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EVALUATION OF PHARMACEUTICAL PROFILE OF *SHARAPUNKHADI YOGA* – REMEDY FOR NON ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT
Non Alcoholic Fatty Liver Disease (NAFLD) is the disease of the era which arises mainly from faulty lifestyle. There is no established treatment for NAFLD in conventional medical science. *Sharapunkhadi Yoga* is a formulated combination comprising of equal quantities of extracts of *Sharapunkha, Bhoomiamalaki and Katuki.* Extracts of all the drugs have shown significant hepatoprotective activity in experimental study in rats. The formulation is one among the best remedies Ayurveda can offer for the management of NAFLD. Present study has been aimed to develop pharmaceutical standards and HPTLC fingerprints for individual ingredients and for *Sharapunkhadi Yoga* itself. Loss on drying, ash value, water and alcohol soluble extract were found to be within the permissible limits of Churna. Phyto-chemical evaluation had shown the presence of carbohydrates, steroids, glycosides, saponins, tannins and phenols. HPTLC study shows 5, 3 and 9 peaks for *Sharapunkhadi Yoga* at 254nm, 366nm & after visualization at 500nm respectively.

KEYWORDS: HPTLC, Pharmaceutics, Physicochemical analysis, *Sharapunkhadi Yoga*, Non-alcoholic fatty liver disease.

INTRODUCTION
Non-alcoholic fatty liver disease(NAFLD) is simply fat deposition in the liver that is not caused by chronic ingestion of alcohol.[1] NAFLD encompasses a spectrum of liver disorders characterized by macro vesicular hepatic fat accumulation alone (steatosis), or accompanied by signs of hepatocyte injury, mixed inflammatory cell infiltrate and variable hepatic fibrosis (Non Alcoholic Steato Hepatitis - NASH), leading to cirrhosis. Urbanisation and associated changes such as sedentary lifestyle, fat rich diet and a higher inherited tendency for Diabetes mellitus make Indians more prone to Metabolic Syndrome or insulin resistance and its manifestations, such as NAFLD and NASH. There is no established treatment for NAFLD in conventional medical science. *Sharapunkhadi Yoga* is a formulated combination comprising of equal quantities of extracts of *Sharapunkha, Bhoomiamalaki and Katuki.* Extracts of all the drugs have shown significant hepatoprotective activity in experimental study in rats. Lack of standardization of poly-herbal formulations creates difficulty in validating the efficacy and maintaining quality of the product. Therefore, it is important to ensure the standard and quality right from the raw drugs to the finished product.

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. There are two types of chemical analysis – qualitative (identification) and quantitative (estimation). Qualitative analysis is performed to establish composition of natural or synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not. Quantitative analytical techniques are mainly used to quantify any compound or substance in the sample.[2]

Qualitative investigation is important part to be done prior to a pharmacological evaluation since it provides a fundamental idea regarding the drug's probable mode of pharmacological activity. Physicochemical analysis provides the objective parameters to set the standards for quality of raw drugs as well as finished products. Generally, analytical study of drugs help to interpret the

pharmacokinetics and pharmacodynamics involved. With the help of analytical studies, it is possible to standardize the drug and differentiate the adulterants. In the present study, an attempt has been made to analyze *Sharapunkhadi Yoga* by preliminary phytochemical and physico-chemical parameters and to develop HPTLC (High-Performance Thin Layer chromatography study) fingerprints of the compound formulation *Sharapunkhadi Yoga.*

MATERIAL AND METHODS

Procurement of raw materials

Extracts of the formulation, *Sharapunkha, Bhoomiamalaki and Katuki* were purchased separately from Konark Herbals and Healthcare, Daman. Extracts were stored in separate air–tight containers. *Sharapunkhadi Yoga* was prepared by mixing the three powders in equal proportions in mass mixing machine till the homogeneous mixture was obtained.

Pharmacognostical Study

Raw drugs were identified and authenticated by the Pharmacognosy laboratory, I.P.G.T & R.A., Gujarat Ayurved University, Jamnagar, Gujarat.

Organoleptic Evaluation

The organoleptic characters of ingredients and the compound formulation like colour, taste, odour and consistency were recorded by sensory knowledge.

Pharmaceutical Study

For the present study, physico- chemical analysis, preliminary phytochemical investigations and HPTLC (High Performance Thin Layer Chromatography) were carried out. The common physico-chemical parameters mentioned for *Churna* in Ayurvedic Pharmacopeia of India and C.C.R.A.S guidelines are total Ash value, pH value, water and methanol soluble extracts.[3] Presence of increased moisture contents in a sample can create preservative problems of *Churna*. Hence loss on drying was also selected as one of the parameter[4,5] For finding out the chemical constituents, preliminary phyto-chemical investigations[6] like Molish's test, Salkowski test, Keller-killiani test, Foam test, Flavonoid test, Dragendroff's test, test for tannins and phenols were performed.

