

# Assessment of in vitro diffusion of *Chincha Lavan Taila*: An Ayurveda medicated oil

## Research Article

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### Abstract

Medicated oils are used for external as well as internal administration to treat various disorders. In Ayurvedic literature *Murchhana* is a procedure carried out on crude oil as treatment to enhance properties of oil. In this study, *Chincha Lavan Taila* a medicated formulation is prepared by two procedures *Murchhita Chincha Lavan Taila* (MCLT) and *Amurchhita Chincha Lavan Taila* (ACLT). This study aims to assess the in-vitro diffusion of *Chincha Lavan Taila* formulated by processed sesame oil and crude sesame oil. The in-vitro rate and extent of permeability of drug dosage forms are good markers to assess the absorbance of the drug. The objective of this study is to evaluate the drug release of both oils by using in-vitro absorbance methodology of Franz diffusion cell apparatus. The in-vitro absorbance was studied in Franz diffusion cell apparatus at pH 3 and pH 8. The samples were collected and analysed under UV spectrophotometer to note absorbance of oil at different wavelength. It was noted that in the buffer solution of pH 8, there was maximum absorbance of all the samples. This experimental study shows that both formulations have well sustained absorption through gastrointestinal tract. The results indicate that the rate of absorption exhibited to be higher in MCLT compared to ACLT. This work helps to develop a new method to evaluate intestinal absorption and compare formulation for therapeutic efficacy and drug absorbance.

**Keywords:** *Chincha Lavan Taila*, Drug pH, Franz diffusion study, Intestinal absorption, Standardization, *Taila Murchhana*.

### Introduction

Ayurveda a holistic health care system prescribes usage of different medicated oil for providing health benefits along with assorted methods of preparation. Medicated oils are used for external as well as internal administration to treat various disorders. *Chincha Lavan Taila* is one such formulation which is widely used by traditional healers. *Chincha Lavan Taila* is used to relieve chronic constipation, cramps of gastrointestinal tract, prevents spasms, flatulence etc (1). Prior to the preparation of any type of medicated oil, *Murchhana* a kind of pharmaceutical process on crude oil is essential (2). *Murchhana* is a pre procedure carried out as treatment to enhance its properties (3-4). This practice has aided significantly at enhancing the acceptability and potency of medicated oil. The process ensures the absorption of the active therapeutic properties of the ingredients by oil (5). Earlier studies have revealed that more active principles are absorbed in to the oil if *Murchhana* (pre procedure) is done (6). Here, in this

study *Chincha Lavan Tail* is prepared from preprocessed oil called *Murchhita Chincha Lavan Taila* (MCLT) where as *Chincha Lavan Tail* prepared from crude oil which is called *Amurchhita Chincha Lavan Taila* (ACLT). This study was designed to determine the importance of *Murchhita* and *Amurchhita Chincha Lavan Taila*. In Ayurvedic literature this pre-process is done by using specific herbs in appropriate proportion and by applying heat to it, later this preprocessed oil is used to prepare other medicated oils. The ingredients used for *Murchhana* has both pharmaceutical and therapeutic implication. This study aims to assess the in-vitro diffusion of *Chincha Lavan Taila* formulated by *Murchhita* and *Amurchhita* oil. Without any substantial proof it cannot be claimed that whether MCLT and ACLT have more or less efficacy. There emerges the need to know the exact result so that its actual efficacy and utility can be acknowledged. In this present research work, this widely used formulation is been evaluated for its in-vitro diffusion study.

It became important to know about the rate and extent of absorption of drug along with its therapeutic efficacy to ensure the quality of the drug for its consumption. Absorption often refers to the overall transport of a drug and related substances into the body. The gastrointestinal (GI) tract plays a major role in determining the rate and extent of drug absorption. To be absorbed a drug must pass through one or more biological membrane. The GI epithelial lining is a membrane concerned with absorption and secretion (7).

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In this study the GI epithelial membrane of animal was used as it can be related to human physiology (8). For this purpose the in-vitro diffusion (drug release) of MCLT and ACLT was analysed by using the methodology of Franz diffusion cells (9). The use of Franz diffusion cell to assess bioavailability has evolved into a major research methodology. The in-vitro rate and extent of permeability of drug dosage forms are good markers to access the bioavailability of the drug. This in-vitro diffusion study is to find out the dosage form influence on biological performance of the drug; sensitivity to detect differences on the rate and extent of absorption (10). In this way this study may help to access the therapeutic effectiveness of both the oil for its consumption. This work helps to develop a methodology which allows the evaluation of formulations including therapeutic efficacy and drug absorbance by using modest recourse like Franz cell test.

