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STANDARDIZATION OF POLYHERBAL AYURVEDIC FORMULATION: CHANDANASAVA

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ABSTRACT

The present study deals with standardization of chandanasava, known to be effective in karsya (malnutrition), sukrameha (presence of semen in urine), mutrakrcchra (painful micturation), hrdroga (heart diseases), balaksaya (astringent), agnimandya (loss of appetite). Formulation has been standardized by modern scientific quality control procedures for the finished product. Standardization of chandanasava was achieved by organoleptic study, physic-chemical analysis, thin layer chromatography, high performance thin layer chromatography (HPTLC). The obtained values of physical and chemical parameters can be adopted to lay down new pharmacopoeial standards for analysis of chandanasava to check batch to batch variation. Quantitative evaluation of gallic acid and apigenin in chandanasava by HPTLC had shown 25.12 µg/ml and 0.4 µg/ml respectively.

KEY WORDS: Chandanasava, Standardisation, HPTLC, *Santalum album*.

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INTRODUCTION

Chandanasava is one of the ancient, commonly used Ayurvedic formulations. The herbal formulation is made up of *Santalum album* L. and other 24 plant ingredients (**Table 1**). Chandanasava is prescribed for the treatment of karsya (malnutrition), sukrameha (presence of semen in urine), mutrakrcchra (painful micturation), hrdroga (heart-diseases), balaksaya (astringent), agnimandya (loss of appetite)[1].

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Botanicals constitute of major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of traditional medicines in the healthcare[2,3]. The plant species mentioned in the ancient texts of Ayurveda and other Indian systems of medicine may be explored with the modern scientific approaches for better leads in the healthcare. Standardization of herbal formulations is essential in order to assess the quality of drugs[4-6].

The main requirement in polyherbal formulation is the presence of each ingredient has to be established. The microscopic characters of each ingredient are very difficult to identify and also some time these are overlapping with the character of other ingredient[7]. Standardized Ayurvedic formulations of uniform quality are essential for beneficial therapeutic use. Quality assurance of herbal medicine is an important factor and basic requirement for herbal drug industry and other drug development organization. Due to lack of standards and quality control methods, there are batch to batch variations in the same formulation as well as variation amongst the same formulation procured from different sources[8].

Standardized Ayurvedic formulations of uniform quality are essential for beneficial therapeutic use. Asavas and aristas are medicinal preparations made by adding the drugs, either in powder form or in the form of decoction (Kasaya), in a solution of sugar or jaggery, as the case may be, for a specified period of time. Plant material it undergoes process of fermentation generating alcohol, thus facilitating the extraction of the active principle present in the drugs. Self generated alcohol

also serves as a preservative. Preparations made using kasaya are called aristas and the other without making decoction are called as asavas[1]. In order to develop standards for the asavas and aristas, the study was undertaken for Chandanasava .

MATERIALS AND METHODS

Authentic sample of Chandanasava formulation was procured from local market of Chandigarh, India. Standards gallic acid and apigenin were used from standard compound library, natural product Lab, NIPER. Chandanasava was subjected for analysis of the parameters such as organoleptic study, total solid content, pH, alcohol soluble extractives and specific gravity as per Ayurvedic formulary of India and also for TLC finger printing and HPTLC quantification. The herbal formulation is made up of 24 plant ingredients and *Santalum album* is the important component of formulation[1]. Active phytochemical constituents like glycosides, flavonoids, alkaloids, acids, gums, tannins were identified through qualitative chemical analysis (**Table 3**). Chandanasava was sequentially extracted with different solvents starting from petroleum ether, dichloromethane (DCM) and ethyl acetate for thin layer chromatography (TLC) study. Quantification study was done for gallic acid and apigenin standards (**Figure 1**) in ethyl acetate extract. The standard solution of gallic acid and apigenin were used for the generation of calibration curves. The solution of ethyl acetate extract (5 mg/ml) for gallic acid and (10 mg/ml) for apigenin were prepared in methanol and 5 µl was applied to the TLC plate in triplicate along with standard. The chromatographic chamber was equilibrated with the developing mobile phase toluene: ethyl acetate: acetic acid: water (3:3:0.8:0.2, *v/v*) for 10 min. The plates were developed and air dried. The plates were derivatised with Natural product: Polyethylene glycol (NP: PEG) reagent. The spots were visualised at 366 nm and the chromatogram was scanned with spectrodensitometer. The calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration was plotted and the regression equation was computed.

RESULTS

Physicochemical parameters

As the part of standardization procedure formulation was tested for relevant physicochemical parameters. pH and non-reducing sugar content were found to be in range. Alcohol soluble extractives, total solid content and specific gravity were found out of range specified in Ayurvedic formulary of India **(Table 1)**.

