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PHYTOCHEMICAL ANALYSIS AND STANDARDIZATION OF *Lashuna churna* (A HERBAL POWDER DRUG OF *Allium sativum)* THROUGH HPTLC

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ABSTRACT

Lashuna (Allium Sativum) is a popular medicinal plant in Ayurveda, India's ancient medical system.The present study was aimed to screen the organoleptic analysis, physico-chemical analysis and phytochemical constituents through preliminary phytochemical tests of *lashuna churna* (powdered form of *Allium sativum*) and to standardize this herbal powdered drug through High Performance Thin Layer Chromatography fingerprinting. The preliminary phytochemical screening of the extract revealed the presence of bioactive compounds like alkaloids, saponins, flavonoids, steroids and terpenoids. The HPTLC fingerprint profile, obtained from this study, of the same herbal formulation may be used for furthur authenticity and quality analysis.

Keywords: Ayurveda, Bioactive compounds, *Lashuna*, Herbal powdered formulation, HPTLC, Phytochemical analyses, Standardization

INTRODUCTION

Ayurveda is the traditional Indian system of medicine being practiced for thousands of years. In Ayurveda natural products like plants, animals and minerals are used for the

treatment of various diseases, mostly the plants are used to derive therapeutic materials. Indian medicinal plants also provide a rich source for antioxidants that are known to prevent/delay different diseased states. India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health *viz*. Ayurveda[1]. *Lashuna* (*Allium sativum*) is such medicinal plant having various medicinal properties and used to treat various diseases[2]. In this study Extract of *lashuna churna i.e.* powdered form of useful parts (Table no.1) of this herb is used for preliminary phytochemical screening and standardization through High Performance Thin Layer Chromatography (HPTLC). The present study was aimed to determine the phytochemical constituents of *lashuna churna* as well as to standardize this particular formulation which will provide another useful resource for future.

MATERIALS AND METHODS

Plant material

The ingredient (Table-1) was procured from the local market. The collected drug was identified and authenticated at the teaching pharmacy of Department of Dravyaguna, Parul Institute of Ayurved, Limda, Waghodia, Vadodara,Gujarat.

Methodology of preparation of *lashuna churna*[3]

Lashuna churna was prepared at GMP Certified- Parul Ayurved Pharmacy, Parul University,Limda,Vadodara,Gujarat.The Bulb of *Lashuna(Allium sativum)* was collected and peeling was done . Then the peeled bulb of *Lashuna(Allium sativum)* was soaked in *Takra* (Butter milk) for one night.The next day the bulb was separately collected.Then it was grinded and made into fine *kalka* (Paste) form and dried in shade.After drying the fine powders was prepared as *lashuna churna.* This mixture of powders was stored in a closed vessel for future use.

Phytochemical analysis:

Preliminary Organoleptic analysis,Physico-Chemical analysis and phytochemical screening and phytochemical studies through HPTLC were carried out at Vasu Research Centre, Makarpura, Vadodara-390010, Gujarat, India as per the standard procedures.

Table 1: Ingredients (Plant materials)

Sl. No.	Ingredient	Latin Name	Part Used
1	*Lashuna*	*Allium sativum*	Bulb

Preliminary phytochemical tests[4, 5]

The preliminary phytochemical screening was performed according to the standard procedure. The procedures are as follows:

Test for alkaloids

Wagner's test: About 1ml of extract and 1ml of Wagner's reagent (dilute iodine solution) are added and mixed. Formation of reddish-brown precipitates indicates the presence of alkaloids.

Dragendroff's Test

To a few milligrams of extract dissolved in alcohol, a few drops of acetic acid and dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Mayer's Test

To a few milligrams of extract dissolved in acetic acid, a few drops of mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's Test

To a few milligrams of extract dissolved in acetic acid, 3 ml of hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Test for carbohydrates

Molisch's Test

To the extract, 1 ml of α-naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's Test

A few milligrams of extract were mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's Test

To 5 ml of Benedict's reagent, a few milligrams of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates

Test for steroids

Libermann Burchard Test

To the extract dissolved in choloro form, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of con.H_2SO_4 were added along the sides of the test tube. Appearance of bluish green color indicates the presence of steroids.

