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Research Article

HPTLC Analysis and Standardization of *Linum usitatissimum* L.

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ABSTRACT
The main objective of the study was to formulate the herbal tablets and standardizing the same for quality and purity of the formulation. In the present investigation flaxseed (*Linum usitatissimum* L**)** extract was used for preparation of tablets. From the available literatures it's evident that there is a need for development of proper medication and dosage form for the treatment of constipation. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, and WIN CATS-4 software were used. HPTLC finger printing of crude drug, extract and formulation of linseed seed was carried out with mobile phase, which showed different Rf values. HPTLC finger printing of crude drug, extract and formulation of linseed in the aqueous extract of *L. usitatissimum* showed 7 peaks by HPTLC analysis. The Rf values of standard arginine, glutamic acid and glycine were found to be 0.15, 0.62 and 0.73 respectively. The Rf values of arginine, glutamic acid and glycine of *L. usitatissimum* was, 0.15, 0.63 and 0.75 and mached with the standard peaks which confirmed the presence of arginine, glutamic acid and glycine in *L. usitatissimum.* The content of amino acid was estimated by comparing the peak area of standard and those present in the formulation. Each 10 ml of *L. usitatissimum* was found to contain 0.05 mg, 1.4 mg and 1.82 mg of arginine, glutamic acid and glycine respectively. *Linum usitatissimum* seed has a tremendous scope on further studies mainly in the area of Nutraceutical and dietary supplements.

Keywords: HPTLC, *Linum usitatissimum*, Standardization.

INTRODUCTION

Linseed Linum usitatissimum, cultivated flax[1] which is an annual plant. Flaxseed, called ('Tisi' or 'Alsi') in northern India Cultivated flax plants grow to 1.2 m, with slender stems. The leaves are green, slender lanceolate, 20–40 mm long and 3 mm broad. The flowers are pure pale blue, 15–25 mm diameter, with five petals. The fruit is a round, dry capsule 5–9 mm diameter, containing several glossy brown seeds shaped like an apple, 4–7 mm long. It is regularly used as a laxative in ayurvedic medicine, in ayurveda linseed pacifies vatta, pitta and increase kappa. In ayurveda linseed is treated as daily food, in ancient times linseed was used as food. There is a lot of fibre in linseed therefore it provides relief to patients of constipation. Flaxseed provides relieves from day one in constipation.

Flaxseed contains omega 3 fatty acids, alpha linolenic acid, lignen, protein and fiber. Flaxseed has 30-40% fixed oil, fiber, protein, vitamins, calcium, chromium, copper, iron, magnesium, Potassium, selenium, zinc, etc., flaxseed increases good cholesterol (HDL) Cholesterol and reduces bad cholesterol (LDL) cholesterol. Flaxseed is also used in the treatment of diseases like hypertension, cardiac arrest and stroke. Flaxseed is effective in treatment of skin diseases, like acne, eczema. Flaxseed controls blood sugar and treats diabetes; it also provides relief from obesity. Flaxseed is very effective in the treatment of cancer. The Ayurvedic specifically approves flaxseed external use as a poultice for boils and internal use as a demulcent or laxative[2]. From the available plethora of literatures, it's evident that there is a need for development of the proper medication and dosage form for the treatment of constipation.

HPTLC is a sophisticated instrument. The advantages of HPTLC are automation, scanning, full optimization, selective detection principle, minimum sample preparation, and so on enable it to be a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products[3]. In this present study the Preliminary phytochemical screening of *Linum usitatissimum L.* has been done to identify the chemical constituents and HPTLC fingerprinting provide quantitative information about the marker compounds or active constituents present in the crude herbal products and it can be used as a diagnostic tool for the correct identification of phytoconstituents and detect adulterants of the plant.

MATERIALS AND METHODS

Plant material

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Table 1: HPTLC Analysis of Standard Arginine, Crude Drug, Extracts and Formulation.