## Material & Methods

### Collection and authentication of the raw drugs

Raw drugs required for the preparation of oil were collected from trusted herbal raw drug provider from local market, Nagpur and authenticated from taxonomist from the Department of Dravyaguna, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha.

### Preparation of (*Murchhita*) pre-processed sesame oil (11)

**Table 1: Ingredients used for the preparation of pre-processed oil**

Sr. No.	Name of Ingredients	Part used	Quantity
1	<i>Manjistha</i> ( <i>Rubia cordifolia</i> Linn.)	Root	95gms
2	<i>Haridra</i> ( <i>Curcuma longa</i> Linn.)	Rhizome	31gms
3	<i>Lodhra</i> ( <i>Symplocos racemosa</i> Roxb.)	Stem bark	31gms
4	<i>Musta</i> ( <i>Cyperus rotundus</i> Linn.)	Tuber	31gms
5	<i>Nalika</i> ( <i>Cinnamom tamala</i> Linn.)	Steambark	31gms
6	<i>Amalaki</i> ( <i>Phyllanthus emblica</i> Linn.)	Pericarp	31gms
7	<i>Bibhitaki</i> ( <i>Terminalia bellerica</i> Roxb.)	Pericarp	31gms
8	<i>Haritaki</i> ( <i>Terminaliachebula</i> Retz.)	Pericarp	31gms
9	<i>Ketaki Mula</i> ( <i>Pandanus odoratissimus</i> Linn.)	Root	31gms
10	<i>Vatankura</i> ( <i>Ficus Bengalensis</i> Linn.)	Leaf buds	31gms
11	<i>Tila Taila</i> ( <i>Sesamum indicum</i> Linn.)	Sesame oil	1500ml
12	Water	Potable water	6000ml

All ingredients listed in (Table1) were cleaned and pounded and made into bolus by adding little quantity of water. Sesame oil was taken into the vessel and heated for some time; bolus was added to lukewarm

oil and stirred well. Later water was added to it and heated on mild fire with stirring to avoid bolus to adhere to the vessel and boiling continued till all water evaporates and the moisture in the pasty mass also evaporates. Precaution was taken that the well cooked oil should not have any residual moisture. The oil was strained while warm through muslin cloth and allowed to cool and stored.

### Preparation of *Chinchā Lavan Taila*

1. *Chinchā* (Tamarind) was cleaned by removing foreign matter present in it.
2. In steel vessel *Chinchā* was soaked in potable lukewarm water for an hour.
3. After an hour *Chinchā* attained soft consistency which was then macerated and filtered through clean muslin cloth.
4. Above obtained filtrate (*Chinchā Drava*) + *Tila Taila* (Sesame oil) + *Saindhav Lavan* (Rock salt) was taken in mentioned quantity and heated on mild fire till obtaining completion test of oil.
5. Prepared oil was allowed to self-cool, filtered through doubled layered muslin cloth and stored in air tight container.

### Preparation of MCLT and ACLT

Ingredients with their proportions used for the preparation of MCLT and ACLT are listed in table 2 and 3 respectively.

**Table 2: Proportion of ingredients used for the preparation of MCLT**

Sr. No.	Name of Ingredients	Part used	Quantity
1	<i>Chinchā</i> ( <i>Tamarindus indica</i> Linn.)	Fruit pulp	100gm
2	<i>Saindhav Lavan</i> (Rock salt)	-	6.25gms
3	<i>Tila Taila- Murchhita</i> (Prep-rocessed)	Sesame seed oil	400ml
4	Water	Potable water	1600ml

Tamarind was taken, cleaned and soaked in lukewarm water for about half an hour. Later macerated well and filtered to obtain tamarind water and pulp was removed. In a vessel macerated tamarind was mixed and heated along with pre-processed oil on low flame till the water completely evaporates. The oil was strained while warm through muslin cloth and allowed to cool and stored.

