



Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Quality Audit and Anti-inflammatory Activity of Marketed Formulations of Ashokarishta Syrup and Chandraprabha Vati

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ARTICLE INFO

Article history:

Received: 09 July, 2022

Revised: 23 August, 2022

Accepted: 03 September, 2022

Published: 30 September, 2022

Keywords:

Ashokarishta syrup,
Anti-inflammatory,
Chandraprabha vati,
Herbal formulations,
Standardization, Quality audit.

DOI:

10.25004/IJPSDR.2022.140513

ABSTRACT

Herbal formulations play an important role in medicines; however, their suboptimal standardization, in terms of identity, purity, quality, efficacy and safety, has questioned their efficiency in treating various disorders. The present study was conducted to perform a quality audit of two herbal formulations, i.e., ashokarishta syrup and chandraprabha vati, of three different marketed brands. The quality auditing procedures included analysis of moisture content, ash value, pH, presence of heavy metals, pesticide residue, radioactive substances and microbial contamination. The formulations were pharmacologically evaluated for anti-inflammatory activity using the rat paw edema model. All the formulations passed the microbial contamination test. None of the heavy metals was detected with higher than permissible limits in any formulations. Moreover, all the formulations were found free from pesticides and radioactive substances. In addition, the formulations also possessed anti-inflammatory activity; however, their activity varied among brands. The present study concluded that all the formulations tested were successfully quality audited. This study can be used as a standardizing tool for the future perspective.

INTRODUCTION

Herbal plants have been used as medicines for ages. However, it has taken thousands of years to accumulate knowledge related to drugs. Due to man's perpetual quest, we today have natural and effective means to ensure better health care.^[1,2] Due to advancements and the interest of scientists in exploring the therapeutic potential of plant products, herbal formulations have gained more importance.^[3,4] The major positive point associated with such formulations is their high efficacy and minimal adverse effects. However, the variation associated with the growth and development of plants, concerning their geographical, climatic, harvesting, irrigation, etc., affect the quality, efficacy and safety related to the plants. Therefore, there is a need to standardize the plants/herbs

and the herbal formulations prepared by using their combinations. Standardization of the plant material involves a comprehensive quality audit of the plant/herb, from its collection to its pharmacological evaluation including pharmacognostic and phytochemical analysis (qualitative and quantitative). It also takes care of the microbial load, toxicity, and biological activity.^[5,6]

Ashokarishta, as the name suggests, is a fermented decoction (Arishta) of the main ingredient (herb) asoka (*Saraca asoca* de wilde) in boiling water.^[7] The syrup is used to treat various diseases such as fever, heavy periods, painful menstruation, bleeding disorders like bleeding hemorrhoids, nasal bleeding, inflammation, lack of taste and indigestion.^[8-10] In addition, chandraprabha vati is one of the effective and popular ayurvedic tablets consisting

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table 1: Physicochemical properties of marketed formulations of ashokarishta syrup and chandraprabha vati.

Physicochemical parameters	Ashokarishta syrup			Chandraprabha vati		
	AS1	AS2	AS3	CV1	CV2	CV3
Specific gravity (1.02 to 1.12% W/V)	Pass	Pass	Pass	-	-	-
pH (3.5 to 4.4)	Pass	Pass	Fail	-	-	-
Total solids (Not less than 11.0% W/V)	Fail	Fail	Fail	-	-	-
Alcohol content (5 to 10% V/V)	Pass	Pass	Pass	-	-	-
Total ash value (20 to 25% W/W)	-	-	-	Pass	Pass	Fail
Acid insoluble ash (Not more than 5% W/W)	-	-	-	Fail	Fail	Fail
Assay for iron (4 to 6% W/W)	-	-	-	Pass	Pass	Fail

Table 2: The alcohol content of Ashokarishta syrup.

Formulation	% Ethanol (W/W)	Result
AS1	8.30	Pass
AS2	7.98	Pass
AS3	7.12	Pass

Table 3: Microbial contamination of the formulations of ashokarishta syrup and chandraprabha vati.