PHYSICO CHEMICAL ANALYSIS[7]

1. Determination of loss on drying[8]

Excess of water content in drug encourage microbial growth i.e. fungi or insects and leads to deterioration. Therefore, limit of water content should be set for every given plant material. This is especially important for materials that absorbs moisture easily and deteriorate quickly in presence of water.

The loss on drying was determined by taking 2 gm, accurately weighed sample of each specimen ie. *Sharapunkha, Bhoomiamalaki, Katuki* and *Sharapunkhadi Yoga* in dried and previously weighed four petri-dishes. Samples were spread evenly and dried in an oven at 110°C till constant weight was noted. The weight after drying was noted and loss on drying was calculated.

The percentage was calculated on the basis of air-dried sample.

Percentage of L.O.D = (Loss on drying/ Weight of Sample × 100) %w/w

2. Determination of water soluble extractive value[9]

This method determines the amount of active constituents extracted with water from a given amount of medicinal plants. It also gives some idea about the amount of water soluble constituent present in a particular drug such as sugar, mucilage, glycosides, tannin etc.

About 5 gm, accurately weighed sample of four specimens were macerated with 100 ml of distilled water in four different closed flasks for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was filtered, taking precaution against loss of solvent and 25 ml of the filtrate was evaporated to dryness in a previously weighed dried evaporating dish. First dried over water bath and then at 110°C in hot air oven, to constant weight which was noted. From the weight of the residue the percentage of water-soluble extractive was calculated with reference to air-dried sample.

Percentage of W.S.E. = [Weight of residue x volume made/ (Weight of Sample x volume taken) × 100] %w/w

3. Determination of alcohol soluble extractive value[10]

Alcohol soluble extractive value was determined by same procedure as described in water soluble extractive value by taking 95% alcohol instead of water.

Percentage of A.S.E. = [Weight of residue x volume made/ (Weight of Sample x volume taken) × 100] %w/w

4. Determination of pH

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration. Five percent aqueous solution of the samples were prepared, filtered and pH of the filtrate was noted.

QUALITATIVE CHEMICAL TESTS[11]

Qualitative tests for various functional groups like alkaloids, glycosides etc., were carried out by using the methanol soluble extracts of the samples and by following standard procedures.(Table no.1).

Table no. 1: Qualitative tests of *Sharapunkhadi Yoga* and ingredients.

Sr. No.	Phyto-constituents	Performed test
1.	Flavanoids	Ammonia test
2.	Tannins + Phenolic compounds	Lead acetate test
3.	Alkaloids	Dragondroff's test
4.	Carbohydrates	Molish's test
5.	Protein	Biurate test
6.	Steroid	Salkowski reaction
7.	Glycoside	Keller Killiani test
8. .	Saponin	Foam test

High Performance Thin Layer Chromatography[12]

Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds even from complicated matrix. Principle of HPTLC remains the same as of Thin Layer Chromatography(TLC) i.e. adsorption. One or more compounds were spotted in a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The component with more affinity towards stationary phase travels faster. Thus the components were separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

Steps involved in H.P.T.L.C were as follows:
1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample
6. Chromatographic development
7. Detection of spots
8. Scanning and Documentation

Methanol extract of *Sharapunkhadi Yoga* and the ingredients were spotted on pre-coated silica gel GF CO254 aluminum plate as 5 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of camag, linomate V sample applicator fitted with a 100 µL. Hamilton syringe was used as the mobile phase. After development, densitometry scanning was performed with a camage TLC scanner III reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 manufactured by CAMAGE Switzerland). The slit dimensions were 6.00 x 0.45 mm and the scanning speed was 20 mm per second.[13] After spraying visualizing agent Vanillin Sulfuric Acid, the samples were observed at 500nm.

OBSERVATIONS AND RESULTS

Organoleptic evaluation: Organoleptic characters of ingredients and the compound formulation like colour, taste, odour and consistency are enlisted in **Table No.2.**

Table No. 2: Organoleptic characters of *Sharapunkhadi Yoga.*[14]

	Colour	Odour	Taste	Consistency
Sharapunkha	Light chocolate brown	Slightly aromatic	*Madhura, Tikta* ends with *Kashaya*	Fine powder
Bhoomiamalaki	Light brown	Light astringent	*Kashaya*	Fine powder
Katuki	Light yellow	Light astringent	*Tikta*	Fine powder
Sharapunkhadi Yoga	Light brown	Astringent	*Madhura, Kashaya* ends with *Tikta*	Fine powder

Physicochemical parameters: Physico-chemical parameters like loss on drying, ash value, water and alcohol soluble extract, pH etc were carried out and the results are depicted in **Table No. 3**.