**Table 3: Proportion of ingredients used for the preparation of ACLT**

Sr. No.	Name of Ingredients	Part used	Quantity
1	<i>Chinchā</i> ( <i>Tamarindus indica</i> Linn.)	Fruit pulp	100gm
2	<i>Saindhav Lavan</i> (Rock Salt)	-	6.25gms
3	<i>Tila Taila- Amurchhita</i> (crude oil)	Sesame seed oil	400ml
4	Water	Potable water	1600ml

Same method of preparation was followed for ACLT as that of MCLT oil.

**In vitro Franz Diffusion test determination of MCLT & ACLT (12-13)**

A Franz diffusion test was carried out as per the method proposed by Bonferoni MC*et al.* in brief. Franz diffusion cell apparatus was used to analyse the medication diffused from GI epithelial membrane of animal. The assembly was made up of adsorbing material which was inert. The assembly was autoclaved each time before using. Gastrointestinal epithelial membrane of goat was used as absorption membrane of pore size 0.45 which relates to human physiology (14). Membranes were separated carefully and one was attached to receptor compartment first. Later donor compartment of about 0.2cm to 2cm square of surface area was exposed to membrane by placing over receptor compartment. The assembly was made ready. A receptor compartment of 0.5 to 10 ml volume was fixed. The buffer solution of pH 3 and pH 8 was prepared to match the pH of stomach as well as intestine. Sample of MCLT & ACLT was added to donor compartment each separately. The receptor fluid compartment was filled with adequate quantity of buffer to dissolve the test substance. The test substance is maintained in contact with other side of the skin from the time of initial start to end of the collection of receptor fluid. The donor compartment was placed over the receptor compartment properly. The two compartments were sealed with placing the skin membrane in between two joining spaces and the assembly was set for running. The temperature of the skin surface in the diffusion cell was maintained at  $\pm 37^{\circ}\text{C}$ . Samples were collected from receptor compartment after diffusion of drug to receptor compartment through the membrane. With the help of syringe at pH 3 in 30, 60, 90 and 180 minutes first. Later the buffer was changed to pH 8 from donor compartment and the same samples were collect at 240, 360, 480, 600 and 660 minutes. Both the samples were analysed under UV spectrophotometer and absorbance was noted at 254nm.

**U.V. Spectrophotometric Analysis: (15)**

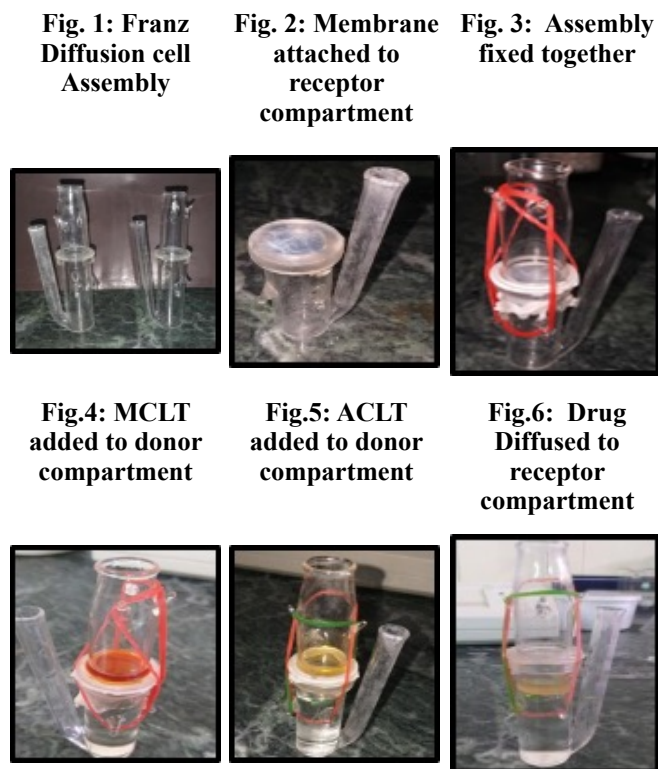
The UV spectrum was captured using an Elico SL 244 dual beam recording UV visible spectrophotometer. The stock solution was prepared from the samples of oil, which was collected from receptor chamber at particular time. The above stock solutions were used for the study. The comparative spectra of all the samples were also recorded. (Table 4) A Graph of absorbance against time was plotted. (Graph 1 and 2)

