results of Anti-Epileptic activity of Saraswataristam

Group	Group Names	Time (Sec)				
		Flexion	Extension	Clonus	Stupor	Recovery
I	Control	5±0.48	12.1±0.10	12.3±1.42	5.84±1.01	179
II	Phenytoin	3.1±0.48***	0	7.4±1.68***	1.27±0.64***	175.1
III	SA (0.5 ml)	2.92±0.32***	1.43±0.32***	5.32±0.32***	25±1.76***	135.73
IV	SA (1.0 ml)	3.29±0.26***	1.06±0.19***	5.17±0.1***	24±1.86***	131.54

n= 6, values are expressed as mean ± SEM, *** P< 0.001 when compared with control

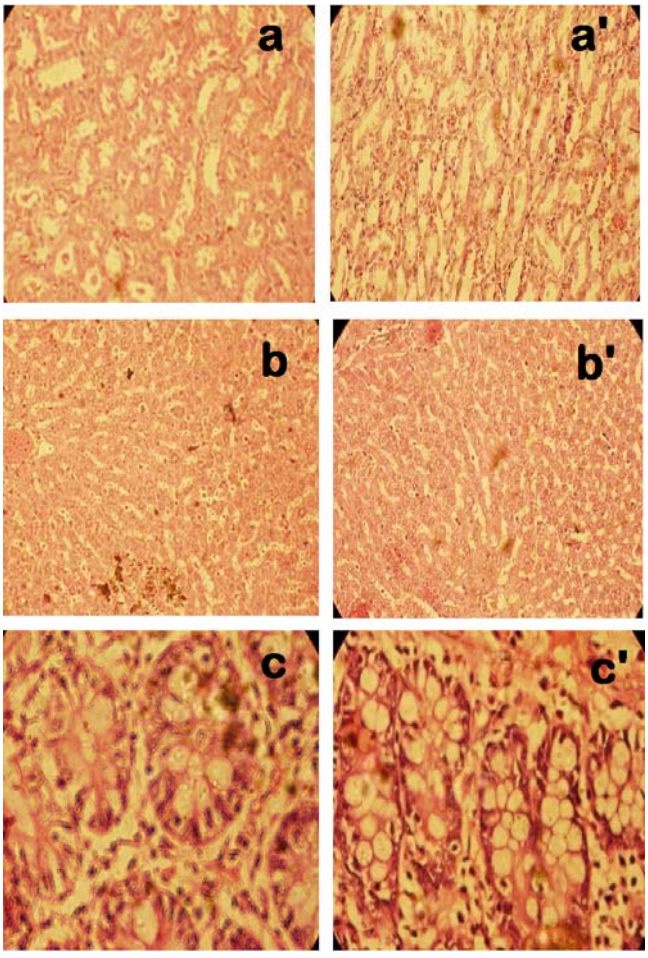

Fig. 4: Histopathology of
(a) Normal Kidney (a´) Kidney treated with Saraswataristam
(b) Normal Liver (b´) Liver treated with Saraswataristam
(c) Normal Stomach (c´) Stomach treated with Saraswataristam

DISCUSSION

In the present investigation Saraswataristam, an Ayurvedic formulation, had been prepared. The quality of formulated arista had been accessed for physical, physico-chemical, chemical and biological characteristics by intervention with modern analytical methods like HPTLC, FTIR and AAS. The findings of the study indicated that all parameters were within limits prescribed by Ayurvedic Pharmacopoeia. The pharmacological evaluation of SA had also been performed and the results exhibited anti-epileptic activity comparable with that of the standard drug, Phenytoin. The results obtained may be used to lay down a set of new Pharmacopoeial standards for the formulation of Saraswataristam to achieve optimal efficacy of the medicine. Further, clinical assessment is to be carried out to completely mark the product as safe and effective.

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