



## Research Article

# In Vitro Diffusion and Bioavailability Assessment of Gandharva Haritaki Churna: An Ayurvedic Herbal Formulation Study

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## Abstract

Medicated oils are used both externally and internally to treat a variety of illnesses. In Ayurvedic literature, *Murchhana* is a method used to improve the qualities of crude oil. In this study, *Gandharva Haritaki Churna* (Powder), a medicinal formulation, is made using two procedures: processed (*Murchhita*) *Erand Taila* (PET) and unprocessed (*Amurchhita*) *Erand Taila* (AET). The purpose of this study is to evaluate the in-vitro diffusion of *Gandharva Haritaki Churna* prepared using processed and crude castor oils. The in-vitro rate and permeability of a pharmacological dosage form are excellent indicators of the drug's absorption. The aim of this study is to assess the drug release of both powders utilizing in vitro absorbance methods using the Franz diffusion cell apparatus. The in-vitro absorbance was measured in a Franz diffusion cell equipment at pH 4, 7, and 9.2. The samples were collected and tested with a UV spectrophotometer to determine the absorbance of Churna (Powder) at various wavelengths. It was discovered that the buffer solution with a pH of 9.2 had the highest absorbance of all samples. This experimental study demonstrates that both formulations have good sustained absorption via the gastrointestinal tract. The results show that the rate of absorption was greater in PGHC compared to UGHC. This work contributes to the development of a new approach for evaluating intestine absorption and comparing formulations based on therapeutic efficacy and drug absorbance.

**Keywords:** Castor Oil, *Erand Taila*, Drug pH, Franz diffusion study, *Gandharva Haritaki Churna*, Intestinal absorption, Standardization, *Taila Murchchhana*.

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## Introduction

Diffusion is a "Mass transfer of individual molecules of a substance caused by random molecular motion, associated with a driving force such as the concentration gradient" OR "A physical process that refers to the net movement of molecules from a region of high concentration to a region of low concentration under the influence of the concentration gradient." Diffusion study play very significant role in Drug release from dosage form, Drug permeation and distribution in live tissues, Polymer molecular weight estimation, Prediction of medication absorption and excretion(1).

The evaluation of the bioaccessibility and bioavailability of bioactive substances is mostly done using four methods: in vitro models, ex vivo models, in skin models, and in vivo models.(2).

Bioavailability refers to the extent and pace at which the active moiety (drug or metabolite) enters systemic circulation and

reaches the site of action(3). It is an in-vivo study that looks into the drug's gastrointestinal digestion, absorption, metabolism, tissue distribution, and bioactivity. Before bioactive compounds may be absorbed, they must be released from the drug matrix and transformed in the GI tract(4). Thus, bioavailability includes the concept of bioaccessibility. Before making any conclusions about potential health consequences, it is necessary to understand whether the digesting process alters bioactive compounds' stability.

The amount of a material released from its matrix in the gastrointestinal tract and made available for absorption (e.g., entering the bloodstream) is referred to as bioaccessibility. This term refers to the digestion of foods into assimilation-ready material, absorption/assimilation into intestinal epithelial cells, and pre-systemic, intestinal, and hepatic metabolism. Bioaccessibility is frequently tested using in vitro digestive procedures that simulate GI and small intestinal digestion, followed by CaCO<sub>2</sub> cell uptake.

The particular result of a substance's exposure is known as its bioactivity. It covers tissue absorption and the ensuing physiological reaction (such as anti-inflammatory, antioxidant, etc.). It also provides details on the bioactive compounds' transportation and penetration into the target tissue, their interactions with biomolecules, the features of metabolism and biotransformation, the creation of biomarkers, and the ensuing physiological reactions.