**Observations and Results**

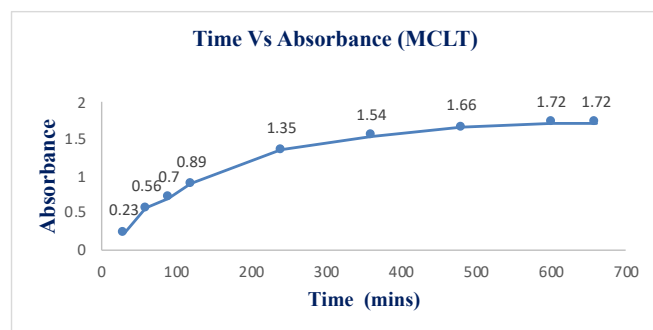
The in vitro diffusion (drug release) of MCLT and ACLT was analysed by using the methodology of Franz diffusion cells. The results obtained by the analysis of samples under UV spectrophotometer shows absorbance at 254 nm. (as shown in table 4) The absorbance in MCLT was found to be highest than that of ACLT. The study showed that the both formulations

have well sustained absorption through gastrointestinal tract; however the rate of absorption is higher in MCLT compared to ACLT.

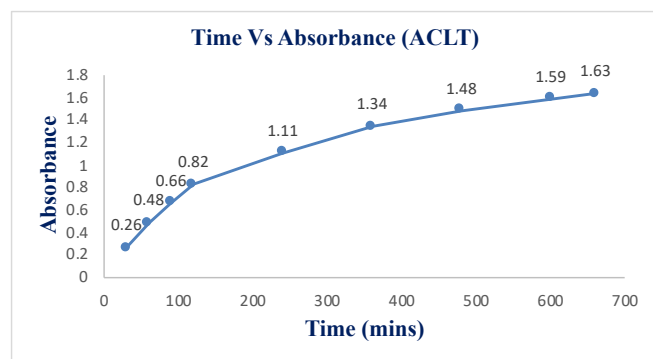
**Images of Franz Diffusion cell**



**Graph 1: Time Vs Absorbance of MCLT**



**Graph no. 2: Time Vs absorbance of ACLT**



**Table 4: Result of Franz Diffusion test of MCLT & ACLT**

Buffer pH	Time in minutes	Absorbance MCLT	Absorbance ACLT
3	30	0.23	0.26
	60	0.56	0.48
	90	0.7	0.66
	120	0.89	0.82
8	240	1.35	1.11
	360	1.54	1.34
	480	1.66	1.48
	600	1.72	1.59
	660	1.72	1.63

**Table 5: Result of pH value of MCLT & ACLT**

Sr. No.	Name of Sample	pH value
1	MCLT	8.25
2	ACLT	8.19

**Inference of Result**

Two buffer pH solutions were utilized for the test: pH 3 and pH 8. It's because the stomach has pH 3 and the intestine has pH 8. The measurement of samples with a UV spectrophotometer revealed absorbance at 254 wavelength. The absorbance in MCLT was 1.66, 1.72, and 1.72 after 480, 600, and 660 minutes, respectively. In ACLT, it was significantly lower, at 1.34, 1.48, and 1.59 after 480, 600, and 660 minutes respectively. The absorbance in MCLT was found to be higher than in ACLT. It was found that the buffer solution with pH 8 had the highest absorbance of all the samples. The rate of absorption increased over time from 360 to 660 minutes in all samples containing the medication in the small intestines. This experimental study found that both formulations had well-sustained absorption through the gastrointestinal system; however, the rate of absorption is higher in MCLT than in ACLT. This study also demonstrates that adding *Murchhana* (pre-process) to the oil causes a considerable difference in the chemical makeup of the final product, as seen by the oil's absorbance.

**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. Oil preparations possess an important place in Ayurveda. Medicated oils are in practice from Vedic period in different forms. Oil preparations facilitate to transfer the active principles from the herbs and later enrich the fluid oil by shifting the active principles. These phenomena can be explained by the theory of mass transfer (16). Sesame oil is widely used as base to prepare many medicated oils and as an ingredient in various other formulations. According to scholars of Ayurveda sesame oil is best among all the vegetable oils (17). Sesame oil when processed with other drugs acts assimilates the properties of other drugs added to it without losing its own properties (18). Considering the above benefits, *Chinchalavan Taila* was prepared by using sesame oil as the base.

*Murchhana* is a procedure carried out on crude oil before subjecting it to actual preparation. In *Murchhana* crude oil was boiled with the coarse powder of selected medicinal drugs and desired quantity of water. This increases potency of the oil by imparting special capability to the crude oil to extract more active principles from the added drugs(19).