S.No.	Marker compounds	Standard R_f value	Samples	Sample R_f values	Amount of marker compound
1.	L-Arginine	0.15	Crude drug	0.14	0.03
			Extract	0.15	0.05
			Formulation	0.14	0.02

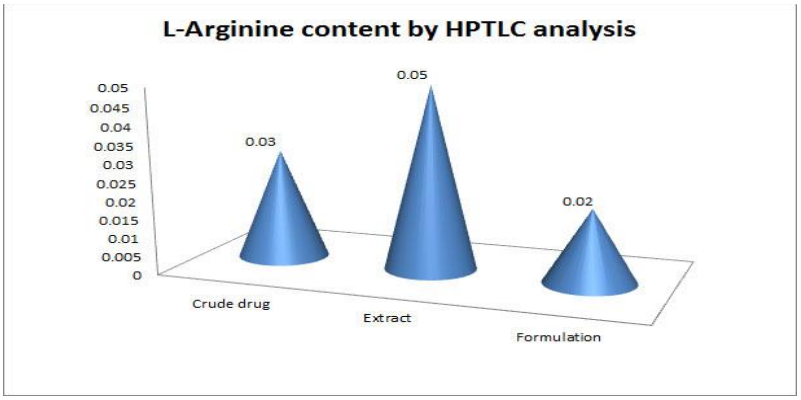

Figure 1

Table 2: HPTLC Analysis of Standard Glutamic Acid, Crude Drug, Extracts and Formulation.

S.No.	Marker compounds	Standard R_f value	Samples	Sample R_f values	Amount of marker compound
1.	Glutamic Acid	0.62	Crude drug	0.14	0.02
			Extract	0.61	0.90
			Formulation	0.63	0.82

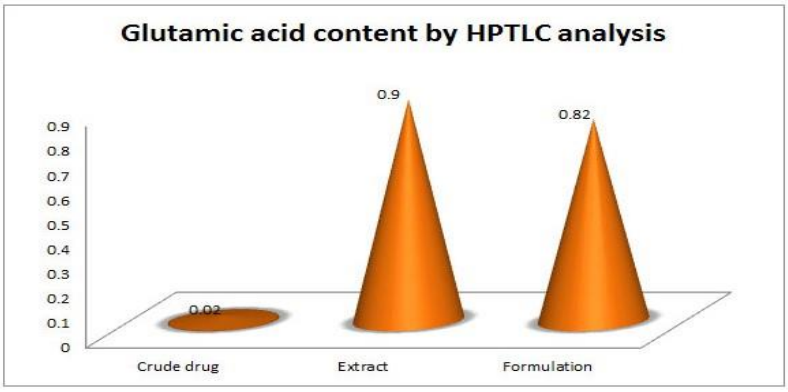

Figure 2

Linum usitatissimum Linn was purchased from Yucca Enterprises Mumbai. The flax seeds were coarsely powdered and stored in a suitable container for further studies.

Preparation of Linseed extract by Pilot Scale Extraction Plant and Spray drying Method[4]

The extraction of linseed seed extract was carried out by pilot scale extractor. The flaxseed of 1kgwas taken and boiled in 8 liters of water for 2hrs in ratio the 1:8 and filtered and the filtrate was spray dried in a spray drier and the yield obtained was 5 mg for every 20 liters of linseed.

Qualitative Phytochemical Screening[5,6]

The different qualitative chemical tests were performed for establishing profile of a given extract for its nature of chemical composition. The chemical tests were carried out on the extract to detect various phytoconstituents. The extract was subjected to various quantitative phytochemical tests and the results reveal the presence of amino acids, carbohydrates, fatty acids, vitamins and minerals etc.,

HPTLC (High Performance Thin Layer Chromatography)[7-10]

HPTLC is a modern sophisticated and automated form of thin layer chromatography and the most simple separation technique today available to the scientist. The HPTLC system was introduced in 1975 and it has the following advantages over conventional TLC. As a powerful separation tool for quantitative analysis with high sample throughput and it is referred as HPTLC. This technique is widely used in pharmaceutical industries. It is unequalled for content uniformity and other quality control analysis.

Preparation of Sample solution for HPTLC analysis

Table 3: HPTLC Analysis of Standard Glycine, Crude Drug Extracts and Formulation.