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Since it was discovered that phytochemicals biotransform in bodily tissues and provide a number of health benefits, interest in them has grown along with their use. Numerous in vivo biotransformations that take place after phytochemical application may have an effect on the ingested herbal and lipid components' bioaccessibility and bioavailability (5). For the purpose of predicting the phytochemical alterations that take place during gastrointestinal digestion, numerous prospective investigations have been carried out utilising in vitro techniques (6-7). Since it offers valuable information on the interactions between skin, drug, and formulation, the Franz diffusion cell test for skin permeability has grown to be a prominent research tool (8-9).

Such testing is very helpful in toxicity screening as well as in the design and development of novel formulations(10). and management of quality (11–13). Franz diffusion cells are typically used using animal or human skin that has been removed. In situations where biological skin is not easily accessible, synthetic membranes are used. Two functions of the synthetic membranes utilised in Franz cell drug diffusion studies are quality control (15) and skin emulation (14). Due to its hydrophobic properties and similar rate-limiting characteristics to skin, polymethyl siloxane (PDMS) is a synthetic membrane that is widely used to mimic skin(16).

In contrast, synthetic membranes used in quality control should merely serve as a support to keep the formulation apart from the receptor media and have the least amount of diffusion resistance to medications. As a "continuous" medium of the receptor media, the synthetic membrane is suitable. Henceforth referred to as "porous membranes," these artificial membranes typically have pores.

Processing, or *murchhana*, is a preprocessing step used as a therapy to improve its properties(17). The potency and acceptability of medicinal oil have been greatly improved by this technique. The procedure guarantees that the oil absorbs the compounds' active medicinal qualities (18). Previous research has shown that performing a *Murchhana* (pre process) results in the oil absorbing more active ingredients (19). Here, in this study *Gandharva Haritaki Churna* is prepared from preprocessed oil called *Processed Gandharva Haritaki Churna* (PGHC) where as *Gandharva Haritaki Churna* prepared from crude oil which is called *Unprocessed Gandharva Haritaki Churna* (UGHC). The goals of this study was to evaluate the relative merits of processed and raw Gandharva Haritaki Churna. This preprocessing is described in Ayurvedic literature as involving the use of particular herbs in the right amounts and heating the oil; this oil is then used to make further medicinal churnas. There are medicinal and pharmaceutical implications to the ingredients utilized in *murchhana*. With the use of processed and unprocessed castor oil, this study attempts to evaluate the in-vitro diffusion of Gandharva Haritaki Churna. It is impossible to determine the relative efficacy of PGHC and UGHC in the absence of strong evidence.

The need to know the precise outcome arises so that its authentic significance and efficacy may be recognized. This popular formulation is assessed for its in-vitro diffusion study in the current study.

It became crucial to understand the drug's rate and degree of absorption as well as its therapeutic effectiveness in order to guarantee that the medication was fit for ingestion. The entire process of a drug and its related components entering the body is referred to as absorption. The GI tract is a crucial factor in affecting the rate and degree of medication absorption. Any medication that wants to be absorbed needs to cross one or more

biological membranes. The membrane that lines the gastrointestinal tract is responsible for secretion and absorption (20). Since the GI epithelial membrane of animals can be connected to human physiology, it was chosen in this study(21).

For this purpose, the in-vitro diffusion (drug release) of PGHC and UGHC was investigated using the Franz diffusion cells methodology (22). The use of Franz diffusion cells to measure bioavailability has become a popular research tool. The in-vitro rate and amount of permeability of drug dosage forms are useful indicators of the medication's bioavailability. This in-vitro diffusion investigation aims to determine the effect of dose form on biological performance of the medication, as well as the sensitivity to detect changes in the rate and amount of absorption (23). In this way, this study may aid in determining the medicinal efficacy of both Churna for ingestion. This work contributes to the development of a methodology that enables the evaluation of formulations, including therapeutic efficacy and drug absorption, using modest recourse like Franz diffusion study.

