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COMPARATIVE PHYTO-CHEMICAL SCREENING OF LASHUNA KALKA PREPARED WITH AND WITHOUT SHODHANA

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ABSTRACT
Acharya Sharaghadhara states the internal administration of Lashuna kalka along with tilataila is beneficial in Vatavyadhi. He also mentions that before preparation of Lashuna kalka, shodhana of Lashuna is must to make it free from its extreme pungent odour and taste. This study was conducted to know the difference between properties of Lashuna kalka prepared with and without shodhana. Lashuna shodhana was done by peeling the outer covering, inner green sprout and soaking it in buttermilk for one whole night and kalka was subjected to organoleptic evaluation, preliminary phytochemical screening, pH and TLC. The organoleptic evaluation has resulted in reduced pungent taste and odour of shodhitaLashunakalka. Phytochemical evaluation showed the addition of two chemical components i.e., Triterpinoids and Steroids. Whereas pH and TLC did not had any marked variation between shodhita and ashodhitaLashunakalka. Therefore the concept put forthby AcharyaSharangadharais said to be precise.

KEYWORDS-Lashuna, Shodhana, Organoleptic evaluation, Phytochemical screening, P^H, TLC.

INTRODUCTION
In Ayurveda Lashuna(Allium Sativam/ Garlic) was highly valued for its numerous health benefiting properties, which are still followed today. It is regarded as a highly effective and most commonly available herb that acts as an excellent natural cure without the possibility of any side effects.

Use of Lashunakalka (Fresh paste) in the treatment of Vataja disorders is mentioned in Sharaghadhara Samhita.[1] While explaining the preparationof Lashunakalka, it is said to soak Lashuna in butter milk overnight after removing its outer covering and green sprout present in the inner part of the clove, to make it free from extreme pungent odour and taste. Later authors mentioned this preoperative procedure as shodhana of Lashuna.

The phyto-chemicals responsible for the sharp flavor of Lashuna are produced when the plant's cells are damaged. When a cell is broken by chopping, chewing or crushing,enzymes stored in cell vacuoles trigger the breakdown of several sulphur- containing compounds stored in the cell fluids(cytosol). The resultant compounds are responsible for the sharp or hot taste and strong smell of Lashuna.[2] The green sprout in the center of clove is hard to digest and especially pungent.[3]

As per modern science, Lashuna is known to cause bad breath (halitosis) and body odour, described as a pungent "garlicky" smell to sweat. This is caused by Allyl Methyl Sulphide(AMS). AMS is a volatile liquid which is absorbed into the blood during the metabolism of Garlic derived sulphur compounds; from the blood it travels to the lungs and skin, where it is exuded through skin pores.[4] Studies have shown sipping milk at the same time as consuming Lashuna can significantly neutralize bad breath. Mixing Lashuna with milk in the mouth before swallowing reduced the odour better than drinking milk afterward.[5] Similar effect might be expected in Lashunakalka when it prepared by soaking in buttermilk for overnight, as butter milk also a milk product. To assess the benefit of shodhana as a preoperative procedure in the preparation of Lashunakalka this study has been conducted.

Objectives
- To do the preliminary phytochemical screening of Lashunakalka prepared with and without shodhana.
- To compare the results of phytochemical screening of Lashunakalka prepared with and without shodhana.

MATERIALS AND METHODS

Source of data

- Lashuna (Allium Sativam) was collected from the local market and butter milk was prepared in teaching pharmacy of SDM Institute of Ayurveda and Hospital, Bengaluru.

Method of data collection

- Sample -1,Lashunakalkawas prepared by subjecting Lashuna to grinding after removing the outer covering, green sprout in the center of the clove and soaking in butter milk for overnight.
- Sample-2 Lashunakalkawas prepared by subjecting Lashuna to grinding after removing only its outer covering.

METHODS

- Both the samples were subjected to organoleptic evaluation, phytochemical screening, pH and TLC.

Organoleptic evaluation

It refers to evaluation of the drug by colour, odour,taste, consistency, texture etc.It is a technique of qualitative evaluation based on the study of sensory profiles of the drug. Organoleptic characters are very useful parameters to determine and compare the quality of the samples.In this study parameters like colour, odour,taste and consistency were considered.

