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Pharmaceutical analysis of *Kanchanara* & Nano *Kanchanara* stem bark powder in Hypothyroidism

Research Article

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Abstract

Ayurveda is a holistic science dealing with all aspects of human life. *Bauhinia variegata* (*Kanchanara*) is a medicinal tree that is widely used in Ayurvedic indegenious medicine system. Herbal medicines have a long therapeutic history; serving many of the health needs of large population of the world. Maintaining quality standards of herbal drugs is the need of today because of its increasing demand. However, due to the wide range of chemical components involved, quality management and assurance remain a concern for herbal medicines. In *Ayurvedic* texts, several formulations have been mentioned in *Galganda* and *Gandamala* where *Kanchanara* being the main component. As most of the formulations mentions the use of stem bark, the pulverized powder of the same was prepared. Later by the use of High Energy Ball Milling Methodology (HEBM) fine powder of *Kanchanara* was converted to Nano *Kanchanara* stem bark powder. This Nano Kanchanara stem bark powder was seal packed to maintain its shelf life. *Kanchanara* and Nano *Kanchanara* stem bark powder were subjected to organoleptic analysis, phytochemical and qualitative analysis to detect the presence of various functional groups, and to high performance thin layer chromatography (HPTLC) examination by optimizing the solvent systems. The investigations are revealed mainly.

Key Words: *Bauhinia variegata Linn., Kanchanara, Nano Kanchanara,* Hypothyroidism, HPTLC.

Introduction

Hypothyroidism is one of the most common functional disorders. Hypothyroidism refers to the common pathological condition of thyroid hormone deficiency (1). Hypothyroidism can be due to primary disease of the thyroid gland itself (primary hypothyroidism) or lack of pituitary TSH (2). Prevalence of Hypothyroidism is 4.6% in developed countries. In urban India it is 10.95%. Incidence of hypothyroidism is more in females (15.86%) than males (5.02%) (3).

Thyroid hormone replacement with levothyroxine is the standard treatment for patients with hypothyroidism (1). Stem bark of *Bauhinia variegata* Linn. (*Kanchanara*) is proven anti-goitrogenic and as antitumor (4).

Ayurveda emphasizes on maintenance and promotion of health along with a vivid description of diseases and its management. Herbal drugs- singularly and in combinations are used (5). It contains several compounds in complex matrices where a single active constituent is not responsible for its overall efficacy. The Standardization of herbal formulations plays a pivotal role in the assessment of drug quality, its active principles and chemical constituents. Quality of raw materials along with preparation plays an important role in the acceptability and safety of the drug (6). In India, the Department of AYUSH has launched a central scheme to develop standard operating procedures for the manufacturing process for developing the pharmacopoeia standards of Ayurvedic preparations.

In this study, Nano *Kanchanara* stem bark powder was prepared by following the standard operating procedures in a GMP certified ayurvedic pharmacy. This formulation is used as an anti-hypothyroid medicine. The pharmaceutical analysis of this formulation has been done for its therapeutic values and efficacy. Analytical study of *Churna* was performed with the following parameters: Organoleptic parameters (Appearance, color, odor, taste), physio-chemical parameters (Loss on drying, Total ash, Acid insoluble ash, Water soluble extract, Alcohol soluble extract, pH) and HPTLC.

Aims and Objectives

- To evaluate the organoleptic characters of the *Kanchanara powder* and Nano *Kanchanara* powder.
- To assess the physicochemical, phytochemical and HPTLC parameters of *Kanchanara powder* and Nano *Kanchanara* powder.

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Materials and methods

Collection of Plant Material

Kanchanara stem bark was purchased from authenticated resources of Vadodara.

Identification and Authentication of Raw Drugs:

Identification and authentication of raw drug was done by the Department of *Dravyaguna,* Parul Institute of Ayurveda, Parul University, Vadodara.

Method of preparation of Nano *Kanchanara* powder

The raw stem bark was cleansed and shade dried and pulverized to fine powder. The powdered raw material was processed to nanoparticle size (1-200nm) *churna* by High Energy Ball Milling (HEBM) method at Institute of Applied Research (IAR) Pune, Maharashtra. Nano Powder was seal packed and labelled.