Table No. 3: Physico-chemical parameters of *Sharapunkhadi Yoga.*[15]

	Sharapunkha	***Bhoomiamalaki***	***Katuki***	***Sharapunkhadi Yoga***
Loss on drying% w/w	4.25	1.95	1.3	2.7
Ash value % w/w	3.23	2.998	2.21	3.14
Water soluble extract % w/w	98.9	92.4	95.7	96.8
Methanol soluble extract % w/w	37.31	2.36	17.84	13.76
Ph (5% v/w Aqua solution)	6.5	6.5	6.5	6.5

Phyto-chemical analysis

Various preliminary phyto chemical tests were carried out to analyse *Sharapunkhadi Yoga* and the observations are listed in **Table No.4**. These tests reveal the presence of tannin, phenolic compounds, carbohydrates and steroids in the specimens of *Sharapunkha, Bhoomiamalaki, Katuki* and *Sharapunkhadi Yoga.* Glycoside and saponin are present in all the samples except that of *Bhoomiamalaki.*

Table no. 4: Phyto-chemical analysis of *Sharapunkhadi Yoga.*

Functional Group	*Sharapunkha*	*Bhoomiamalaki*	*Katuki*	*Sharapunkhadi yoga*
Flavanoids	Absent	Absent	Absent	Absent
Tannins + Phenolic Compounds	Present	Present	Present	Present
Alkaloids	Absent	Absent	Absent	Absent
Carbohydrates	Present	Present	Present	Present
Protein	Absent	Absent	Absent	Absent
Steroid	Present	Present	Present	Present
Glycoside	Present	Absent	Present	Present
Saponin	Present	Absent	Present	Present

High performance thin layer chromatography (HPTLC)

On performing HPTLC the chromatogram showed 6 peaks with R_f values 0.03, 0.43, 0.47, 0.61, 0.87, 0.94 for *Sharapunkha*, 0.03, 0.49, 0.53, 0.75, 0.87, 0.94 for *Bhoomiamalaki*, 4 peaks with R_f values 0.04, 0.38, 0.60, 0.95 for *Katuki* and 5 peaks with R_f values 0.02, 0.39, 0.50, 0.58, 0.94 for *Sharapunkhadi Yoga* at 254nm; while at 366nm the chromatogram showed 4 spots with R_f values 0.03, 0.43, 0.82, 0.94 for *Sharapunkha*, 3 spots with R_f values 0.03, 0.82, 0.94 for *Bhoomiamalaki*, 3 spots with R_f values 0.04, 0.81, 0.95 for *Katuki* and 3 spots with R_f values 0.02, 0.83, 0.94 for *Sharapunkhadi Yoga* (**Table No. 5)**. Commonly seen $R_f$values at both 254nm and 366nm were 0.03, 0.43 and 0.94 for *Sharapunkha*; 0.03 and 0.94 for *Bhoomiamalaki*; 0.04 and 0.95 for *Katuki*; and 0.02 and 0.94 for *Sharapunkhadi Yoga.* After spraying visualizing agent, chromatogram showed 10 peaks with R_f values 0.02, 0.27, 0.30, 0.34, 0.38, 0.56, 0.66, 0.78, 0.84, 0.98 for *Sharapunkha*, 11 peaks with R_f values 0.02, 0.27, 0.34, 0.36, 0.41, 0.51, 0.57, 0.75, 0.86, 0.90, 0.98 for *Bhoomiamalaki*, 12 peaks with Rf values 0.02, 0.21, 0.31, 0.37, 0.39, 0.45, 0.56, 0.63, 0.65, 0.70, 0.77, 0.97 for *Katuki* and 9 peaks with Rf values 0.03, 0.24, 0.32, 0.35, 0.38, 0. 45, 0.51, 0.57, 0.99 for *Sharapunkhadi Yoga* at 500 nm**. (Plate. 2. Fig 1-8) (Plate.3).**