## Materials and Methods

### Material

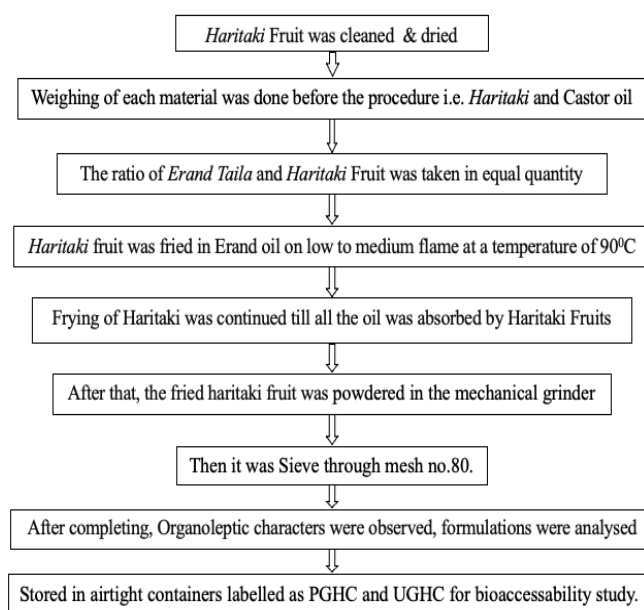
These polymeric layers form a porous structure with a diffusivity and pore size gradient throughout the membrane. The porous structure is impregnated with a proprietary blend of synthetic lipids, which provide the synthetic membrane additional skin-like qualities.

**Buffer Solution Preparation:** Distil water and pH tablets were used to create buffer solutions with pH values of 4, 7, and 9.2. Ionic strength was modified using the procedures outlined in the United States Pharmacopoeia.

### Collection and preparation of PGHC and UGHC

The dried Haritaki Fruit (*Terminalia chebula*) and Castor Oil were obtained from a pharmaceutical unit at Mahatma Gandhi Ayurved College Hospital and Research Centre (MGACH & RC), Wardha. Medicine preparations (PGHC and UGHC) and their analysis were performed at MGACH & RC's pharmaceutical section.

### Method of preparation



**Table 1: Show the ingredients, part and quantity use for the preparation of PGHC & UGHC**

S.No.	Ingredients		Part Used	Quantity
1	Processed Gandharva Haritraki Churna	Uprocessed Gandharva Haritraki Churna	Fruit	100g
2	Haritaki ( <i>Terminalia chebula</i> Retz) Erand Oil (Processed Castor Oil)	Haritaki ( <i>Terminalia chebula</i> Retz) Erand Oil (Unprocessed Castor Oil)	Oil	100g

### Simulated Gastrointestinal Digestion in Dynamic Conditions

#### Preparation of Digestive Fluids

The simulated digestive juices were made fresh daily, as stated below.

**Simulated Gastric Fluid.** By using pH 4 tablet diluted it in 100ml of distilled water.

**Simulated Intestinal Fluid.** By using pH 7 tablet diluted it in 100ml of distilled water.

**Simulated Bile Solution.** By using pH 9.2 tablet diluted it in 100ml of distilled water.

#### Simulated Gastrointestinal Digestion

**Simulated Gastric Digestion.** A 500 mg sample of Gandharva Haritaki Churna was accurately weighted, dried, and powdered. It was incubated with newly made SGF (25 mL, pH 4.0) in a 50 mL Erlenmeyer flask for 60 minutes at 37 °C in a shaking water bath (167 rpm). The gastric digesting phase was completed by inactivating pepsin and elevating the pH of the solution to 7.0.

**Intestinal Digestion Simulated.** The two-step proteolysis model created by Savoie and Gauthier (24–25) was simplified into the dynamic model, which was utilized to estimate digestibility. Following stomach digestion, the entire sample (pH 7.0) was moved to the receptor compartment, which served as a stand-in for the small intestine. About four hours of constant stirring were spent on the digestion process.