Formulation	Bacterial count	Fungal count	Result
AS1	2×10^1	2×10^1	Pass
AS2	4×10^1	2×10^1	Pass
AS3	4×10^1	1×10^2	Pass
CV1	4×10^4	1×10^2	Pass
CV2	4×10^4	1×10^2	Pass
CV3	2×10^4	2×10^2	Pass

Table 4: Heavy metals content observed in ashokarishta syrup and chandraprabha vati formulations.

Heavy metal	Formulation					
	AS1	AS2	AS3	CV1	CV2	CV3
Lead	Nil	Nil	Nil	0.164	0.0105	0.0359
Arsenic	Nil	Nil	Nil	0.022	0.0016	0.9874
Mercury	0.0012	0.0036	0.0067	Nil	0.0001	0.0135
Cadmium	Nil	Nil	Nil	0.189	0.0294	1.7050

Permissible limits for heavy metals: Lead (100 ppm), Arsenic (3 ppm), Mercury (1 ppm), Cadmium (0.3 ppm).

of 37 ingredients and is prescribed for many diseases like cold, cough, diabetes, cancer, urinary tract ailments, rhinitis, bronchitis, asthma, allergic skin conditions, piles, liver, spleen diseases, anemia and fistula.^[11] It is also recommended for dental problems, eye infections and gynecological problems. This medicine is believed to bring glow to the face as well.^[12,13] Quality control of plant and their materials for efficacy and safety of the herbal products is of high importance.^[14-19]

The present study was conducted to standardize marketed preparations of Ashokarishta syrup and Chandraprabha vati concerning moisture content, ash value, pH, specific gravity, alcohol content, heavy metals, microbial

contamination, pesticide residues, radioactive substances and anti-inflammatory activity.

MATERIALS AND METHODS

Collection of Samples

Marketed herbal formulations of Ashokarishta syrup and Chandraprabha vati were procured from three different brands and stored in clean and dry containers.

Standardization of Herbal Formulation

Quantification of Moisture Content

The moisture content of each sample was determined by the gravimetric method. This process was carried out in specific conditions to quantify the moisture or volatile principle. Accurately weighed 2–5 g sample of each brand (of herbal formulation) was spread in a previously dried crucible and placed in an oven at 105°C. The analysis was carried out till the consecutive value doesn't exceed 5 mg.^[8] The %loss on drying (LoD) was calculated using the following formula:

$$\% \text{ LoD} = \frac{(\text{Initial weight of sample} - \text{Final weight of sample})}{\text{Initial weight of sample}} \times 100$$

Quantification of Total Ash

Total ash determination is one of the important parameters of standardization of herbal formulation. Ash value is determined by taking a 2–5 g sample in the crucible by using a muffle furnace under standard temperature conditions. The preparation was then cooled in desiccators and weighed until a constant weight was obtained.^[9] The %total ash was calculated using the following formula:

$$\% \text{ Total ash} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

Quantification of Acid-insoluble Ash Value

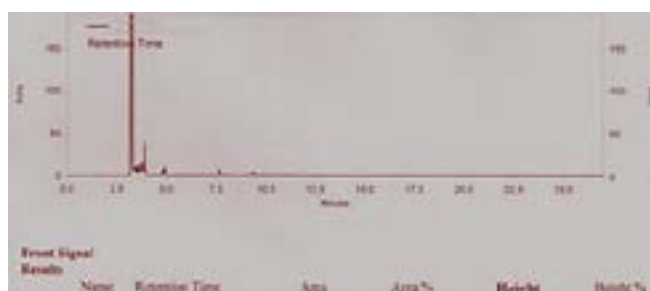
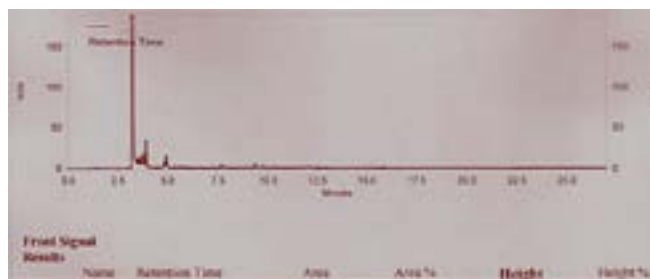
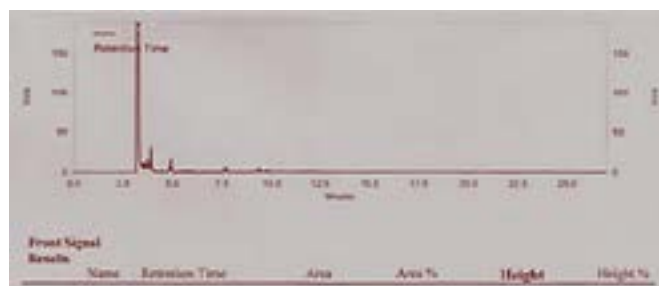
For quantification of acid-insoluble ash, total ash was transferred to a crucible and added 25 mL HCl, covered the crucible, and boiled for 5 minutes. The solution was filtered with the help of ash-less filter paper. We kept the ash-less filter paper in the crucible and placed it in a muffle furnace for incineration using standard parameters. The crucible



