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Pharmaceutical and Analytical Study of *Nilitnaduliyadi Leha*

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ABSTRACT

Nilitanduliyadi leha is one among the formulation explained in Vishavaidya Jyotsnika, a Malayalam Vishachikitsa text (treatment of poisoning). It is explained in the context of formulations used in all types of poisoning. The preparation of this formulation is simple and it contains easily available drugs. Test for reducing and non reducing sugar and also HPTLC was conducted for the standardization of the formulation. In high performance thin layer chromatography (HPTLC) study (ethanol extract) using Toluene: n-Hexane: Ethyl acetate (6:4),visual observation was done under UV light.

Key Words

Nilitanduliyadi Leha, Standardization, HPTLC, *Vishavaidya Jyotsnika*

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INTRODUCTION

The drug (*Dravya*) comes second in the order of the four fundamental components of the treatment[1]. Success of the treatment depends upon proper raw drug selection, proper manufacturing method and proper way of dose administration. Nilitanduliyadi leha is one among the formulation explained in the context of Vishahara yogas[2].It is indicated in all types of poison (vishas)[3] - Sthavara, Jangama, and Kritrima . It is prepared in the form of avaleha. *Avaleha* is a semisolid preparation of herbal drugs prepared in decoction or extracts of different herbs by adding sweetening agents like jaggery, sugar or sugar candy.The qualities of *Avaleha* over other medicaments are easy to administer, safe to use and are accepted by all age groups. They have pleasant and agreeable taste, high therapeutic efficacy and longer shelf life[4]. Standardization is essential in order to assess the quality of drugs for its efficacy. Standardization of Ayurvedic and other poly herbal formulation and analysis of their chemical marker has always been a concern. High-performance thin layer chromatography based on the complete potential of thin layer chromatography is a sophisticated instrumental technique[5].Nilitanduliyadi leha is explained in Vishavaidya Jyotsnika, a textbook of toxicology

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written in malayalam. This formulation is indicated in all vishaja conditions. The main aim and objective of the present study is to prepare the Nilitanduliyadi leha and its analysis through HPTLC method and test for reducing and non reducing sugars.

MATERIALS AND METHODS

Collection of the raw drugs

The raw drugs (table 1) were collected from Kerala and the raw drugs were authenticated at Department of Dravyaguna, SDMCA, Hassan.

Table 1 Ingredients of Nilitanduliyadi Leha and their proportion

Sl No	DRUG	BOTANICAL NAME	PART USED
1	Nili	*Indigofera tinctoria*	Patra
2	Tanduliya	*Amaranthus spinosus*	Patra(Leaves)
3	Tagara	*Valeriana wallichi*	Mula(Root)
4	Shunti	*Zingiber officinale*	Kanda(Rhizome
5	Maricha	*Piper nigrum*	Phala(Fruit)
6	Pippali	*Piper longum*	Phala(Fruit)
7	Saindhava		
8	Sugar		

Method of preparation: Medicine was prepared at the Teaching pharmacy SDMCA, Hassan as per GMP standards. Ingredients of the preparation and the part used is as mentioned in Table 1. Since the quantity of each ingredient was not mentioned, the lehya was prepared according to sharangadhara samhita. Sugar 4 parts, swarasa 4 parts and churna 1 part. Each ingredients were dried well and pounded into choorna separately. Swarasa of nili and tanduliya was prepared according to the method told by sharangadhara(Figure no:1). The coarse powder of nili and tanduliya was boiled in eight times of water. This was then reduced to quarter and used as swarasa. Lehya was prepared by mixing sugar (4 parts) with the extracted swarasa of the leaves of nili and tanduliya (4parts) (Figure no:2). After proper cooking, sukshma choorna of vyosha that is shunti,maricha and pippali (1/3 parts), tagara (1/3 parts) and saindhava (1/3 parts) were added and mixed well(Figure no:3).

Dose: 1 Pala (48g)

Route of administration: Oral

Test of Perfectness: Sinks in water due to proper consistency (Apsumajjana) (Figure no:4)

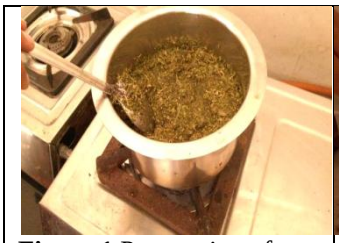

Figure 1 Preparation of Swarasa

Figure 2 Preparation of Leha

Figure 3 Prepared Leha

Figure 4 Test of Perfectness

Physical characteristic of leha

- Appearance: Paste like
- Colour: Black-Brown
- Odour: Smell of Vyosha
- Touch: Smooth
- Taste: Tikta
- Solubility: Dissolves in water

REDUCING AND NON REDUCING SUGAR

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In a 250 ml volumetric flask10 gmsof sample was taken and 200 ml of water was added. Slight excess solid basic Lead acetate was added to remove tannins and made up to the mark without disturbing the solution by adding water. It is thenshaken and filtered. This filtrate was used for the estimation of reducing sugar.