The in vitro diffusion (drug release) of MCLT and ACLT was analysed by using the methodology of Franz diffusion cells. An attempt was made to establish in vitro absorbance study of MCLT and ACLT with the help of Franz diffusion cell apparatus. It is important to know about the rate and extent of absorption of drug along with its therapeutic efficacy to ensure the quality of the drug for its consumption. Absorption often refers to the overall transport of a drug and related substances into the body. The gastrointestinal (GI) tract plays a major role in determining the rate and extent of drug absorption (20). To be absorbed a drug must pass through one or more biological membrane. The GI epithelial lining is a membrane concerned with absorption and secretion. The membranes are primarily composed of a biomolecular lipid matrix, which determines membrane permeability characteristics. Drugs diffuse across a cell membrane from a region of high concentration to one of low concentration (21). Diffusion rate is directly proportional to gradient but also depends on the molecules lipid solubility and area of absorptive surface. Because the cell membrane is lipid, lipid soluble drug diffuses most rapidly than other ones (22).

Franz diffusion cell is widely used methodology which is a simple, reproducible test for measuring the in vitro drug release. This test determines the amount of active drug that has permeated the membrane at each time point (23). The results obtained by the analysis of samples under UV spectrophotometer showed absorbance at 254 wavelength (as shown in table 4) (24).The absorbance is unit less because in UV spectroscopy, the concentration of sample solution is measured in molL-1 and the length of the light path in cm (25). The absorbance in MCLT was found to be highest than that of ACLT. In MCLT the absorbance was 1.66, 1.72 and 1.72 at 480, 600 and 660 minutes respectively. In ACLT it was comparatively less i.e. 1.34, 1.48 and 1.59 at 480, 600 and 660 minutes respectively. It was noted that in the buffer solution of pH 8, there was maximum absorbance of all the samples. The rate of absorption gradually increased from 360 minutes to 660 minutes in all the samples where the drug was present in intestine. It could be because the absorption of weakly basic drugs was found in the intestine, where they exit essentially unionized (26). Most of the drugs are weak organic acid or bases, existing in unionized and ionized forms in an aqueous environment. The unionized form is usually lipid soluble and diffuses readily across cell membranes (27). The pH of MCLT and ACLT was 8.5 and 8.17 which is weakly basic. Also small intestine is the most important region of GIT with respect to active absorption (28). The degree of acidity or basicity of biological fluids at the absorption site is one of the most critical factors in

the absorption of drugs from gastrointestinal tract. A drug may be well absorbed from one portion of track, where a favourable pH exists and poorly absorbed from another portion, where a much less favourable pH is found (29). The intramural pH is 4 to 5 in the duodenum but becomes progressively more alkaline, approaching 8 in lower ileum (30). The mucosal surface area from which absorption can take place in the small intestine is greatly increased by the presence of villi, finger like projection arising from, and forming part of the folds in, the intestinal mucosa. The extremely large surface area in the small intestine makes this site in the GIT well designed for passive absorption (31).

This experimental study showed that both formulations have well sustained absorption through gastrointestinal tract; however the rate of absorption is higher in MCLT compared to ACLT. This study also reveals that *Murchhana* (pre-process) given to the oil results in significant difference in chemical composition of final product which is evident from difference in absorbance of the oil. It may suggest that more active principles of herbs can be absorbed if oil is pre-processed.

## Conclusion

Franz diffusion test reveals that both formulations have well sustained absorption through gastrointestinal tract, the rate of absorption is elevated in pre-processed MCLT compared ACLT. Thus, pre-processed oil may have better therapeutic efficacy. *Murchhana* process plays vital role in Ayurved oil formulations. Franz's diffusion study provides a new process to test and compare oral formulations which allows the prediction of GI absorption process. Therefore, in vitro absorption testing is highly valuable tool in assessment of Ayurveda preparations.

**Conflict of interest:** None

**Acknowledgement:** Authors are thankful to the management for providing all necessary support to conduct this research study. Dr. Bharat Rathi devised the study and was in charge of overall direction. Dr. Anita Wanjari conceived the idea for new process to test. Dr. Bhagyashree Jibkate prepared the formulation, performed experiments and written manuscript with esteem support of Dr. Akshay Pargaonkar. All the authors contributed to discuss the analysis of result and committed on manuscript.

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