Fingerprinting of extracts of chandanasava

Fingerprinting was done for petroleum ether, dichloromethane, ethyl acetate extracts. A solution of 10 mg/ml of each of the extract was prepared in respective solvent. TLC plates of 10x3 cm were used. Various solvent systems were tried and best was selected for final development. The plates were developed up to 80% of the length of plate. Plates were viewed at 254 nm, 366 nm and 560 nm (**Figure 2; Table 2**). The occurrence of spots at same respective locations in TLC obtained for all batches confirmed the batch-to-batch consistency.

Quantification of gallic acid and apigenin

The calibration curve of gallic acid (1 mg/ml) and apigenin (0.1 mg/ml) was plotted and the regression equation were computed i.e. $y = 3225.54x + 2125.35$(r^2 0.9889) and $y = 4845.19 x - 992.27$(r^2 0.9965) respectively.

Quantification of gallic acid and apigenin in ethyl acetate extract were done in Chandanasava by integrating the peak area employing regression equation and were found to be 8.10 µg/ml (**Figure 3**) and 0.4 µg/ml (**Figure 4**) respectively.

DISCUSSION

Ayurvedic formulation, Chandanasava has been standardized by modern scientific quality control measures. The results obtained from HPTLC fingerprinting could be used to analyse ayurvedic formulation to check quality and batch-to-batch variation. Generated analytical method and parameters can be used to analyse the Ayurvedic formulation. Physicochemical parameters like high sugar content in the analysed chandanasava indicates that, during manufacturing process, more sugar content was added to facilitate fermentation purpose and to generate more alcohol. High solid content and specific gravity reflects the presence of more fine content in the fermentation content.

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Table 1: Physicochemical parameters for Chandanasava

Sr No	Physicochemical Tests	Standard Values	Observed Values
1	Description	Fragrant clear liquid with bitter taste	Fragrant brownish black liquid with bitter taste
2	Total solids	5 to 10 % (w/v)	10.656 % (w/v)
3	Specific gravity	1.01 to 1.02	1.0456
4	Sugar Reducing Non-reducing	 4 to 7 % (w/v) NMT 1% (w/v)	 3.88 % (w/v) 0.98 % (w/v)
5	pH	3.5 to 5.0	3.78
6	Alcohol content	5 to 7 % (v/v)	8.55 % (v/v)
7	Dose	15 to 30 ml	-

Table 2: List of solvent systems used for fingerprinting of extracts of Chandanasava

Sr No	Extracts	Solvent system	Derivatising agent
1	Petroleum ether	Pet ether: ethyl acetate (9.5:0.5)	Anisaldehyde-H2SO4 reagent
2	Dichloromethane	Pet ether: ethyl acetate (9:1)	Anisaldehyde-H2SO4 reagent
3	Ethyl acetate	Toluene: ethyl acetate: acetic acid (5:4:1)	NP-PEG reagent

Table 3: Phytochemical constituents of Chandanasava

Constituents	Observations
Alkaloids	+ve
Tannins	+ve
Carbohydrates	+ve
Sugars	+ve
Terpenes	+ve
Sterols	+ve
Saponins	+ve

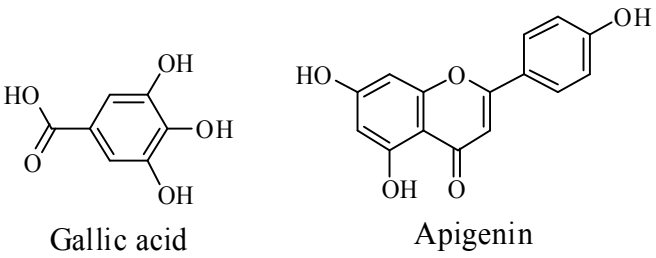

Figure 1: Chemical structures of standard compounds

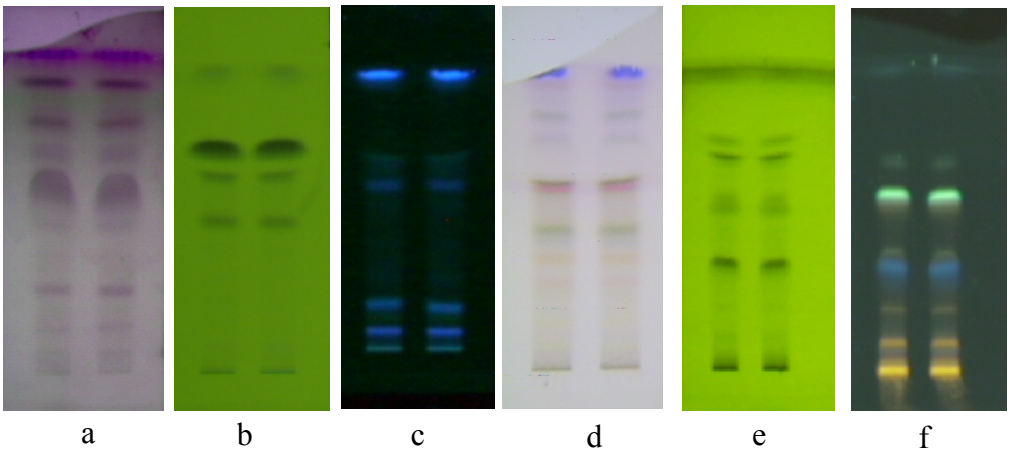

Figure 2: HPTLC fingerprints of Chandanasava extracts

a- Petroleum ether extract at 560 nm (Derivatising agent: Anisaldehyde-H_2SO_4 reagent); **b**- DCM ext. at 254 nm; **c**- DCM ext. at 366 nm; **d**- DCM extract at 560 nm (Derivatising agent: Anisaldehyde-H_2SO_4 reagent); **e**- Ethyl acetate ext. at 254 nm; **f** - Ethyl acetate ext. at 366 nm (Derivatising agent: NP-PEG reagent).