Table no.1 shows the results of organoleptic evaluation.

Organoleptic parameters	Shodhita Lashunakalka	Ashodhita Lashunakalka	Butter milk before soaking	Butter milk after soaking
Colour	Creamy	Blackish cream	White	Yellowish white
Odour	Slightly pungent	More pungent	Sour	Pungent, Sour
Taste	Sour,Astringent, Pungent	Pungent, Astringent	Sour	Pungent, Sour
Consistency	Paste	Paste	Liquid	Liquid

The strong pungent taste and odour, which was present in the ashodhitaLashunakalka was comparatively reduced in shodhitaLashunakalka. Slight sour and astringent taste was appreciated in shodhitaLashunaKalka. Colour of ShodhitaLashunakalka was cream whereas ashodhitaLashunakalka was blackish white. White colour and sour taste of butter milk was changed to yellowish white and pungent, sour respectively.

Preliminary phytochemical screening[6]

Preliminary phytochemical screening is a kind of qualitative analysis done for the identification of various plant constituents present in the drug. The alcoholic and aqueous extracts of shodhita Lashunakalka and ashodhita Lashuna kalka were subjected to qualitative tests for the identification various constituents. Results of the study are mentioned in table no.2&3.

Table no.2 shows the results of preliminary phytochemical screening of sample 1&2.

Tests	Colour if positive	AshodithaLashuna	ShodithaLashuna
Alkaloids			
Dragondroff's test	Orange red precipitate	White precipitate	White precipitate
Wagner's test	Reddish brown precipitate	Brick red precipitate	Brick red precipitate
Mayer's test	Dull white precipitate	Dull white precipitate	Dull white precipitate
Hager's test	Yellow precipitate	Yellow precipitate	
Steroids			
Liebermann- buchard test	Bluish green colour	No bluish green colour	No bluish green colour
Salkowski test	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer	No Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer
Carbohydrate			
Molish test	Violet ring	Violet ring	Intense dark violet ring
Fehling's test	Brick red precipitate	Brick red precipitate	Brick red precipitate
Benedict's test	Red precipitate	Red precipitate	Red precipitate
Tannin			
With $FeCl_3$	Dark blue or green or brown	Yellowish brown colour	Yellowish brown colour
Flavonoids			
Shinoda's test	Red or pink	No pink color	No pink color
Saponins			
With $NaHCO_3$	Stable froth	No stable froth	No stable froth
Triterpinoids			
Tin and thionyl chloride test	Pink/red colour	No red colour	Dark red colour

Coumarins			
With 2N NaOH	yellow	Colourless solution	White precipitate
Phenols			
With alcoholic ferric chloride	Blue to blue black	Yellowish brown color	Yellowish brown color
Carboxylic acid			
With water and $NaHCO_3$	Brisk effervescence	Brisk effervescence	Brisk effervescence
Amino acid			
With ninhydrine reagent	Purple colour	Purple colour	Purple colour
Resin			
With aqueous acetone	Turbidity	Turbidity	High amount of Turbidity
Quinone			
Conc. Sulphuric acid	Pink/purple/red	Colourless solution	Colourless solution

Table no.3 shows the results of preliminary phytochemical screening of sample1&2.

Tests	Shodhita Lashunakalka	Ashodhita Lashunakalka
Alkoloid		
Steroid	+	
Carbohydrate	+	+
Tannin		
Saponins		
Triterpinoids	+	
Coumarins		
Phenols		
Carboxylic acids	+	+
Aminoacids	+	+
Resine	+	+
Quinone		

Results of preliminary phytochemical screening shows the addition of two chemical components named Steroids and Triterpinoids in shodhita Lashunakalka, which was not seen in ashodhita Lashunakalka.

pH:

The pH value conventionally represents the acidity or alkalinity of an aqueous solution.pH is defined as negative logarithm of hydrogen ion concentration, expressed in gms.In this study both shodhitaLashunakalka , ashodhitaLashunakalka, plain butter milk and Lashuna soaked butter milk were subjected to pH estimation and the results are tabulated in table no.4.

Table no.4 shows the pH of samples.