The receptor compartment was submerged in a 1000 mL buffer solution (pH 7.0, comparable to Simulated Intestinal Fluid without pancreatin addition) that was kept at 37°C during mixing. This vessel was attached to both a receiving flask and a buffer feeding reservoir that was set at 37°C. At the same transfer rate, the buffer solution containing the penetrated digested products was moved to the receiving flask. After the intestinal phase of digestion, the buffer solution was gathered in the receiving flask.

#### In vitro Franz Diffusion test determination of PGHC & UGHC (26-27)

A franz diffusion test was conducted using the procedure that Bonferoni MC et al. suggested. The drug which diffused from the GI epithelial membrane, similar to a synthetic membrane, was analyzed using a Franz diffusion cell device. The assembly consisted of inert adsorbing substance. Every time the assembly was used, it was autoclaved. An absorption membrane with a hole size of 0.45µ, related to human physiology, was developed using a synthetic membrane similar to that of the gastrointestinal epithelium (28). Membranes were carefully separated, with the first one being affixed to the receptor compartment. A later donar compartment with a surface area of between 0.2 and 2 cm square

was placed over the receptor compartment to expose it to the membrane. The assembly was prepared. A fixed receptor compartment with a capacity of 0.5 to 10 ml was used. The buffer solutions with pH values of 4, 7, and 9.2 were made to correspond with the intestinal and stomach pH. A separate sample of PGHC and UGHC was added to each donor compartment.

A sufficient amount of buffer was added to the receptor fluid compartment to dissolve the test substance, which was kept in contact with the other side of the skin from the beginning to the end of the receptor fluid collection process. The donor compartment was appropriately positioned over the receptor compartment, and both compartments were sealed by sandwiching a synthetic membrane between the two joining spaces. The assembly was then prepared for operation, and the skin surface temperature in the diffusion cell was kept at  $\pm 37^{\circ}\text{C}$ . After the drug's diffusion through the membrane into the receptor compartment, samples were taken from the receptor compartment. first for thirty minutes at pH 4 with the aid of a syringe. Subsequently, the donor compartment buffer was replaced to pH 7, and the same samples were collected after one and two hours. Subsequently, the donor compartment's pH 9.2 buffer was added, and the same samples were collected at 3, 4, 5, 6, 7, 8, and 9 hours. The samples were examined with a UV spectrophotometer and a photo spectrophotometer, and the absorbance at 254 nm was recorded.

#### U.V. Spectrophotometric Analysis (29)

Using an Elico SL 244 dual beam recording UV visible spectrophotometer, the UV spectrum was recorded. The study used the stock solutions above, which were made from churna (Powder) samples that were collected from receptor chambers at specific times. The comparative spectra of each sample were also recorded. (Table 3) A graph of absorbance against time was plotted. (Graph 11 and 21)

**Fig. 1: Images of Franz Diffusion Test**



**Fig.: Franz diffusion cell Assembly with Donar & receptor compartment**



**Fig.: Membrane attached to receptor compartment**



**Fig.: Assembly fixed together**



**Fig.: PGHC added to donar Compartment**



**Fig.: UGHC added to donar Compartment**



**Fig.: PGHC Diffused to receptor compartment**

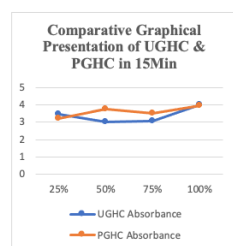


**Fig.: UGHC Diffused to receptor compartment**

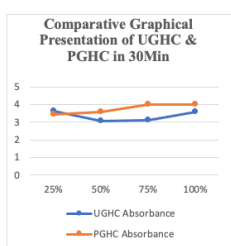
## Observations and Result

Franz diffusion cell approach was used to study the in vitro diffusion (drug release) of PGHC and UGHC. The samples' analysis under a UV spectrophotometer obtained data that

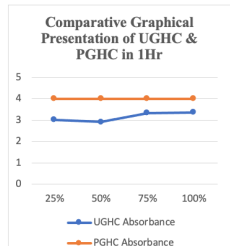
indicated absorbance at 254 nm. (As indicated in Table 3) It was discovered that PGHC had a higher absorbance than UGHC. According to the study, both formulations exhibit good sustained absorption through the gastrointestinal tract; however, PGHC absorbs significantly higher rates than UGHC.