S.No.	Marker compounds	Standard R_f value	Samples	Sample R_f values	Amount of marker compound
1.	Glycine	0.73	Crude drug	0.74	0.45
			Extract	0.75	1.40
			Formulation	0.75	0.46

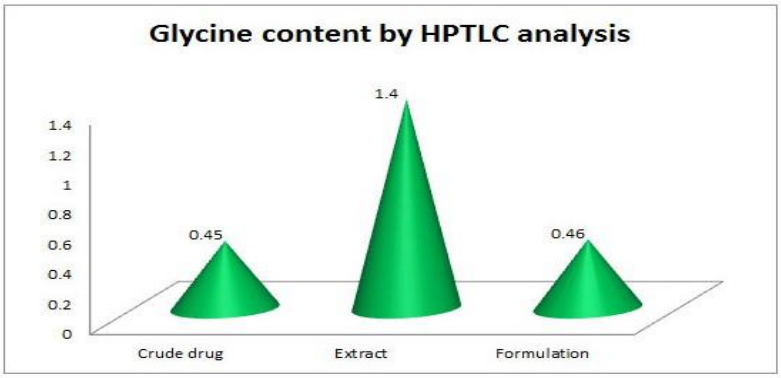

Figure 3

Formulation : 20 mg / ml in methanol

Amino acid Estimation in Linum usitatissimum and Formulation

Stationary Phase: Silica gel F_{254} plates
Mobile phase: n-butanol: acetic acid: water (50:10:40)
Standard 1: L- Arginine (10 µl)
Standard 2: Glutamic acid (10 µl)
Standard 3: Glycine (10 µl)
Sample 1: Crude drug 20 mg / ml (12 µl)
Sample 2: *Linum usitatissimum* extract 20 mg /ml (10 µl)
Sample 3: Linseed Formulation 20 mg / ml (8 µl)
Detection wavelength: 366 nm
Mode of scanning: Absorption (tungsten)

Preparation of Standard Extract

1 mg of arginine, glutamic acid and glycine amino acids were dissolved in 1 ml of water individually, filtered and these solutions were used as the standard solutions (1 mg / ml) for the estimation of amino acids arginine, glutamic acid and glycine in L. usitatissimum.

Standard Solution

Amino acids: 1. L- Arginine -1 mg / ml in water, 2. Glutamic acid -1 mg / ml in water, 3. Glycine - 1 mg / ml in water.

Development of solvent system

A number of solvent systems were tried for extract, but the satisfactory resolution was obtained in the solvent systems. n-butanol: acetic acid: water (50:10:40) (All the reagents of Analytical grade. E. Merck)

Application of Sample

Commercially available Precoated HPTLC aluminium sheets of silics gel F 254 (Merck) were used for the study. The different fractions were applied on plates with band width of 6 mm. Application rate was maintained at 10 µl / min, using Linomat IV applicator (automatic TLC applicator, Camag, Switzerland). Sample volumes of 5 – 15 µl were applied. 10 mcl of aqueous solution of arginine, glutamic acid, Glycine and L. usitatissimum extract (1 mg / ml) were applied as 7 mm bands, using Linomat IV – Applicator.

Chromatogram Development

The plates were developed in Camag HPTLC Twin trough linear development chamber using the solvent system as used in the TLC of different fractions. Camag Scanner III, combined with integration software, (Switzerland) CATS 4.06 (Switzerland). After developing, the plates were air dried and observed under UV light chamber (Camag UV chamber 3, Model no:" 022.9120).

Densitometry Scanning

The developed plates were scanned using densitometer at 254 and 366 nm (Camag TLC scanner – III, combined with integration software, CATS 4.06 (Switzerland). Migration distance was 85 mm, the development chamber was saturated with mobile phase for 1 h at 20-25^0

RESULTS AND DISCUSSION

From the available Literatures survey reveals that this plant *Linum usitatissimum* contains more number of amino acids. There is no research work on the standardization of the active constituent in this plant. The phytochemical test on aqueous seed extract showed the presence of amino acids, carbohydrates, fatty acids, vitamins and minerals etc., were previously reported. So, the present investigation is an attempt made to study its HPTLC studies.

HPTLC analysis of crude drug, aqueous seed extract and formulation of Linum usitatissimum

The mobile phase selected for the HPTLC studies of crude drug, aqueous seed extract and linseed formulation was n-butanol: acetic acid: water in the ratio of (50:10:40). The Rf values of standard arginine, glutamic acid and glycine were found to be 0.15, 0.62 and 0.73 respectively. The Rf values of arginine, glutamic acid and glycine of *L. usitatissimum* was, 0.15, 0.63 and 0.75 and mached with the standard peaks which confirmed the presence of arginine, glutamic acid and glycine in *L. usitatissimum.*