Table 1: Kanchanara Powder

No.	Name of Drug	Botonical Name	Family	Part Used
1	**Kanchanara**	*Bauhinia variegata Linn.*	Leguminosae	Stem Bark

Methods of evaluation of *Kanchanara* and Nano *Kanchanara* stem bark podwer

Kanchanara and Nano *Kanchanara* stem bark powder was analysed by using the entire standard qualitative and quantitative parameters conducted at Laboratory of G.M.P certified Parul Ayurveda Pharmacy, Vadodara.

Physico-Chemical Analysis

It includes parameters like colour, taste, pH, loss on drying (7), total ash value (8), ash value (9), alcohol soluble extract (10), and water soluble extract (11).

- **pH:** A solution of the sample drug is prepared with distilled water and then the filterate is used to measure its pH value. Calibration is performed using pH Meter.

- **Water soluble extract:** A solution of the sample drug is prepared with water and allowed to settle for 18 hours, 2ml Filtrate is taken in evaporating dish and after it dries off, percentage of dry drug left is measured. The same procedure is done with Alcohol soluble extract.

- **Ash value:** Heat is applied to the sample drug until it starts burning, then it is allowed to cool down. Later it is placed in muffle furnace for 1 hour at 550˚C – 650˚C. Lastly the percentage of weight loss is measured.

- **Chromatography:** High-Performance Thin Layer Chromatography (HPTLC) a refined form of TLC. It works on the same principles as TLC i.e. the principle of adsorption and separation but with a better resolution by separation of components than normal TLC. High performance thin layer chromatography (12) uses chromatographic stationary phases with superior separation efficiency, employs with the state of the art and instrumentation for all steps in the procedure. All the methods and procedures followed for HPTLC of *Kanchanara* and Nano *Kanchanara* stem bark powder have been discussed below.

Results and Discussion

Kanchanara and Nano *Kanchanara* stem bark powder were prepared by following all the standard operating procedures in GMP certified pharmacy and was subjected to qualitative and quantitative analysis. The pharmaceutical analysis results have been discussed below.

Organoleptic evaluation

The organoleptic parameters are one of the basic criteria for the selection of raw ingredients and confirming the quality of the finished formulation. The color was reddish-brown, astringent in taste and the smell was slightly bitter due to the special properties of the components.

Table 2: Organoleptic Characteristics of Nano *Kanchanara* and *Kanchanara* stem bark powder

Sr. No.	Characters	Observation	
		Nano Kanchanara Powder	Kanchanara Powder
1	Color	Reddish-Brown	Reddish-Brown
2	Odor	Bitter	Bitter
3	Taste	Astringent	Astringent
4	Consistency	Very Fine Powder	Fine Powder

Loss on drying

It is referred to determine the moisture contents of the sample i.e. water and volatile contents. In this sample of *Kanchanara* and Nano *Kanchanara* stem bark powder loss of drying was 4.5 % and 2.5%, i.e. the sample is having a good shelf life and will not decay when stored for a long period.

Total ash and acid insoluble ash

Total ash value is a method used to quantify the amount of total inorganic compound. Acid insoluble ash test we can know only about silica availability. It is helpful in determining the quality and purity of crude drugs and also about contamination, substitution and adulteration. In the given sample, the ash value of Kanchanara was 16.27% and of Nano *Kanchanara* powder was 18.34%. Acid insoluble ash was 0.7% and 0.2% for *Kanchanara* and Nano *Kanchanara* respectively.

Water and Alcohol soluble extracts

The given sample of *Kanchanara* and Nano *Kanchanara* stem bark powder conatins 0.1% and 0.11% respectively of Water soluble extract and 0.11% and 0.13% respectively of Alcohol soluble extract. High water solubility indicates that the drug is best suited for extraction with water or water-based preparations.

pH

The pH is expedient to detect the acidity or basicity of the aqueous solution of the drug. It helps to understand the drug absorption and metabolism. In this sample of *Kanchanara* and Nano *Kanchanara* stem bark powder the pH was 7 and 7.5 which clearly indicates that the drug tested was slightly alkaline in nature.