Table 5: Anti-inflammatory potential of ashokarishta syrup and chandraprabha vati in rat paw oedema model.

Time (hours)	Mean paw volume								
	Control	-ve control	Std.	AS1	AS2	AS3	CV1	CV2	CV3
0	0.79 ± 0.03	0.77 ± 0.03	0.72 ± 0.02	0.80 ± 0.01	0.82 ± 0.02	0.75 ± 0.01	0.82 ± 0.02	0.84 ± 0.02	0.82 ± 0.02
0.5	0.81 ± 0.01	0.93 ± 0.03	0.79 ± 0.04	0.82 ± 0.03	0.85 ± 0.02	0.75 ± 0.02	0.86 ± 0.03	0.87 ± 0.03	0.86 ± 0.01
1	0.78 ± 0.03	1.06 ± 0.02	0.83 ± 0.02	0.93 ± 0.04	0.90 ± 0.03	0.80 ± 0.02	0.91 ± 0.02	0.92 ± 0.01	0.91 ± 0.03
1.5	0.76 ± 0.01	1.23 ± 0.01	0.94 ± 0.02	1.02 ± 0.02	1.02 ± 0.01	0.99 ± 0.01	1.01 ± 0.01	1.03 ± 0.02	0.95 ± 0.02
2	0.79 ± 0.02	1.30 ± 0.02	0.97 ± 0.03	1.06 ± 0.03	1.04 ± 0.02	1.03 ± 0.03	1.03 ± 0.03	1.05 ± 0.03	1.03 ± 0.03
3	0.80 ± 0.02	1.34 ± 0.02	0.98 ± 0.03	1.09 ± 0.02	1.06 ± 0.01	1.04 ± 0.02	1.06 ± 0.02	1.07 ± 0.04	1.09 ± 0.02
6	0.78 ± 0.03	1.25 ± 0.03	0.91 ± 0.04	1.02 ± 0.02	1.02 ± 0.03	1.03 ± 0.03	1.02 ± 0.02	1.03 ± 0.01	1.01 ± 0.01
24	0.77 ± 0.01	0.97 ± 0.03	0.78 ± 0.03	0.96 ± 0.03	0.94 ± 0.01	0.98 ± 0.02	0.88 ± 0.03	0.86 ± 0.03	0.84 ± 0.02

*Std.: Diclofenac sodium (+ve control).

**Fig. 1:** Chromatogram of blank sample.**Fig. 4:** Chromatogram of formulation AS2 showing absence of pesticide residues.**Fig. 2:** Chromatogram of standard injection showing peaks for pesticides (heptachlor, aldrin, endosulphan I, endosulphan II and 4,4-DDT).**Fig. 5:** Chromatogram of formulation AS3 showing absence of pesticide residues.**Fig. 3:** Chromatogram of formulation AS1 showing absence of pesticide residues.

was placed in a desiccator and allowed to cool. After some time, the crucible was removed from the desiccator and weighed.^[12] The %acid-insoluble ash was calculated using the following formula:

$$\% \text{ Acid insoluble ash} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

pH Determination

The pH of the 5% aqueous solution of each sample was determined using a pH meter (Model-510, Merck) under standard conditions. The pH meter was first calibrated with buffer solution (pH 7.0) and the pH of each marketed formulation was directly read on the pH meter by dipping the electrode in the aqueous solution of the formulation.^[8,9]

Quantification of Specific Gravity (Relative density)

The density bottle (RD bottle) was first cleaned with purified water and dried in an oven, and the empty dried bottle with a stopper was weighed. The bottle was then



Fig. 6: Chromatogram of formulation CV1 showing absence of pesticide residues.