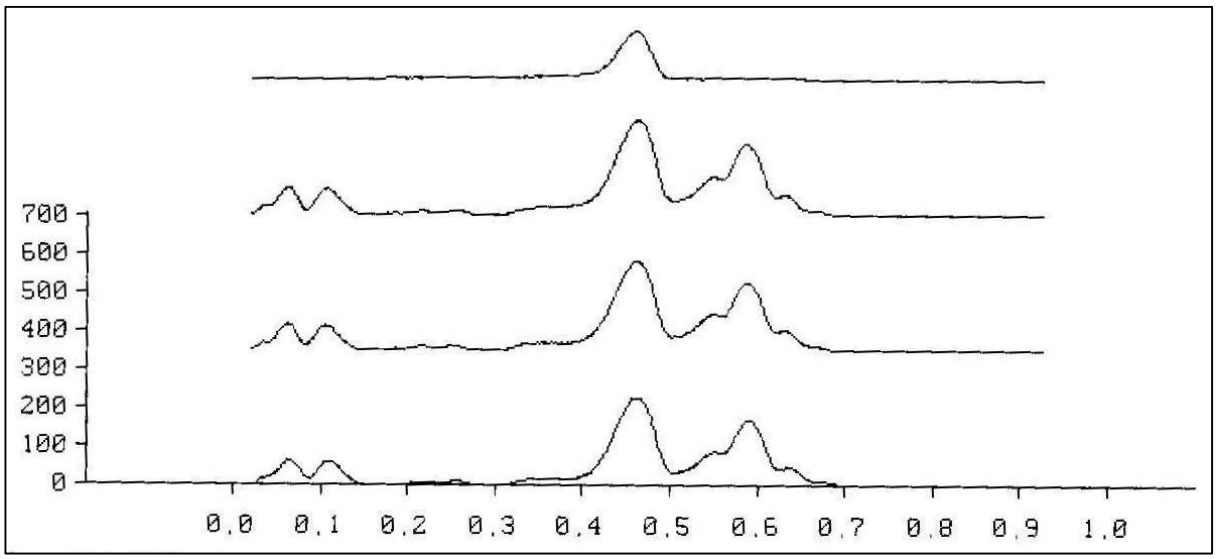

Figure 3: Integrated calibration chromatograms and HPTLC fingerprint of ethyl acetate extract with gallic acid standard

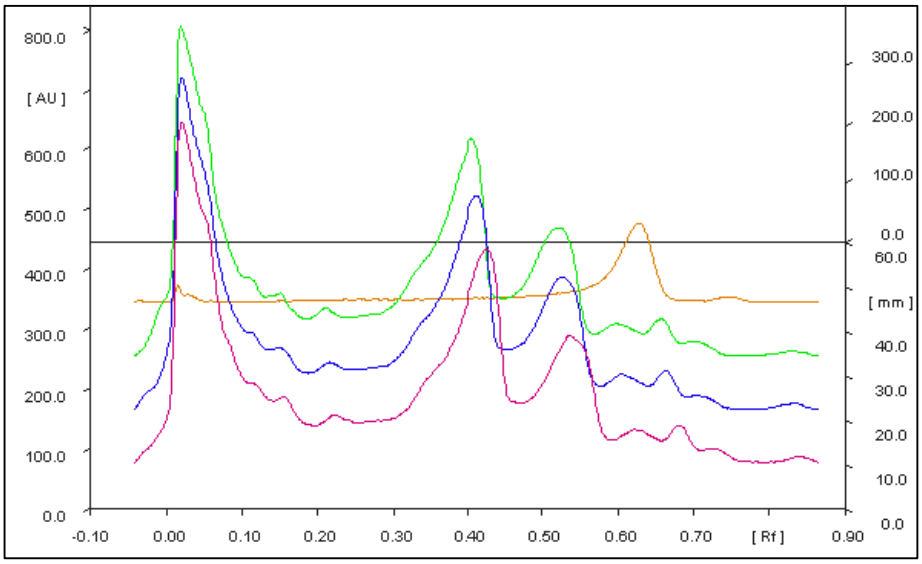

Figure 4: Integrated calibration chromatograms and HPTLC fingerprint of ethyl acetate extract with apigenin standard

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