Table 3: Physico-Chemical Analysis of *Kanchanara* & Nano *Kanchanara* stem bark powder

Sr. No	Parameters	*Kanchanara* stem	Nano *Kanchana*
		Values	Values
1	Loss on drying at 105^0 C	4.5	2.5
2	Total Ash Value (%w/w)	16.27	18.34
3	Acid Insoluble Ash (%w/w)	0.7	0.2
4	Water soluble extractive (%w/	0.1	0.11
5	Alcohol soluble extractive	0.13	0.11
6	pH (5% Aqueous)	7	7.5

Chromatography

It was done by Vasu Research Centre, Vadodara. HPTLC fingerprinting report was done for the analysis of the raw and finished formulation of *Kanchanara.*

High Performance Thin Layer Chromatographic Study:

Sample was prepare by weighing 1g in an iodine flask and applying 20ml of methanol to it. Reflux was done for 30 min in a warm bath. After the timer went off, Whatman filter paper No.1 was used to filter. The test solution was used for HPTLC fingerprinting. Preparation of Spray reagent (Anisaldehyde-Sulphuric acid reagent): A mixture was prepared of 0.5 ml anisaldehyde with 10ml glacial acetic acid followed by 85 ml methanol and 5ml Sulphuric acid (98%).

Table no.5- HPTLC chromatographic condition details have been mentioned below

Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 – Applicator
Filtering System	Whatman filter paper No. 1
Stationary phase	MERCK – TLC / HTPLC Silica gel 60 F_{254} on Aluminium Sheets
Application (Y axis) Starting Position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	10 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene: Ethyl Acetate: Formic Acid (7:3:1 v/v)
Visualization	@ 254nm, @ 366 nm and @ 540 nm (after derivatization)
Spray Reagent	Anisaldehyde Sulphuric Acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 min
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100±5^{0}C for 3 minutes

HPTLC details at different Rf

After derivatization, plate was examined and different bands at different R_f were found. These following were the findings:

Details of HPTLC of all tracks @ 254 nm**:** Under the 254 nm wavelength-Track -T1 of *Kanchanara Churna* and Track-T2 of Nano *Kanchanara Churna* respectively 5 spots and 5 spots were identified and with respect to retardation factor it is 0.24, 0.28, 0.59, 0.74, 0.95 and 0.24, 0.31, 0.59, 0.74, 0.86 respectively.

Details of HPTLC of all tracks @ 366 nm: Under the 366 nm wavelength Track -T1 of *Kanchanara Churna* and Track-T2 of Nano *Kanchanara Churna* respectively, 6 spots and 6 spots were identified and with respect to retardation factor it is 0.12, 0.28, 0.42, 0.59, 0.66, 0.86 and 0.12, 0.28, 0.42, 0.59, 0.66, 0.86 respectively.

Details of HPTLC profile of all tracks @540 nm: Under the 540 nm wavelength- Track -T1 of *Kanchanara Churna* and Track-T2 of Nano *Kanchanara Churna* respectively, 6 spots and 7 spots were detected and with respect to retardation factor it is 0.12, 0.24, 0.28, 0.38, 0.51, 0.59 and 0.12, 0.24, 0.28, 0.38, 0.51, 0.59, 0.74 respectively.

HPTLC generated @ 254 nm, @ 366 nm and @ 540 nm after the derivatization, reveals that the presence of 9 spots, 7 spots and 9 spots at each wavelength respectively. Thus, the formulation *Kanchanara* and *Nano Kanchanara* powder are rich in phytoconstituents.

Conclusion

Any formulation used medicinally requires a detailed study prior to its use. The therapeutic efficacy of the drug depends on the quality of the ingredients used for the preparation of the medicinal product. *Kanchanara* and Nano *Kanchanara* stem bark powder was pharmacologically subjected for physicochemical, qualitative and HPTLC analysis in accordance to standard operating procedures at a GMP certified pharmacy. Raw materials of the drug were identified and authenticated prior to the preparation. The drug was subjected pharmacologically to physicochemical analysis, qualitative and HPTLC analysis. The groundwork for standardization of *Kanchanara* and Nano *Kanchanara* stem bark powder has been attempted in this study. The study concludes the standard organoleptic, physiochemical, phytochemical and HPTLC parameters of *Kanchanara* and *Nano Kanchanara* stem bark powder which will be helpful in future studies regarding to *Kanchanara*.

Conflict of Interest: None

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