Fig. 7: Chromatogram of formulation CV2 showing absence of pesticide residues.

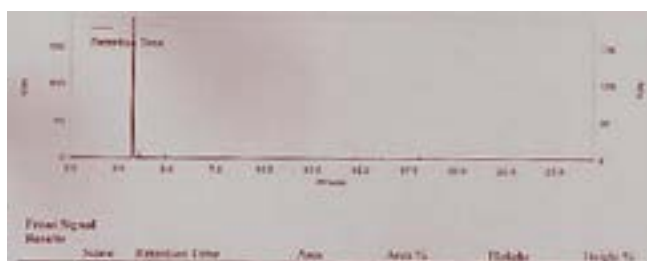


Fig. 8: Chromatogram of formulation CV3 showing absence of pesticide residues.

filled with purified water, placed stopper and weighed. The water was then transferred to a measuring cylinder and volume was noted. An equal volume of the formulation was filled in the same bottle, a stopper was placed and the bottle was weighed. All brands of formulation were treated the same and specific gravity was determined using the following formula.^[20]

$$\text{Specific gravity} = \frac{\text{Density of substance}}{\text{Density of water at 4}^\circ\text{C}}$$

Estimation of Total Solid Content

The formulation of each brand was accurately weighed (2–5 g), spread in a previously dried crucible, and placed in an oven at 105°C. The amount remained in crucible is indicated as total solid content.^[8] The percentage of solid content for each brand sample was calculated using following formula:

$$\% \text{ Solid content} = 100 - \% \text{ LOD}$$

Quantification of Alcohol Content

Alcohol determination was performed using standard and internal controls. The volume of the samples of 1-mL was

pipette into measuring flasks and diluted with redistilled water to a total volume of 10 mL. Next 5 mL of diluted samples were placed into 65 mL vials. After analyzing ethanol content by the external and internal standard methods, 40 µL of ethanol solution (500 mg/mL) was added to each vial and after stabilization, the samples were analyzed by the standard addition method.^[21,22] The following formula was employed for the determination of alcohol content:

A_T = Area of the sample (Test)

A_S = Area of Std.

W_S = Wt. of Std.

W_T = Wt. of the sample (Test)

D_S = Dilution factor of Std.

D_T = Dilution factor of sample (Test)

P_S = Standard potency

Estimation of Total Microbial Contamination

1-g of the formulation was dissolved in a buffered solution of NaCl peptone (pH-7) in a volumetric flask and volume was made up. Applied 0.1-mL of each formulation brand sample in duplicate on the casein soybean digest agar media filled in the sterilized petri plates. Control was also prepared without applying the sample in duplicate. The total number of bacteria was counted in petri plates incubated at standard conditions using a colony counter.^[23,24] The total number of cells was calculated using the following formula:

$$\text{Number of cell/mL} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{Dilution}}$$

Determination of Heavy Metals

Preparation of Sample

Formulation 0.5 g of each brand was placed in a different digestion flask. 3 mL of nitric acid, 1-mL of hydrogen peroxide and 1-mL of hydrochloric acid were added to the flask and sealed.^[25-27] All the digestion flasks were placed in the oven and the reaction was carried out in 3 steps according to the following programmed 5 minutes (80% power), 5 minutes (100% power) and 20 minutes (80% power). In the last phase, flasks were cooled in air, mixed with 4 mL of concentrated H₂SO₄ in each flask, and repeated the digestion programme one more time. Each flask was cooled in air and transferred the clear, colorless solution into of Erlenmeyer flask (50 mL). Rinsed the digestion flask two times (15 mL) with deionized water and transferred the rinsing to the respective volumetric flasks. Add 1.0 mL (10 g/L) mg nitrate solution and 1-mL solution of ammonium dihydrogen phosphate (100 g/L). Volume was made up (50 mL) with deionized water.

Preparation of Blank Solution

3 mL of nitric acid, 1-mL of hydrogen peroxide and 1-mL of hydrochloric acid were mixed in a digestion flask. Digestion was carried out similar to the sample solution.