Table No. 5: HPTLC results of methanolic extract of *Sharapunkhadi Yoga.*

Drug	Under UV radiation				Post chromatographic visualization (After spray of Vanillin sulfuric acid) – 500 nm	
	254 nm		366 nm			
	No. of spots	Rf value	No. of spots	R_f value	No. of spots	Rf value
Sharapunkha [Plate 2 fig 3 & 4]	6	0.03, 0.43, 0.47, 0.61, 0.87, 0.94	4	0.03,0.430. 82,0.94	10	0.02, 0.27, 0.30, 0.34, 0.38, 0.56, 0.66, 0.78, 0.84, 0.98
Bhoomiamalaki [Plate 2 fig 5 & 6]	6	0.03, 0.49,0.53, 0.75, 0.87, 0.94	3	0.03, 0.82, 0.94	11	0.02, 0.27, 0.34, 0.36, 0.41, 0.51, 0.57, 0.75, 0.86, 0.90, 0.98
Katuki [Plate 2 fig 1 & 2]	4	0.04, 0.38, 0.60, 0.95	3	0.04, 0.81, 0.95	12	0.02, 0.21, 0.31, 0.37, 0.39, 0.45, 0.56, 0.63, 0.65, 0.70, 0.77, 0.97
Sharapunkhadi Yoga [Plate 2 fig 7 & 8]	5	0.02, 0.39, 0.50, 0.58, 0.94	3	0.02, 0.83, 0.94	9	0.03, 0.24, 0.32, 0.35, 0.38, 0. 45, 0.51, 0.57, 0.99

Plate 2: HPTLC Study.