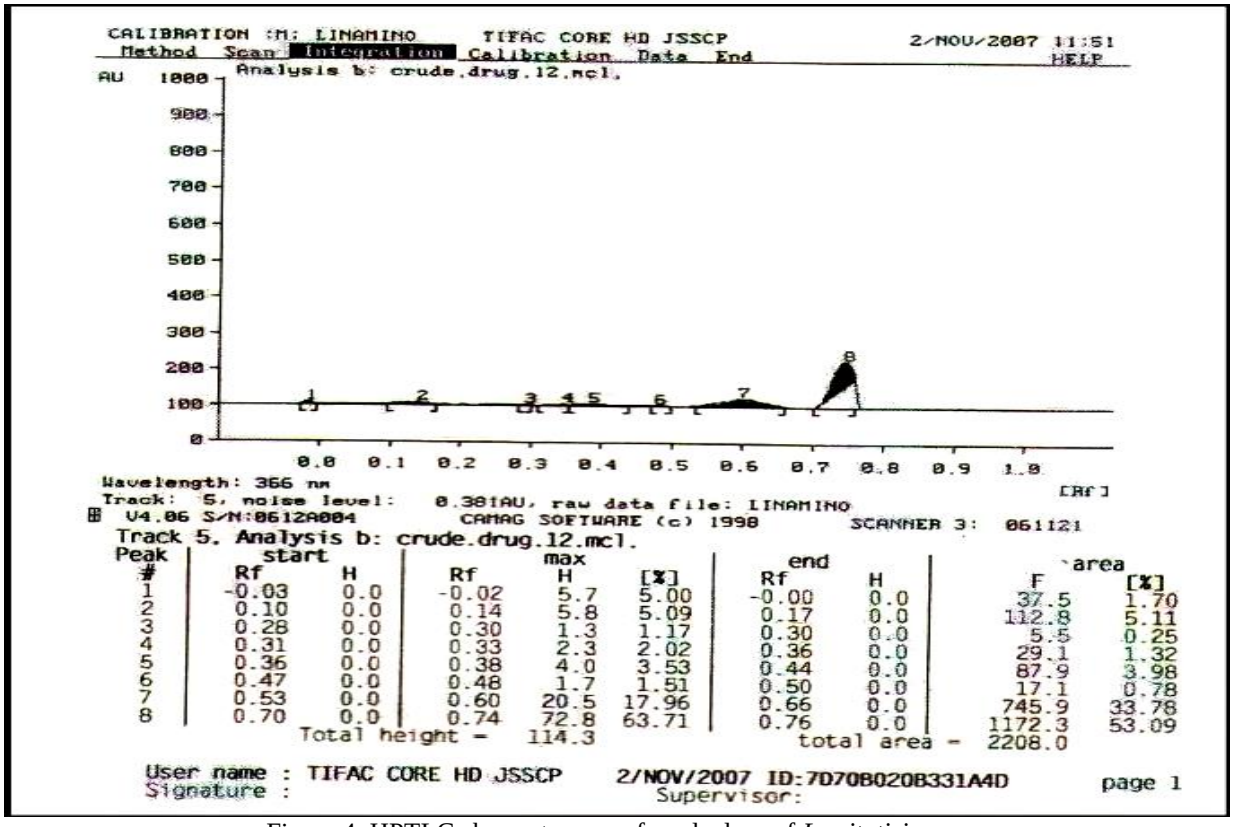

Figure 4: HPTLC chromatogram of crude drug of *L.usitatisimum.*

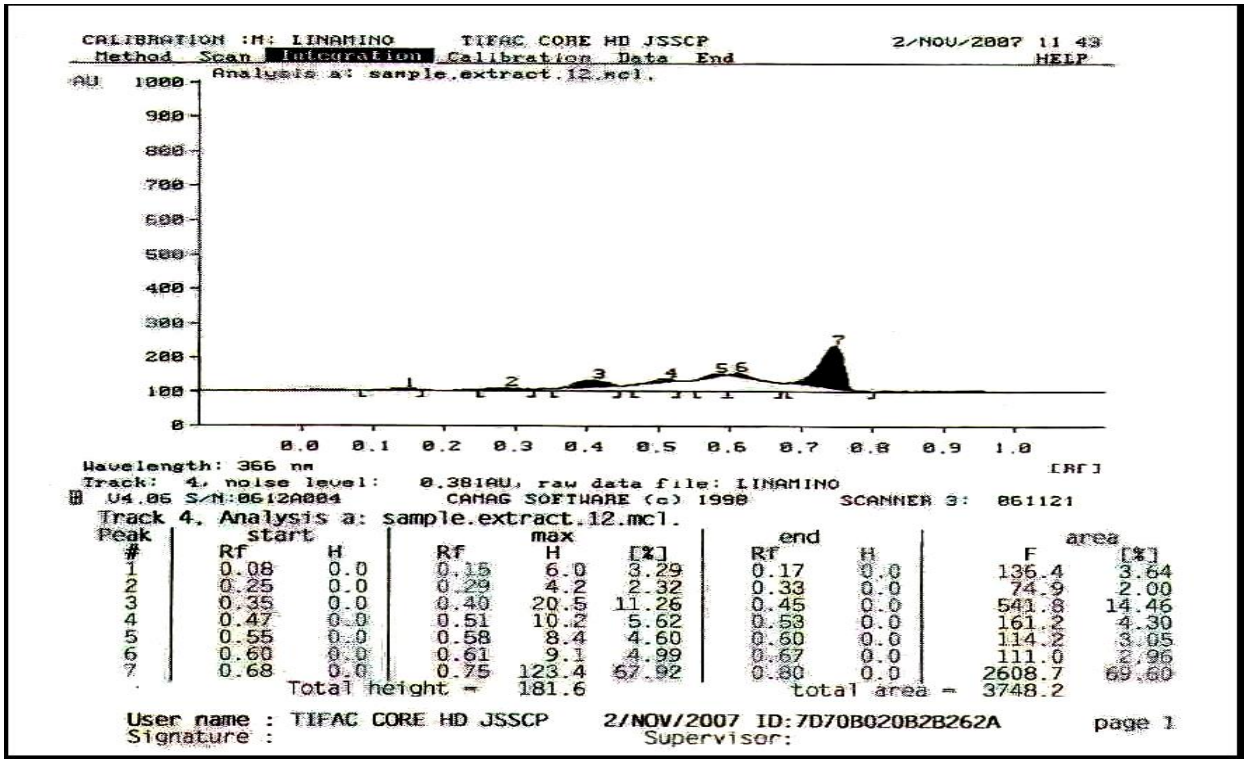

Figure 5: HPTLC chromatogram of Extracts of *L.usitatisimum.*

The content of amino acid was estimated by comparing the peak area of standard and those present in the formulation. Each 10 ml of *L. usitatissimum* was found to contain 0.05 mg, 1.4 mg and 1.82 mg of arginine, glutamic acid and glycine respectively.

The results are reveled in Table 1 & Figure 1. HPTLC Analysis of Standard Glutamic acid, Crude Drug, Extracts and Formulation and the corresponding HPTLC chromatogram was presented in Figure 7. HPTLC chromatogram of standard Glycine. HPTLC Analysis of