Stock solution (100 µg/mL) was prepared for individual pure heavy metals. Further aliquots were prepared by using the stock solution of the different heavy metals. The absorbance of different concentrations of the different heavy metals was measured and the calibration curve was plotted between absorbance vs. concentrations. The test solution was injected and absorbance was measured. The metal concentration was calculated from the calibration curve.

Pesticidal Residue Analysis

In the present study, estimation of pesticide residues (endosulfan I and II, heptachlor, aldrin and 4,4'-dichlorodiphenyltrichloroethane (DDT)) was undertaken using a Gas chromatograph (GC) equipped with electron capture detector and flame thermionic detector. The GC oven temperature for the electron capture detector was programmed for an initial temperature of 170°C with a hold time of 13 minutes and then increased to 270°C at a rate of 3°C/min with a hold time of 20 minutes. Whereas for flame thermionic detector oven temperature was programmed for an initial temperature of 180°C with a hold time of 2 minutes, then increased to 270°C at a rate of 10°C/min with a hold time of 3 minutes and finally to 280°C at a rate of 5°C/min with a hold time of 5 minutes. The injection port temperature was kept at 280°C and the detector's temperature was kept at 310°C. The concentrations of target pesticide residues in formulation samples were quantified by comparing the peak area and retention time of the particular compound in sample extracts to that of the corresponding external standard of pesticide run under the same operating conditions separately.^[28,29]

Radioactive Analysis

Gamma-spectrometric determinations were carried out to detect the presence of radioactive substances such as ⁶⁰Co, ¹³⁷Cs and ²²⁶Ra in all the samples of marketed herbal formulations at the Board of Radiation and Isotope Technology (Mumbai) using a multichannel analyser system with high-purity germanium detectors (30% relative efficiency) in heavy shielding. The gamma lines used for radionuclide determination were 661.6 keV for ¹³⁷Cs, as well as 1173.2 and 1332.5 keV for ⁶⁰Co. ²²⁶Ra was determined both using the gamma line at 185 keV and by determination of the daughter nuclides ²¹⁴Bi and ²¹⁴Pb (609 and 351 keV). The calibration and efficiency of the system were carried out using a multi-gamma ray standard source (MGS-5, Canberra) of Marinelli beaker geometry. A library of radionuclides which contained the energy of the characteristic gamma emissions of each nuclide was analyzed and their corresponding emission probabilities were built from the data supplied in the software. Samples (1.0 g) were placed in Marinelli beaker sealed off and kept for one month. To determine the background distribution due to naturally occurring radionuclides in the environment around the detector,

an empty Marinelli beaker container was counted in the same manner as the samples. After the measurement and subtraction of the background, the activity concentrations were calculated.^[30]

Anti-inflammatory Activity

Carrageenan induces rat hind paw edema method was used to determine *in-vivo* anti-inflammatory potential. Wistar rats weighing between 150–200 g from the animal house of M.M. (deemed to be University), Mullana (1355/AC/10/CPCSEA) were used throughout the work.^[13] The animals were kept under standard conditions (temperature 22 ± 3°C, humidity- 55–65%) and with proper light and dark (12–12 hours) schedule. They had free access to water and were fed up with pellets and distilled water. The animals were randomly divided into groups of five rats each. Edema in the paw was induced by injecting 0.1-mL carrageenan solution (1% w/v) in sterile saline in the sub planar area of the left foot of rats. The standard drug, diclofenac sodium (1-mg/kg), and test formulations were suspended in 2% emulsion of acacia gum and administered p.o. with the help of an oral cannula half an hour before the injection of carrageenan. The volume of paw oedema was noted immediately after regular intervals of time by a digital plethysmometer (Model PTH-7070, medicad system).^[31-33] Mean increase in paw volume up to the tibiotarsal articulation was measured at intervals of 0, 0.5, 1, 1.5, 2, 3, 6, and 24 hours. The percentage protection against inflammation was calculated as follows: $(V_c - V_d)/V_c \times 100$, where V_c is the increase in paw volume in the absence of test formulations and V_d is the increase of paw volume after injection of test formulations. Data were expressed as (Mean ± SD, n = 5).