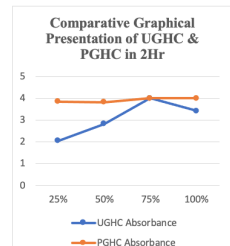
Graph 1



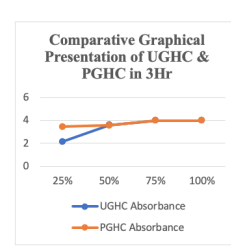
Graph 2



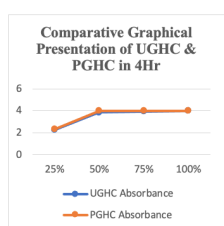
Graph 3



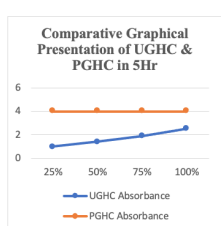
Graph 4



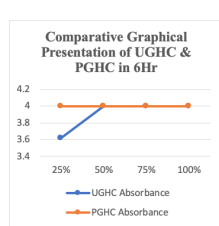
Graph 5



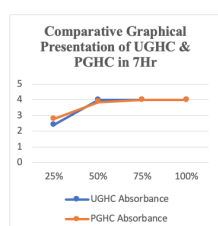
Graph 6



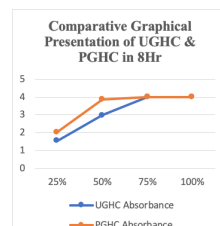
Graph 7



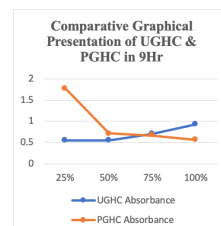
Graph 8



Graph 9



Graph 10



Graph 11

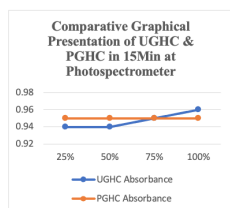
**Table 2: Showing results of Franz Diffusion test of UGHC & PGHC**

Buffer pH	Time	Distribution Absorbance UGHC				Distribution Absorbance PGHC			
		25%	50%	75%	100%	25%	50%	75%	100%
4	15min	3.4427	3.0214	3.0819	4.0000	3.2209	3.7590	3.5014	3.9563
	30min	3.6098	3.0547	3.1152	3.5571	3.4447	3.5720	4.0000	4.0000
7	1Hr	3.0139	2.9177	3.3326	3.3634	4.0000	4.0000	4.0000	4.0000
	2Hr	2.0587	2.8115	4.0000	3.4210	3.8573	3.8119	4.0000	4.0000
9.2	3Hr	2.1464	3.6014	4.0000	4.0000	3.6485	4.0000	4.0000	4.0000
	4Hr	2.2708	3.8464	3.9403	4.0000	2.3355	4.0000	4.0000	4.0000
	5Hr	0.9968	1.4246	1.8822	2.5249	4.0000	4.0000	4.0000	4.0000
	6Hr	3.6161	4.0000	4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
	7Hr	2.4137	4.0000	4.0000	4.0000	2.7751	3.2161	4.0000	4.0000
	8Hr	1.5551	2.9824	4.0000	4.0000	2.0555	3.8577	4.0000	4.0000
	9Hr	0.5482	0.5495	0.7054	0.9321	1.7749	0.7123	0.6666	0.5623

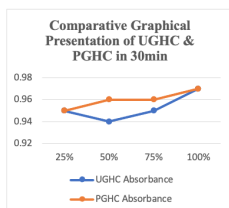
**Table 3: Showing results of Franz Diffusion test of PGHC & UGHC in Photo Spectrometer:**