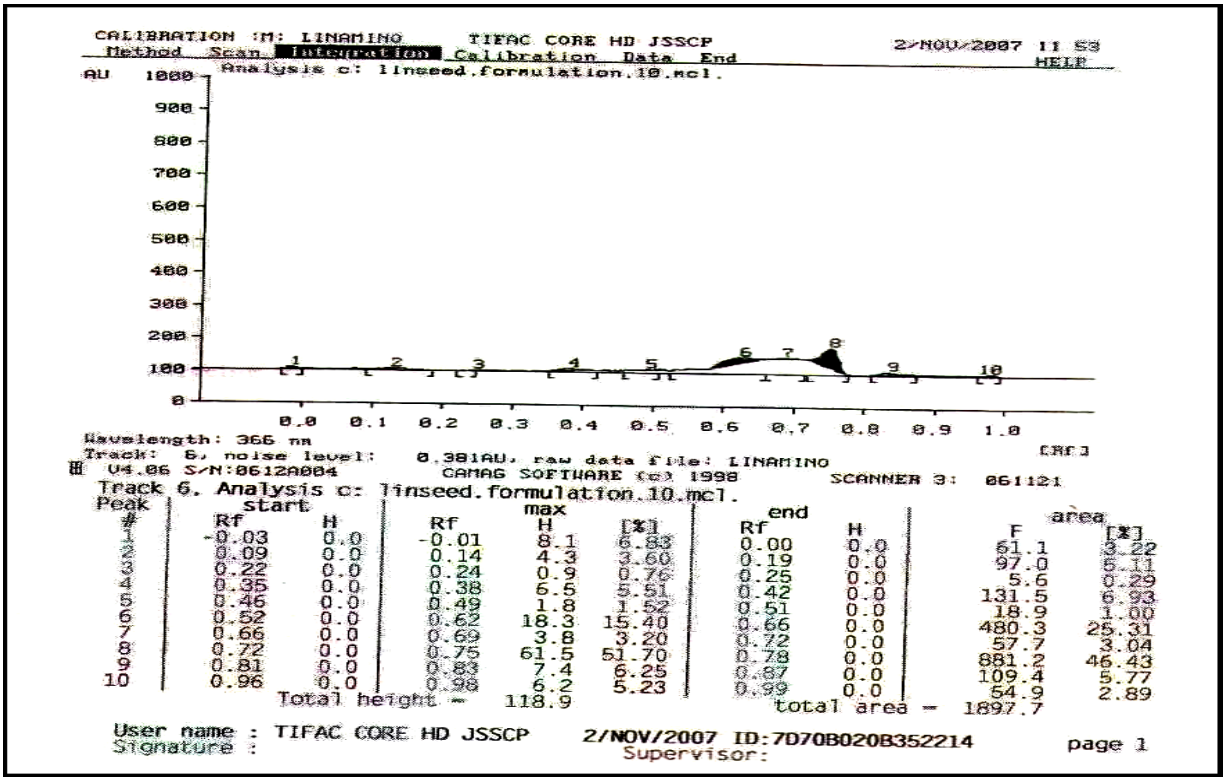

Figure 6: HPTLC chromatogram of *L.usitatisimum* Formulation F3.

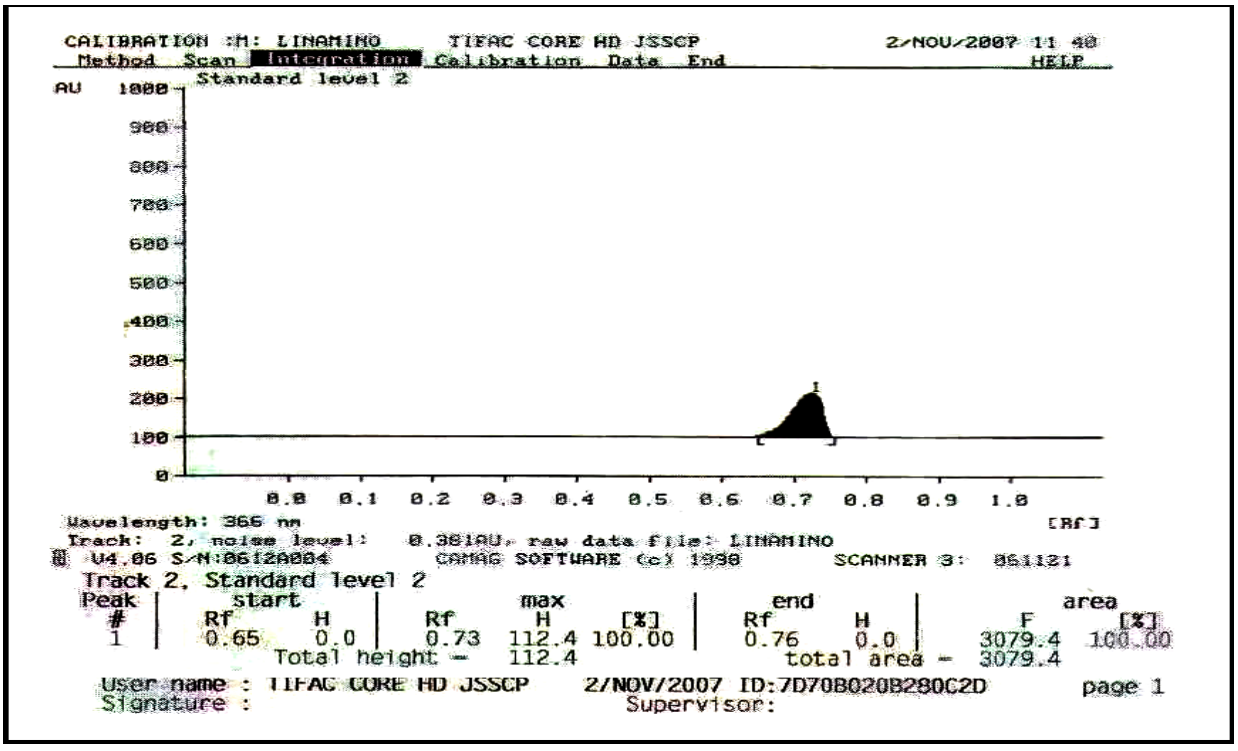

Figure 7: HPTLC chromatogram of standard Glycine.