Salkowski Test

The extract was dissolved in chloroform and equal volume of con. H_2SO_4 was added. Formation of bluish red to cheery red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for Saponins

To a few milligrams of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for Tannin

To the extract a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for Flavonoids

Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. H_2SO_4 were added and heated on a water bath. Formation of red red to pink colour indicates the presence of flavonoids.

Test for Phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for Coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for Triterpenoids

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for Carboxylic Acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

Few milligrams of the sample was mixed with water and acetone. Turbidity indicates the presence of resin.

Test for quinine

A few milligrams of alcohol extract was treated with 0.5% of sodium hydroxide. Deep colouration like pink, purple or red indicates the presence of quinine.

High Performance Thin Layer Chromatography [6, 7]

Preparation of Test Solution

2.5 g of sample was weighed in an Iodine Flask and 50 ml of Methanol is added to it. Vortex the iodine flask for 1 hour to dissolve the sample. Then the sample was filtered through Whatman Filter Paper which futher filtered with Syringe Filter. The filtrate thus obtained was used for HPTLC fingerprinting.

Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]

0.5 ml Anisaldehyde is mixed with 10 mL Glacial acetic acid, followed by 85 mL Methanol and 5 mL Sulphuric acid (98 %). 10.0 µl of the above extract were applied on a pre-coated Silica gel 60 F254 on aluminum sheets to a band width of 10 mm using CAMAG Linomat 5 TLC applicator. The

plate was developed in Toluene: Ethyl acetate: Acetic acid (7: 2:1 v/v/v). The developed plates were visualized in short UV 254nm, 366nm, and then derivatised with Anisaldehyde Sulphuric acid reagent and scanned under UV 254nm, 366nm and 540nm. Rf and densitometric scan were recorded.

Table 2: Organoleptic and Physico-Chemical Analysis

Sr. No.	Parameters	Results
1	Description	Brown coloured powder
2	Odour	Characteristic
3	pH	5.50

Table 3: Phytochemical constituents of Lashuna churna

Sr. No.	Parameters	Results
1	Alkaloid	+
2	Starch	-
3	Tannins & Polyphenols	-
4	Saponins	+
5	Flavonoids	++
6	Carbohydrates	-
7	Proteins	-
8	Steroids	++
9	Terpenoids	++
10	Anthraquinone	-
Key word: "+, ++, +++" indicates Present in increasing intensity and " - " indicates Absent		