Reducing sugar: The sugar solution is taken in a 50 ml burette.

Preliminary titration: 10 ml of Fehling's solution was pipetted into a 250 ml conical flask, from the burette, 15 ml of the sugar solution was added. The liquid boiled on asbestos-covered gauze and further quantities of the sugar solution was added (One ml at a time) at 10 to 15 second intervals to the boiling liquid until the blue colour is nearly discharged. Around 3-5 drops of aqueous Methylene blue solution (1%) was added and continued the titration until the indicator wascompletely decolourised.

Accurate titration: The titration was repeated and before heating, almost all of the sugar solution required to effect reduction of copper was added and gently boiled for two minutes. Added 3-5 drops of methylene blue indicator and the titration was completed within a total boiling time of three minutes. At the end point all the blue colour was discharged and the liquid was red. The proportions of the various sugars, equivalent to 10 ml of Fehling's solution are taken from the table.

Total Sugar: 20ml of reducing sugar solution was taken and 10ml of Concentrated Hydrochloric acid added and kept it aside over night. It was then neutralized with approximately 1M Sodium hydroxide solution and made up to 100 ml in a volumetric flask. Determined the total sugar content by the titrimetric method described above. The experiment was repeated twice and the average value was taken.

HPTLC

Extracted 1g of Sample with 10 ml of *alcohol* and 4,8and 12µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (1:1). The developed plates were visualized under short UV and long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm and 366nm. R_f, colour of the spots and densitometric scan were recorded.

RESULTS AND DISCUSSION

The Nilitanduliyadi leha was prepared using swarasa of Nili and Tanduliya. The color of the Nili swarasa was dark brown to black in color, which gave the brown-black color to the leha. Most of the drugs used in the formulation have tikta rasa(bitter taste), which gives the tikta rasa to the leha. The smooth texture of the leha indicates that the sookshma churna (fine powder) was powdered well and leha is well prepared. The Vyosha/Trikatu (Pippali,Shunti and Maricha) poses intense fragrance, which dominates all other herbs used, thus the leha has the odour of Vyosha.

Table 2 Test for reducing and non reducing sugar

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Parameter	Results n = 3 %w/w
Total Sugar	18.49
Reducing sugar	5.89
Non reducing sugar	12.60

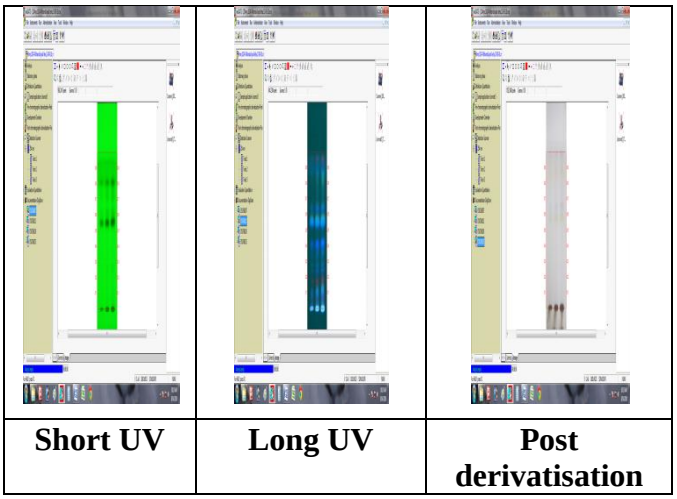
On testing, the total sugar present in the formulation (table 2) was found to be 18.49%w/w. Out of this 5.89%w/w was reducing sugar and 12.60%w/w was non reducing sugar. The type of sugar that acts as the reducing agent due to its free aldehyde or ketone functional groups in its molecular structure and can effectively donate electrons to some other molecule by oxidizing it is called reducing sugar. Sugars which do not possess a free ketone or an aldehyde group are called the non-reducing sugar[6].

Table 3 R_f values of sample of *Nilitanduliyadi leha*

Short UV	Long UV	Post derivatisation
-	0.06 (F. blue)	-
-	0.12 (F. blue)	-
-	0.18 (F. red)	-
0.24 (L. green)	0.24 (F. blue)	-
-	0.28 (F. blue)	-
-	0.41 (F. blue)	-
0.56 (D. green)	0.56 (F. blue)	0.56 (Greenish yellow)
0.62 (L. green)	-	-
-	0.65 (F. blue)	0.65 (Purple)
0.72 (L. green)	-	-
-	-	0.74 (Purple)
-	-	0.77 (Purple)
0.82 (D. green)	0.82 (F. green)	-
-	0.87 (F. blue)	-
-	0.96 (F. red)	-