Standard Glutamic Acid, Crude Drug, Extracts and Formulation are represented in Table 2 & Figure 2 and the corresponding HPTLC chromatogram was presented in Figure 8. HPTLC chromatogram of standard Glutamic Acid. HPTLC Analysis of Standard Arginine, Crude Drug Extracts and Formulation were shown in Table 3 & Figure 3 and the corresponding HPTLC chromatogram was presented in Figure 9. HPTLC chromatogram of standard Arginine. The HPTLC chromatogram of crude drug of *L.usitatisimum* is shown in Figure.4. The HPTLC chromatogram of Extracts of *L.usitatisimum* is shown in Figure.5 and the HPTLC chromatogram of *L.usitatisimum* Formulation F3 is shown in Figure.6:

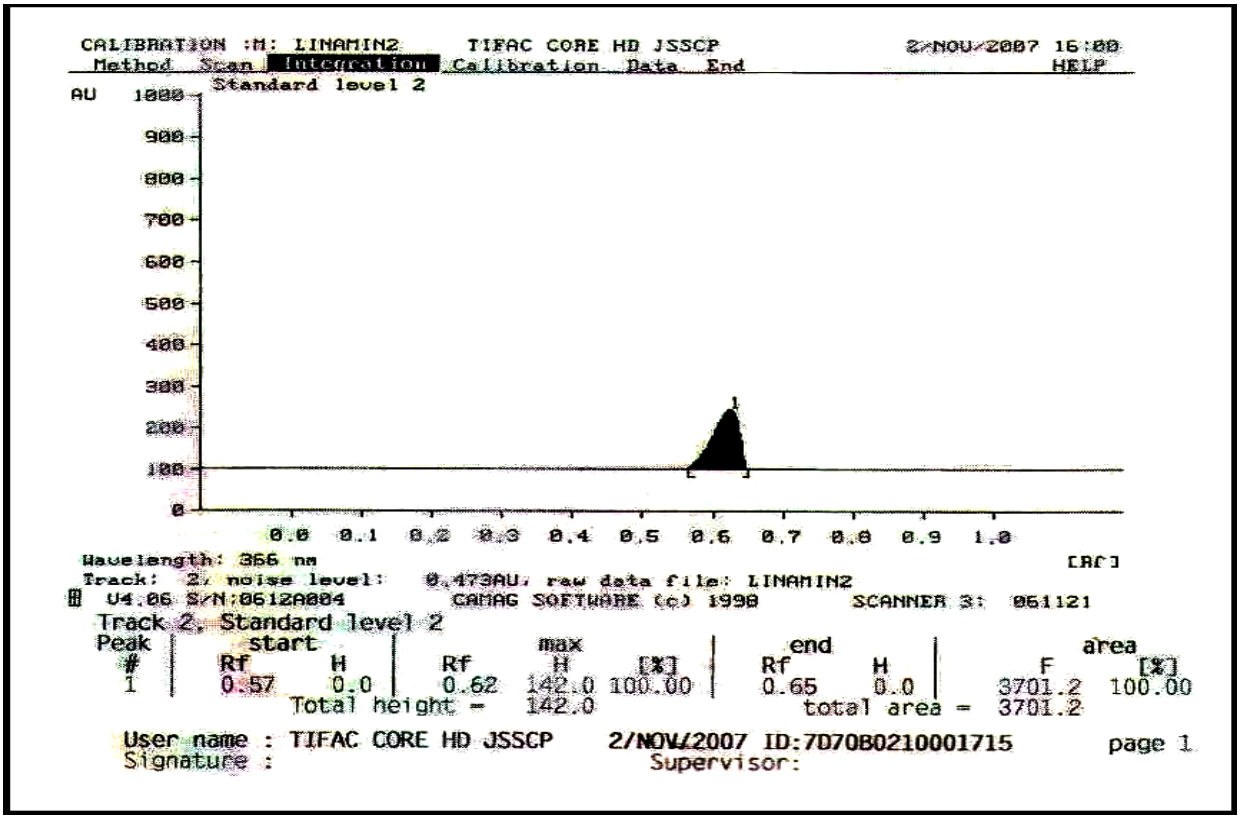

Figure 8: HPTLC chromatogram of standard Glutamic acid.