Table 4: R f value at 254nm

Spot No.	Track T 1
1	0.12
2	0.29
3	0.60
4	0.65

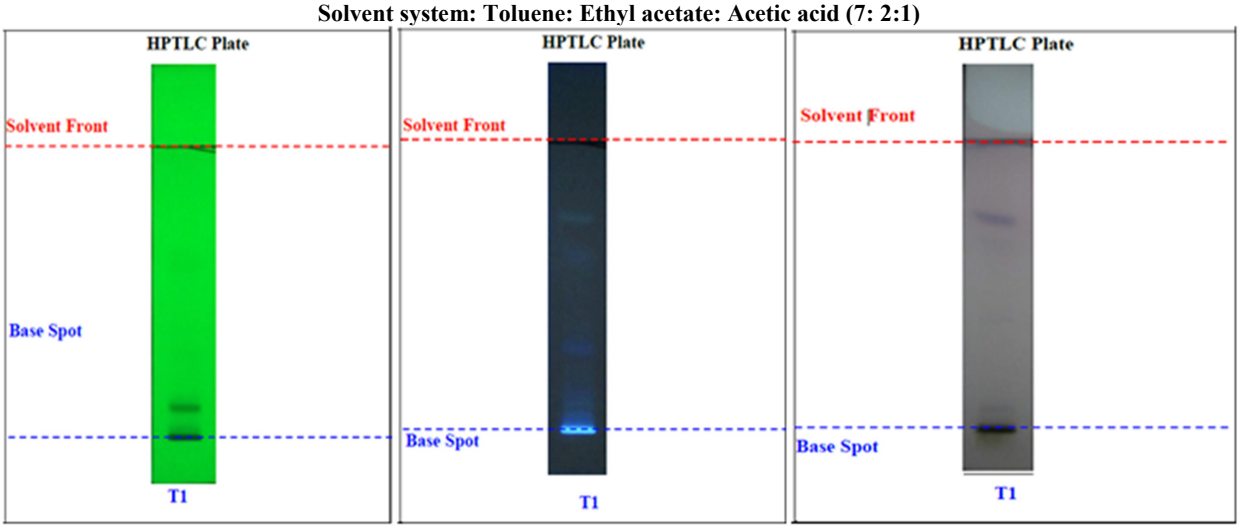
Table 5: R f value at 366nm

Spot No.	Track T 1
1	0.74

Table 6: R f value at 540nm

Spot No.	Track T1
1	0.12
2	0.39
3	0.60
4	0.65
5	0.74

Track T1- *Lashuna churna*
Solvent system: Toluene: Ethyl acetate: Acetic acid (7: 2:1)