Samples	pH
ShodhitaLashunakalka	6.56
AshodhitaLashunakalka	6.4
Plain butter milk	3.97
Lashuna soaked butter milk	4.2

Shodhita and ashodhitaLashunakalka as well as plain butter milk and Lashuna soaked butter milk have shown difference in pH value, but this difference is not significant.

Thin layer Chromatography (TLC)[7]

Thin layer Chromatography is a non –destructive procedure, where mixtures of components are separated in to individual components through equilibrium distribution between two phases. It is an important tool for qualitative and quantitative analysis. ShodithaLashunakalka, ashodithaLashunakalka,Lashuna soaked buttermilk, Plain buttermilk were subjected to Thin Layer Chromatography at Skanda Life Sciences Pvt. Ltd., Bengaluru.

Procedure

10mg/ml samples were prepared 2.5 µl of samples were spotted on TLC plate and allowed to dry. A TLC plate is made up of a thin layer of Silica gel 0.25mm with fluorescent indicator F_{254} with Solvent system Chloroform: methanol (9.5:0.5) was used for TLC analysis. The strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components. This is called as the development of TLC plates. The separation depends on several factors, the plate is removed after an optimal development time and dried and the spots/zones are detected using UV chamber and Rf value is calculated using,

Rf = Distance moved by compound /distance moved by solvent and compared for the changes in Rf value.

RESULT of TLC

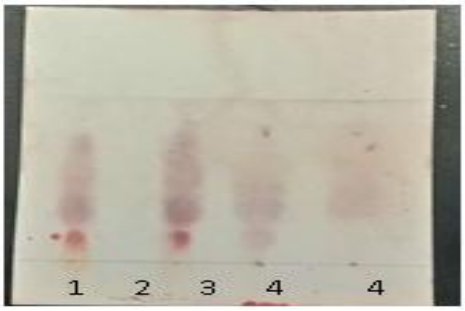

Fig 1: TLC chromatogram for sample at visible (lane 1- 1, lane 2 – 2, lane 3- 3, lane 4- 4.

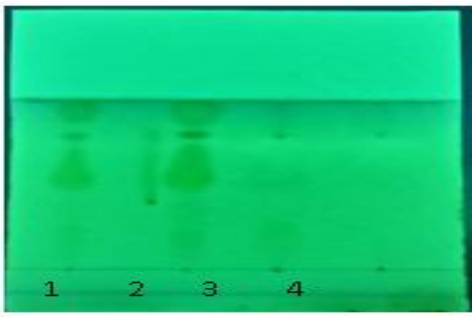

Fig 2: TLC chromatogram for sample at 254nm (lane 1- 1, lane 2 – 2, lane 3- 3, lane 4- 4.

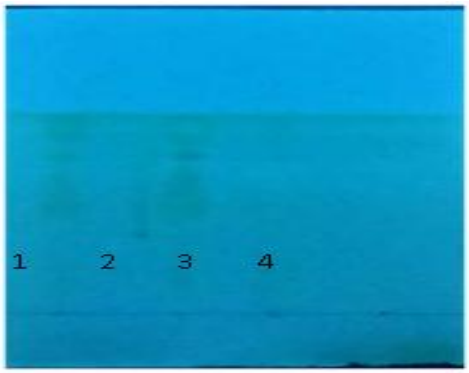

Fig 3: TLC chromatogram for sample at 254nm (lane 1- 1, lane 2 – 2, lane 3- 3, lane 4- 4.

Table no.5 shows TLC characteristic for samples.

Sample	TLC Band at 254 nm	Retention Factor	TLC Profile characteristics		
			366 nm	366 nm	366 nm
1	3	0.50	light blue	light blue	light blue
		0.78	light blue	light blue	light blue
		0.80	light blue	light blue	light blue
		1	-	-	-
2	3	0.50	light blue	light blue	light blue
		0.78	light blue	light blue	light blue
		0.80	light blue	light blue	light blue
		1	-	-	-
3	2	0.783216783	light blue	light blue	light blue
		0.797202797	-	-	-
4	2	0.783216783	light blue	light blue	light blue
		0.797202797	-	-	-

TLC of ashodhita Lashunakalka and shodhita Lashuna kalka does shown much variation in retention factor as well as TLC profile characteristics at visible light, 366nm and 254nm region. TLC of Lashuna soaked butter milk and plain butter milk also does not shown any variation.