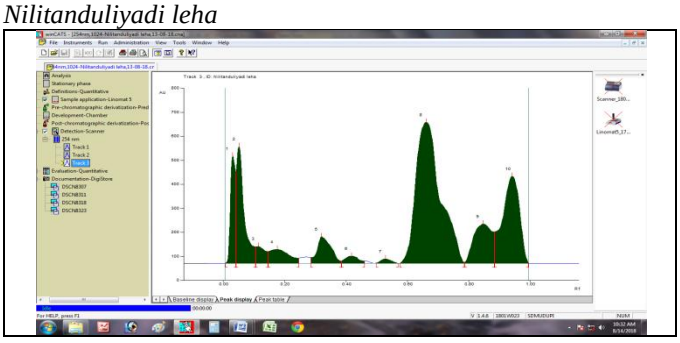
***D- dark; L-light; F- fluorescent**

In High performance thin layer chromatography (HPTLC) study of visha bilwadi gutika (ethanol extract) using Toluene: n hexane: Ethyl acetate (6.0:4.0) visual observation was done under UV light (Figure 5) showed (Table no:3) 5 spots at short UV at Rf 0.24,0.56,0.62,0.72 and 0.82. At long UV, chromatogram showed 11 prominent spots at Rf 0.06, 0.12, 0.24, 0.28, 0.41, 0.56, 0.65, 0.82, 0.87 and 0.96. At post derivatisation showed 4 spots at R_f 0.56,0.65,0.74,0.77. The Densitometric scan of the sample of Nilitanduliyadi leha is shown in Figure 6 (Figure 6a and Figure 6b).



Track 1: *Nilitanduliyadi leha* - 4µl
Track 2: *Nilitanduliyadi leha* - 8µl
Track 3: *Nilitanduliyadi leha* - 12µl
Solvent system: Toluene: Ethyl aetate (1.0: 1.0)

Figure 5 HPTLC Photo documentation of sample of *Nilitanduliyadi leha*



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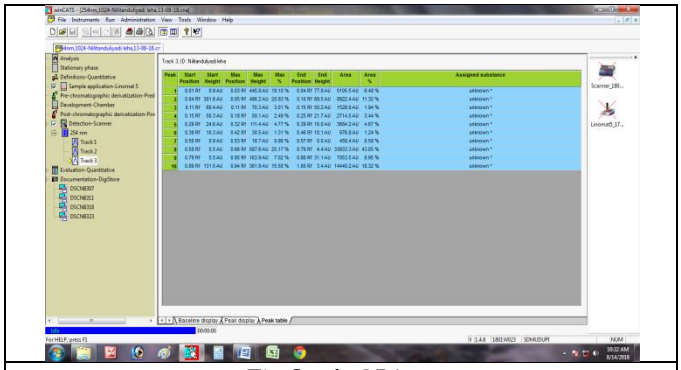
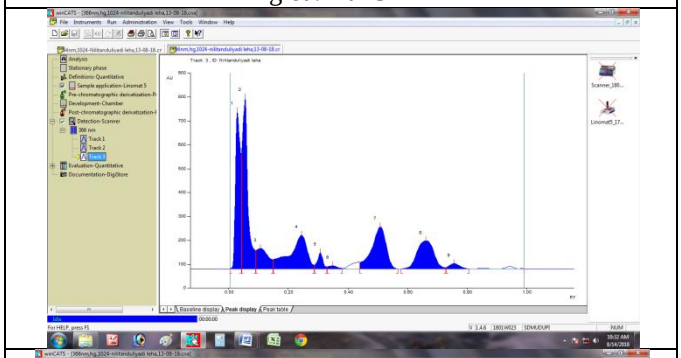


Fig 6a. At 254nm



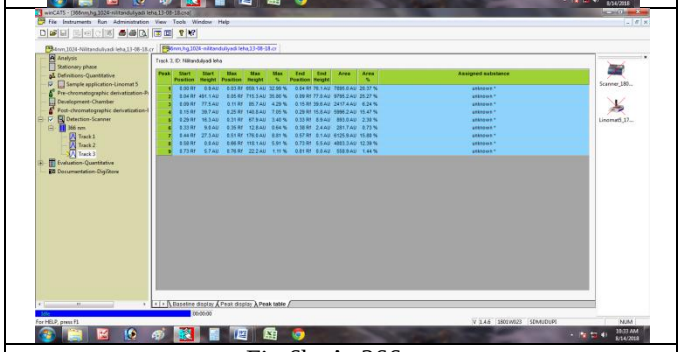


Fig 6b. At 366nm

Figure 6 Densitometric scan of the sample of *Nilitanduliyadi leha*

CONCLUSION

Nilitanduliyadi leha that contains drugs which are easily available, is a formulation that can be used in all cases of visha. Further studies can help to put the formulation in clinical uses. In this study, the test for reducing and non reducing sugars and HPTLC was conducted for the standardization of the formulation, and hence this can be considered as a preliminary standard of Nilitanduliyadi leha.

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