At 254nm **at 366nm** **at 540nm (after Derivatization)**

Fig- 1: HPTLC Photo Documentation of Alcohol Extract of *Lashuna churna*

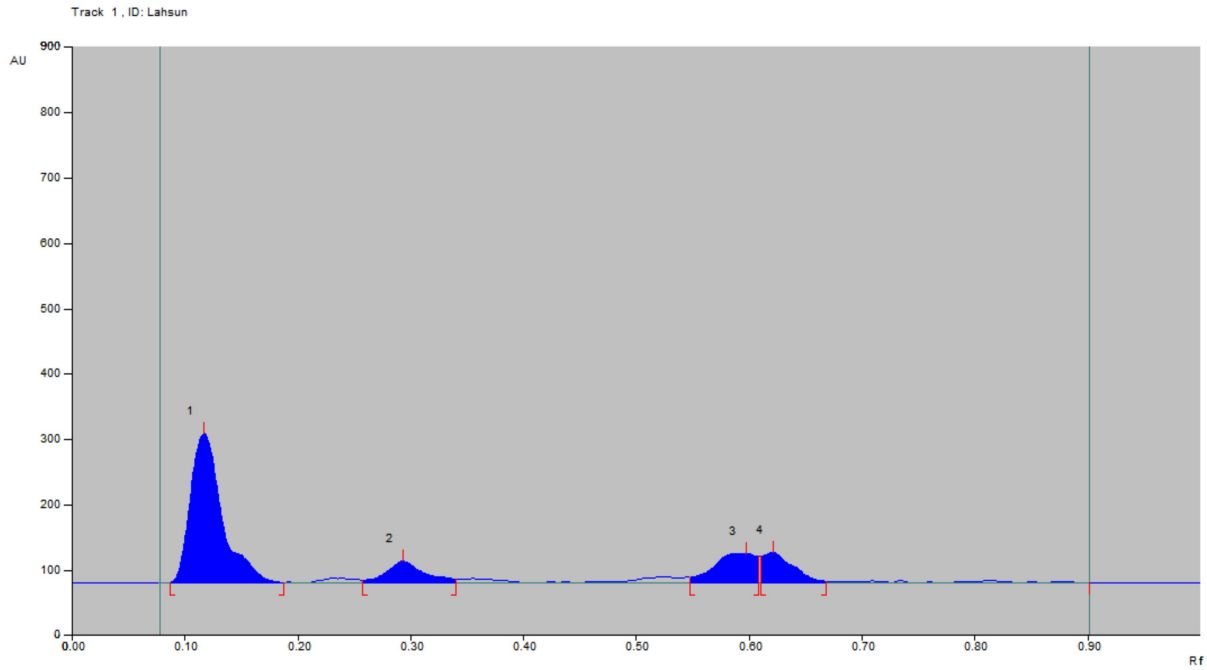


Fig- 2: HPTLC 2D Chromatogram at 254 nm

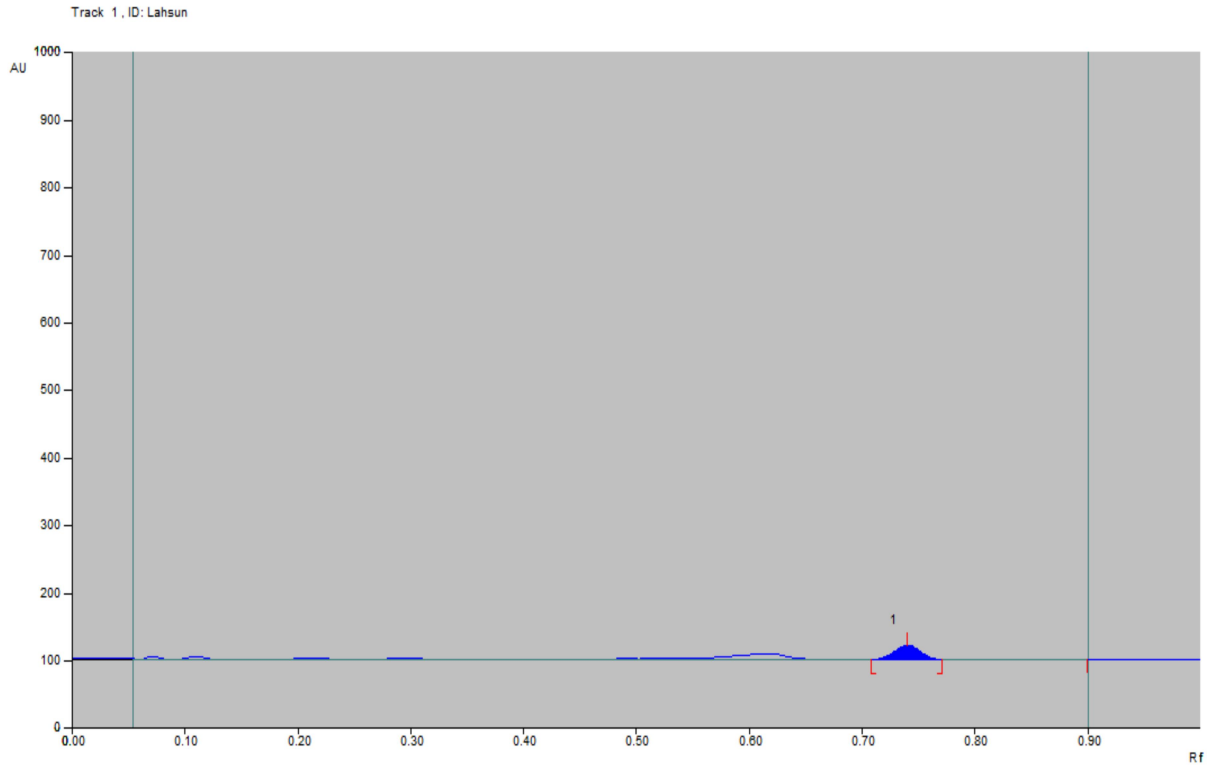


Fig- 3: HPTLC 2D Chromatogram at 366 nm

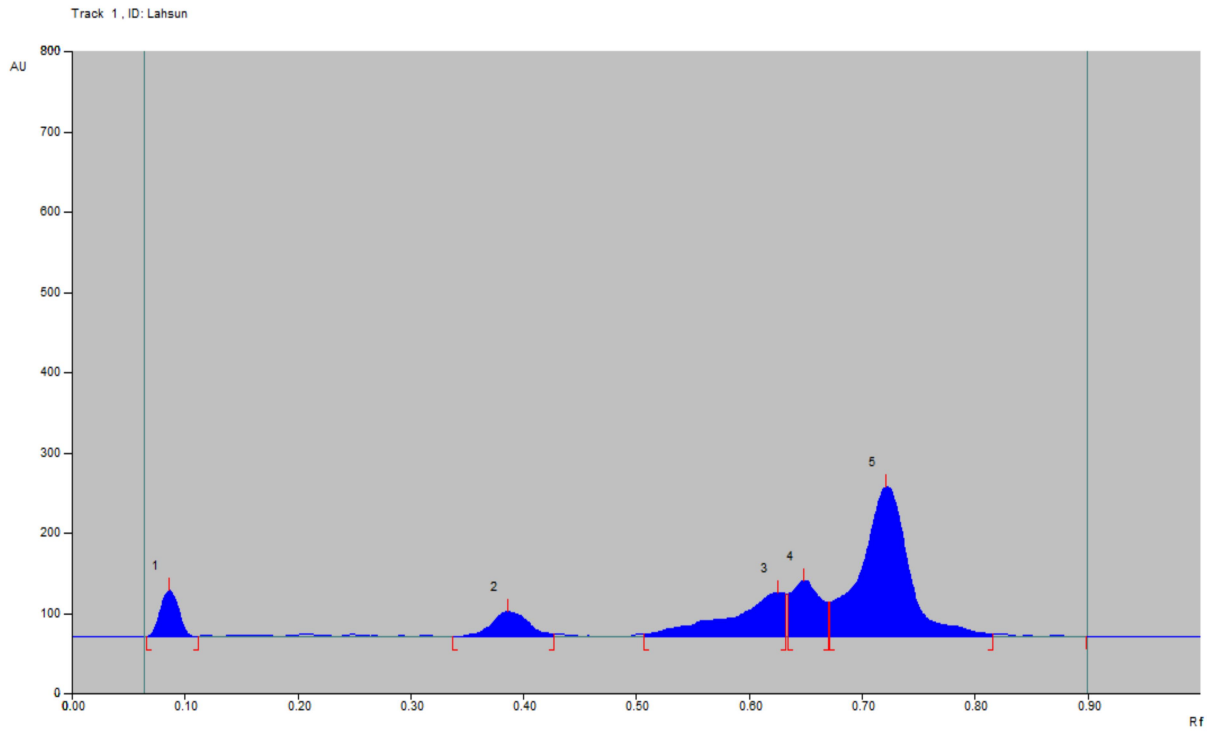


Fig - 4: HPTLC 2D Chromatogram at 540 nm

RESULTS AND DISCUSSION

Organoleptic and Physico-chemical Analysis

Organoleptic and Physico-chemical characters are illustrated in (Table 2). The description provides as brown coloured powder having characteristic odour and taste and pH value as 5.50.

Preliminary Phytochemical Tests

The phytochemical screening results showed the presence of alkaloids, saponin, flavonoids, steroids, terpenoids, in the extract of *lashuna churna* (Table 3). Most of the identified phytochemical compounds have been reported to have various biological activities *viz*. the alkaloids is a multi target and multi path plant extract and can interfere with the development of PCOS and relate to pathological process from many aspects with less adverse reactions. It can alleviate insulin resistance, reduce the level of serum androgen regulate lipid metabolism and pacify chronic inflammation[8]. Saponins alleviate the inflammatory burden, insulin resistance and adipokine expression in obesity and hence prevent the secondary gonadal complications in female subjects and also control reproductive system pathologies[9] .Flavonoids have good effects on anti –inflammatory, analgesic, immunomodulator and could restore the ovulation function ,it has anti-proliferative and antioxidant activities[10]. Steroids possess anti-inflammatory properties[11]. Terpenoids possess cytotoxic (may cause tumours to shrink in size) and anti-inflammatory activities.[12].