DISCUSSION

In Sanskrit, garlic is commonly known as Lashuna or Rasona. The species that is commonly available in India is botanically known as Allium sativum This is extensively used as medicine all over the world. In spite of its therapeutic excellence for which it is compared to ambrosia, as a common ingredient of food, it is not held in high esteem in smruti shastras, perhaps, because of its bad odour. Chemical constituent named allicin is mainly responsible for pungent odour. When garlic cells are injured(i.e.cut, crused etc.) the enzyme alliinase converts alliin contained in raw garlic to allicin to protect itself from bacteria and other disease causing organisms[8]. Hence to make the Lashuna kalka free from pungent odour, Acharya Sharangadhara might have introduced this Lashuna shodhana as a preoperative procedure in the preparation of it.

In the present study, two samples of Lashunakalkawereprepared and both were subjected to organoleptic characters,phytochemical screening, pH estimation and TLC. In the first sample, before grinding Lashuna, it was subjected to shodhana procedure i.e soaking Lashuna in butter milk for 12 hours, after peeling off its outer covering and separating green sprout present in the center of the clove. Second sample was prepared just by grindingthe peeledLashuna. A variation in organoleptic characters was observed in between two samples. Strong pungent odour and taste ofLashuna was found reduced in shodhita sample, when compared to ashodhita sample. Separation of green sprout from the center of Lashuna clove and soaking in butter milk is responsible for this variation. A kind of chemical reactions takes place between sulpher-containing compounds which will be released during grinding of Lashuna and the buttermilk. Buttermilk helps in neutralizing the strong smell of Lashuna. Shodhita Lashuna kalka was creamish in colour, whereas ashodhita Lashuna was blackish white .Sulpher containing compounds which are released during grinding is responsible for the blackish white colour of the ashodhita Lashuna kalka. Whereas in shodhita Lashuna kalka colour was maintained, indicating the absence or reduction in percentage ofsulphurcontaining compounds. White and sour taste of butter milk was changed to yellowish white and pungent sour respectively due to soaking of Lashuna in it. This might be because of diffusion of components betweenLashuna and the buttermilk.

The Triterpenoid and Steroids which were not seen initially in the ashoditaLashunakalka were seen after the shodhana through the phytochemical analysis test. These two chemical components might be imparted from buttermilk. Triterpinoid and Steroids both are known for its analgesic, anti-inflammatory property. And also, it helps to maintain blood sugar level. ShodhitaLashunakalka is indicated in Vatavyadhi as per Sharangadhara. Inflammatory conditions predominating with pain is the usual signs of Vatavyadi. Lashuna is well known for its vatahara property, since shodhita Lashuna kalka which contains Triterpenoids and Steroids helps to conquer the symptoms of Vatavyadhi better than ashodhita Lashuna kalka.. Henceforth shodhana of Lashuna is must as a pre-operative procedure before administration in the form of Kalka. Therefore, the concept put forth by Acharya Sharangadhara is precise.

By removing the outer covering and green sprout from the middle of clove, easy digestion in the Gastro-intestinal tract can be expected and hence better absorption as these are hard to digest. All together shodhana procedure might help in reducing the garlicky belching/nausea after the consumption of Lashuna. Therefore, the reference which is present in the SharangadharaSamhita can be accepted.

CONCLUSION

The Shodhana of Lashuna by peeling its outer covering, removing the inner green sprout and soaking in buttermilk reduces the pungent taste and odor of Lashunakalka which has been proved by the organoleptic evaluation. The shoditha Lashuna kalka shows the presence of Triterpenoids and steroids which were not present in the ashoditha Lashuna kalka which was confirmed by the phytochemical analysis. Hence the concept of preoperative procedure of Lashuna kalka preparation putforth by AcharyaSharanghadhara can be accepted. Quantitative analysis can give the better results to prove the variation in the properties of shodhita and ashoditha Lashunakalka. Clinical study on Vatavyadhi by using shodhita and ashoditha Lashunakalka may help in proving its efficacy as well as reduction in helitosis.

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