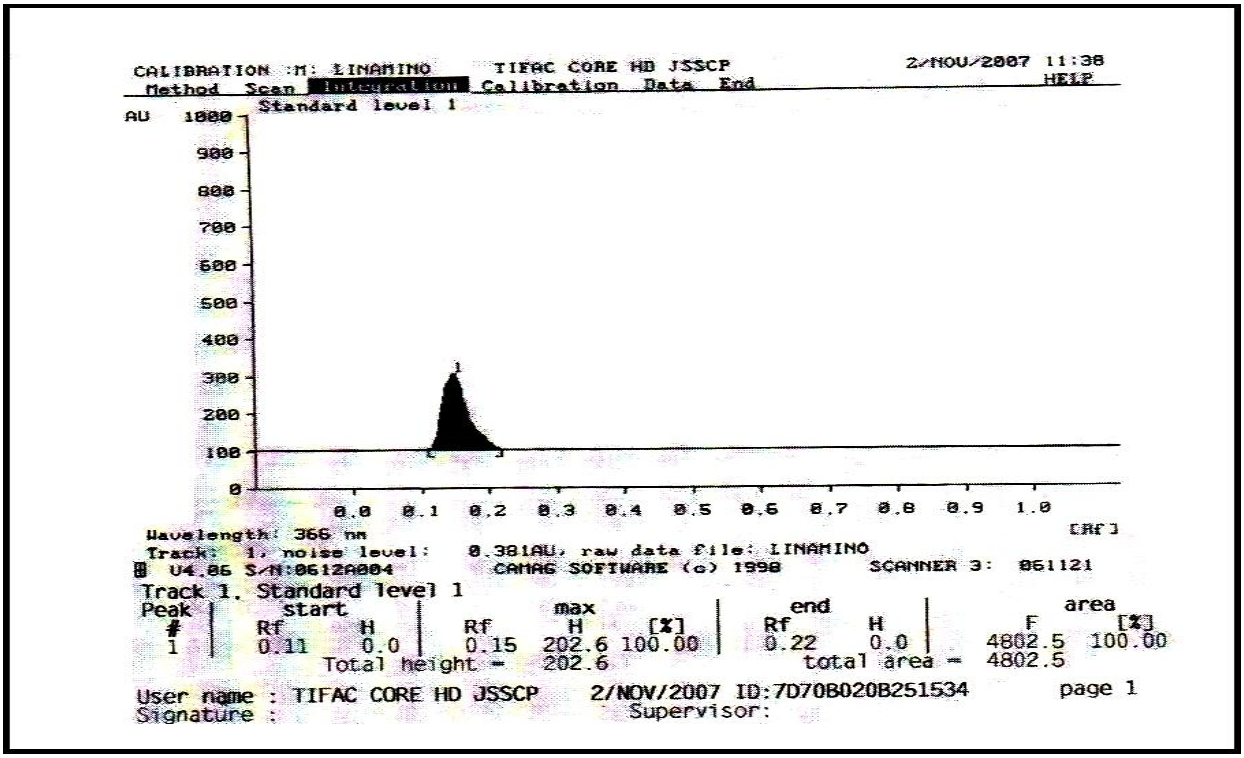

Figure 9: HPTLC chromatogram of standard L-Arginine.

The present studies on preliminary phytochemical screening and HPTLC provide useful information which may help in identifying the plant along with nature of phytoconstituents present in it. HPTLC studies show clear separation of components present in the aqueous seed extract of *L. usitatissimum L.*

CONCLUSION

This estimation method would be very useful for the analysis of various extracts and formulations containing aminoacids. *Linum usitatissimum* seed has a tremendous scope on further studies mainly in the area of Nutraceuticals and dietary supplements; because it contains many amino acids, carbohydrates, fatty acids, vitamins and minerals etc., therefore further research work to be carried out on this plant towards above said field. More research work is recommended on the plant for isolation and characterization of bioactive compounds.

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