High Performance Thin Layer Chromatography (HPTLC)

HPTLC photo documentation of *Lashuna churna* (Fig -1) showed four, one and five spots under 254 nm, 366 nm and 540 nm after derivatization respectively. Spot with Rf 0.12, 0.60, 0.65, and 0.74 were commonly detected in any two detection methods. All the three methods gave optimum separation of different bands and hence all of them may be used as HPTLC fingerprint pattern to identify the composition of the mixture (Table no. 4, 5, 6). Densitometric scan at 254 nm revealed 1 high peak and 3 peaks corresponding to 4 different compounds in the ethanol extract, compounds with Rf 0.12, 0.29, 0.60 and 0.65 were the peaks (Fig- 2). At 366 nm there was one high peak, with Rf 0.74, being the major peak detected (Fig- 3). At 540 nm there were five peaks and three high peaks, with Rf 0.12, 0.60 and 0.65 being the major peaks detected (Fig- 4).

CONCLUSION:

Preliminary phytochemical tests of the extract of *Lashuna churna* showed the presence of alkaloids, saponins, flavonoids,

steroids, terpenoids, which are reportedly bioactive in nature and may add up to the therapeutic effect of this herbal drug. HPTLC fingerprint profile of the same herbal formulation may be used for authentication and quality control. The analytical data and HPTLC finger print profile obtained in the present study for Lashuna churna will help to develop SMP (Standard manufacturing process) of *Lashuna churna* which will become a standard for further study and other remedies in future.

Conflict of interest: None Declared.

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