Buffer Ph	Time	Distribution Absorbance PGHC				Distribution Absorbance UGHC			
		25%	50%	75%	100%	25%	50%	75%	100%
4	15min	0.94	0.94	0.95	0.96	0.95	0.95	0.95	0.95
	30min	0.95	0.94	0.95	0.97	0.95	0.96	0.96	0.97
7	1Hr	0.96	0.98	0.97	0.98	1.04	1.11	1.15	1.18
	2Hr	0.97	0.98	1.00	1.01	1.07	1.07	1.11	1.15
9.2	3Hr	1.10	1.15	1.22	1.33	1.08	1.16	1.17	1.19
	4Hr	1.07	1.18	1.26	1.30	1.05	1.18	1.18	1.20
	5Hr	0.95	0.97	0.98	0.99	1.26	1.42	1.54	1.69
	6Hr	0.72	0.96	0.89	0.92	1.26	1.42	1.54	1.70
	7Hr	0.58	0.72	0.82	0.89	1.08	1.09	1.16	1.23
	8Hr	0.52	0.63	0.75	0.88	1.01	1.05	1.10	1.12
	9Hr	0.43	0.43	0.45	0.47	0.52	0.58	0.60	0.66

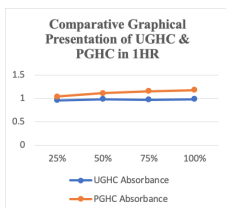




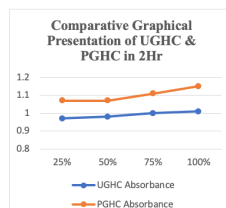
Graph 12



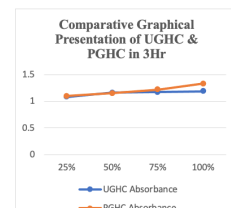
Graph 13



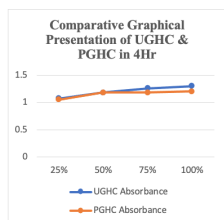
Graph 14



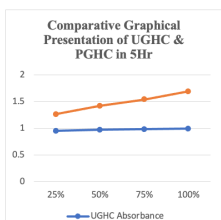
Graph 15



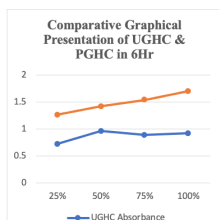
Graph 16



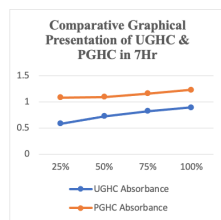
Graph 17



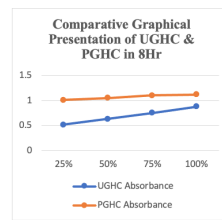
Graph 18



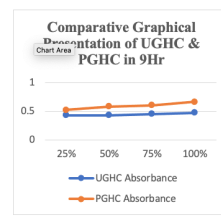
Graph 19



Graph 20



Graph 21



Graph No.22

## Discussion

In this present research work it is attempted modestly to discuss scientifically the whole study in the light of fundamentals of the Ayurvedic and modern basic science. Churna (Powder) preparations possess an important place in Ayurveda. Medicated Powders are in practice from Vedic period in different forms. By shifting the active principles, oil preparations help to move the active principles from the herbs and subsequently enrich the fluid oil. The mass transfer theory provides an explanation for these phenomena(30). Crude oil undergoes a process called *murchchhana* before being put through real preparation. Crude oil was cooked in *Murchchhana* together with the appropriate amount of water and coarsely ground medicinal medication powder. This gives the crude oil a unique ability to extract more active ingredients from the added medications, increasing the oil's potency(31).