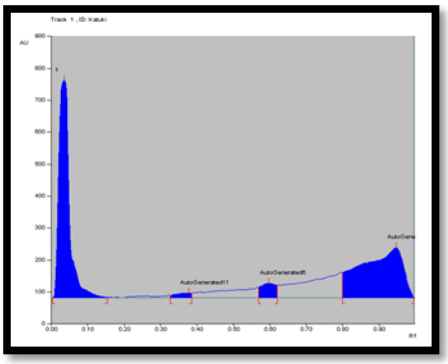


Fig.1

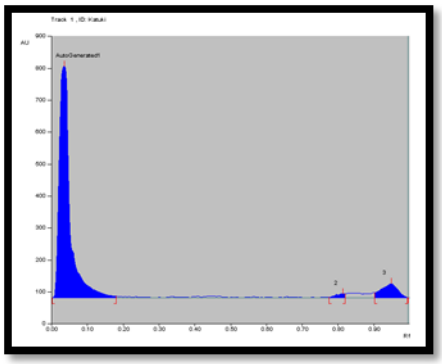


Fig.2

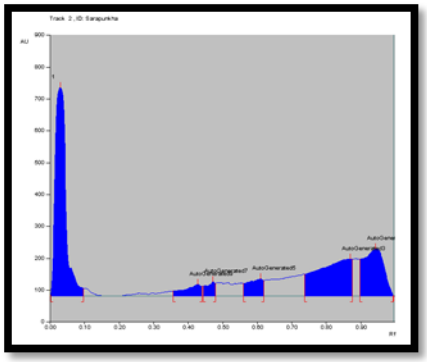


Fig.3

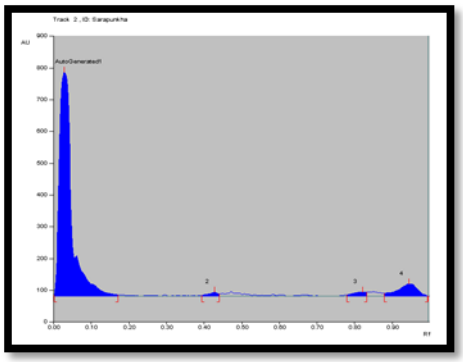


Fig. 4

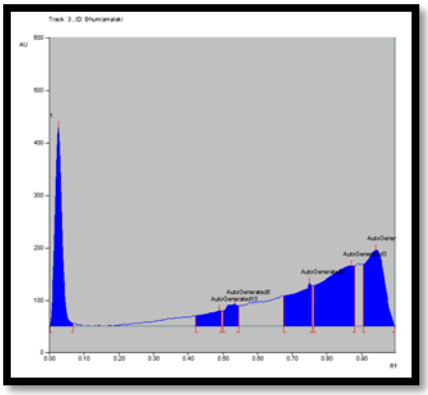


Fig.5

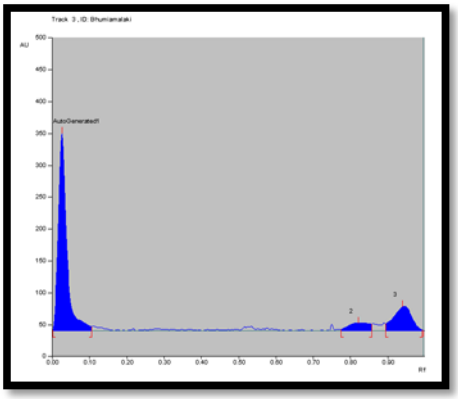


Fig.6

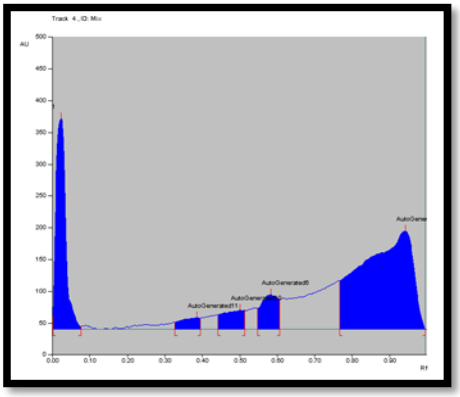


Fig.7

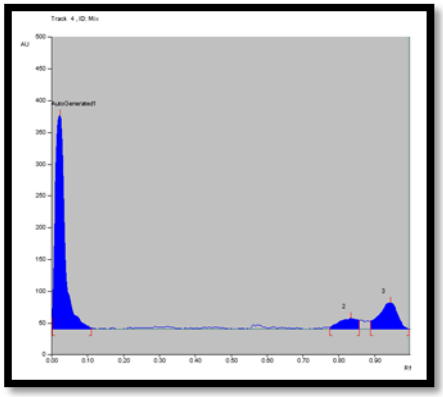
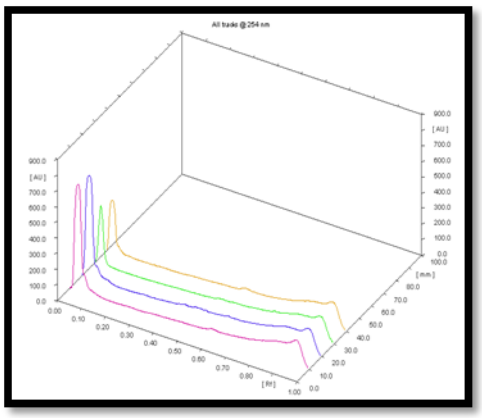
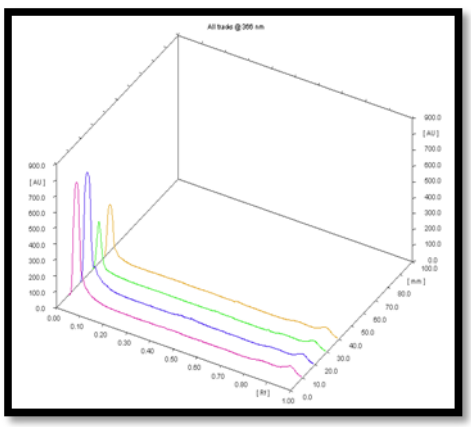


Fig.8

Plate 3: Three Dimensional Spectra Comparison.



Spectra at 254nm



Spectra at 366nm

DISCUSSION

Pharmaceutical analysis of *Sharapunkhadi Yoga*, containing the extracts of *Sharapunkha*, *Bhoomiamalaki* and *Katuki* in equal proportions, found to be effective in the management of NAFLD has been conducted. This was an attempt towards pharmaceutical standardization of the drug. All the pharmaceutical parameters analysed were within the permissible limits for *churna*. Percentage of water soluble extract was found to be more than alcohol soluble extract. Ash value of the final product was very less ie. 3.14% showed that the presence of inorganic material was negligible. The phyto-chemical evaluation of *Sharapunkhadi Yoga* was done and it showed the presence of carbohydrates, steroids, glycosides, saponins, tannins and phenols. Thus it can be inferred that the drug may yield desired pharmacological action. Solvent system showed good separation of components in post visualization chromatograph having 9 spots at 500nm, so it can be used for further analysis. Further the HPTLC results can also be compared with standards of individual raw material for obtaining and concluding standards for *Sharapunkhadi Yoga.*

CONCLUSION

Physico-chemical analysis and HPTLC studies on *Sharapunkhadi Yoga* inferred that the formulation meets the minimum quality standards at a preliminary level. Qualitative analysis has helped to develop an overall idea about the probable mode of action of the formulation. Further analysis and investigations are essential for determining the active ingredients of the test drug and to provide scientific background for the validation of its clinical efficacy. Observations from the present study may be used as reference standard in further quality analysis studies.

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