RESULTS AND DISCUSSION

Physicochemical Parameters

All the samples underwent observation of physicochemical properties. It was observed that the specific gravity and alcohol content of Ashokarishta syrup was within range whereas all three brands of Ashokarishta syrup failed in total solid contents (Table 1). In addition, ashokarishta syrup AS3 did not pass the pH range test. On the other hand, for chandraprabha vati, only CV1 and CV2 brands could pass the total ash content and iron assay, while none of the brands could pass the acid insoluble ash assay (Table 1). These results verified the quality of the formulations under study.

Estimation of Alcohol Content

The alcohol content was determined in all three samples of ashokarishta syrup using GC. It was observed that ethanol content was least in the AS2 brand while it was highest in the AS1 brand. However, all the brands of Ashokarishta syrup passed the ethanol content test (Table 2).

Detection of Microbial Contamination

Bacterial and fungal contamination (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella*) in all the marketed formulations of ashokarishta syrup and chandraprabha vati was detected, and it was observed that bacterial count was lesser than 10^5 while the fungal count was less than 10^3 in all the samples, which indicated the passing of these formulations of ashokarishta syrup and chandraprabha vati for microbial contamination examination (Table 3). These results confirmed the stability of these formulations of ashokarishta syrup and chandraprabha vati.

Estimation of Heavy Metals

It was observed that lead, arsenic and cadmium were not detected in all formulations of ashokarishta syrup while mercury was found in all brand formulations; however, it was under permissible limits. Hence, all the formulations passed the test for toxic heavy metals. For chandraprabha vati, lead, arsenic, cadmium and mercury were observed in all brands of chandraprabha vati under study except cadmium which was not detected in the CV1 brand. However, the detected amount of heavy metals in all the samples was found under permissible limits (Table 4).

Detection of Pesticide Residues

Endosulfan I and II were not detected in all studied samples of ashokarishta syrup and chandraprabha vati. Heptachlor, aldrin, and 4,4'-dichlorodiphenyltrichloroethane (DDT) were also not found in all the samples compared to the standard injection (Figs. 1-8). In standard injection, 5 peaks were observed in the chromatograms (Fig. 2). Fig. 1 presents a chromatogram of the blank sample. Peaks A, B, C, D, and E in Fig. 2 represent heptachlor, aldrin, endosulphan I, endosulphan II and DDT, respectively. These peaks showed the presence of these pesticide residues in the standard injection (Fig. 2).

Detection of Radioactive Substances

All the samples of ashokarishta syrup and chandraprabha vati were found free from radioactive substances (^{60}Co , ^{137}Cs and ^{226}Ra) as evaluated by the Board of Radiation and Isotope Technology (Mumbai).

Anti-inflammatory Activity

The anti-inflammatory potential of ashokarishta syrup and chandraprabha vati of different brands were investigated using the rat hind paw edema method, as depicted in Table 5. The result of the present investigation shows that both ashokarishta syrup AS2 and chandraprabha vati CV3 represent maximum anti-inflammatory activity. All the results were compared using diclofenac sodium as a standard drug.

CONCLUSION

Ashokarishta syrup and chandraprabha vati have been used to cure several ailments and are currently manufactured by more than hundreds of companies. In our study, we selected three brands for each herbal formulation. The results conclude that all the formulations were safe as no radioactive agents were found in them. Moreover, the formulations contained heavy metals but were found within limits. The pesticide residue was also absent in all the formulations. All the herbal formulations did not show any harmful microbial contamination. The formulations also possessed anti-inflammatory activity. The present study was the first study conducted to standardize these marketed preparations. This study can be used as a standardizing tool for future prospects and by the researchers willing to work on these herbal formulations. This study also forms the basis for niche researchers to extend the work on these herbal formulations and work to explore the pharmacological potential of these formulations.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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HOW TO CITE THIS ARTICLE: Deswal G, Dhingra AK, Saini V, Grewal AS. Quality Audit and Anti-inflammatory Activity of Marketed Formulations of Ashokarishta Syrup and Chandraprabha Vati. *Int. J. Pharm. Sci. Drug Res.* 2022;14(5):595-601. **DOI:** 10.25004/IJPSDR.2022.140513