Franz diffusion cell approach was used to study the *in vitro* diffusion (drug release) of PGHC and UGHC. Using a Franz diffusion cell equipment, an attempt was made to establish an *in vitro* absorbance analysis of PGHC and UGHC. To guarantee the quality of the medication for ingestion, it is critical to understand the rate and degree of absorption of the medication in addition to its therapeutic efficacy. The term "absorption" generally describes how a medicine and its constituents are transported throughout the body. The rate and degree of medication absorption are significantly influenced by the gastrointestinal (GI) tract (32). A medicine needs to cross one or more biological membranes in order to be absorbed. The membrane that lines the gastrointestinal tract is responsible for secretion and absorption. The biomolecular lipid matrix that makes up the majority of the membranes controls the permeability properties of the membranes. From a region of high concentration to one of low concentration, drugs disperse across a cell membrane (33). In addition to being directly correlated with gradient, diffusion rate is also influenced by the lipid solubility and absorptive surface area of the molecule. Lipid cell membranes allow lipid-soluble drugs to spread more quickly than other types. (34).

The Franz diffusion cell is a commonly employed methodology that offers a straightforward and repeatable assay for quantifying drug release *in vitro*. The amount of active medication that has

penetrated the membrane at each time point is ascertained by this test (35). As can be seen in tables 2 and 3, the results of the analysis of the materials using a UV spectrophotometer revealed absorbance at a wavelength of 254(36). Because the sample solution concentration in UV spectroscopy is measured in mol/L-1 and the light path length is measured in cm, the absorbance is unit less. (37). It was discovered that PGHC had a higher absorbance than UGHC. In PGHC the absorbance was 4, 3.9173, 3.9121, 3.5838, 4 at 1, 2, 3, 4, 5Hrs respectively. In UGHC it was comparatively less i.e. 3.1566, 3.0728, 3.4369, 3.5050 at 1, 2, 3, 4 Hrs respectively. It was observed that every sample exhibited its maximum absorbance in the pH 7 buffer solution. In all of the samples where the medication was present in the intestine, the rate of absorption grew steadily over the course of one hour to four hours. The reason for this could be that the intestine is where weakly basic medicines are absorbed and ultimately leave, virtually unionized. (38). The majority of drugs are weak organic acids or bases that exist in aqueous solutions as unionized and ionized forms. The unionized form is often lipid soluble and easily diffuses across cell membranes. (39). The pH of PGHC and UGHC was 6 and 6.7 which is weakly basic. Also small intestine is the most important region of GIT with respect to active absorption (40). The acidity or basicity of biological fluids at the absorption site is one of the most important elements influencing drug absorption from the gastrointestinal system. A medication may be readily absorbed from one section of the track with a favorable pH but poorly absorbed from another with a significantly lower pH (41). The duodenum has an intramural pH of 4 to 5, which gradually increases to 8 in the lower ileum (42). The presence of villi, finger-like projections that arise from and constitute part of the folds in the intestinal mucosa, significantly increases the mucosal surface area from which absorption can occur in the small intestine. The small intestine's extremely vast surface area makes it well-suited for passive absorption. (43).

This experimental study discovered that both formulations have well-sustained absorption via the gastrointestinal tract; however, the rate of absorption is faster in PGHC than in UGHC. This study also reveals that Processed Gandharva Haritaki Churna (Powder) results in a significant difference in the chemical contents of the final product, as evidenced by the Churna absorbance. It is likely that more active components of herbs will be absorbed if pre-processed oil is used in churna preparation.

## Conclusion

The Franz diffusion test demonstrates that both formulations had well-sustained absorption into the gastrointestinal system, with Processed GHC having a higher rate of absorption than Unprocessed GHC. Thus, Processed Churna (Powder) may have a higher therapeutic efficacy. Franz's diffusion research is a novel method for testing and comparing oral formulations, allowing the prediction of the GI absorption process. As a result, in vitro absorption testing is an extremely useful approach for assessing Ayurvedic medications.

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**Conflict of interest:** Nil

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