Original Research

A Comparative Physicochemical and Pharmacognostical Evaluation of Nishamalaki - An Ayurvedic Antidiabetic Formulation

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Abstract	Article Information
Most of the traditional systems of medicine are effective but they require standardization. Standardization of an herbal formulation is essential in order to assess the quality of drugs, based on the amount of their active principles, physicochemical standards and pharmacognostical parameters. This article reports on standardization and comparative study of the prepared herbal formulation nisamalaki churna which is used as anti-diabetic ayurvedic formulation with standard formulation (IMPCOPS). These two batches of *nisamalaki* churna are standardized and comparative study was done on the basis of organoleptic, Physicochemical properties, TLC, macro and microscopic powder studies.	 ***Corresponding Author:** **Venkateshwarlu G** **E-mail:** nadri.bengaluru1@gmail.com

INTRODUCTION

India is in rich heritage of traditional medicine and Ayurveda is one of the well-developed ancient systems of medicine. The quality assessment of ayurvedic/herbal formulation is of a very importance in order to justify their acceptability in modern system of medicine. The World Health organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to formulate the national policies on traditional medicine. In India, the department of AYUSH, Government of India, framed protocols to develop standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurvedic preparations

Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Cooke and Plotnick, 2008).

Very few reputed companies produce Nisha Amalaki in India and IMPCOPS (Indian Medicinal Practitioner's Co-Operative Pharmacy & Stores Ltd) based in Chennai (South India) produces this formulation in the form of a tablet. The Amalaki (fruits of embelica officinalis) and Nisha (rhizomes of curcuma longa) are also native to Karnataka (South India). Till now there has been no documentation on Physicochemical and Pharmacognostical evaluation to set standards for this polyherbal formulation and compare the classically prepared nisha amalaki with the market available sample using this parameters and understand whether there are any differences.

Hence this study presents methods of preparation of a Ayurvedic polyherbal antidiabetic formulation called as Nisha Amalaki as per classical method, preliminary standardization of the preparation employing physicochemical, Pharmacognostical and TLC parameters according to WHO protocol and comparing the same with that of available market sample [Indian Medicinal Practitioner's Co-Operative Pharmacy & Stores Ltd (batch-APT 227)] of the same preparation.

Ayurveda prescribes a poly-herbal formulation called Nisha Amalaki churna (available in powder form or tablet form) for diabetes. It consists of fine powders of Curcuma longa (Turmeric) and Emblica officinalis each in equal proportion (Vaidya Yoga Ratnavali, 2000).

Amalaki (Indian Goose Berry) is one of the ingredients of *nisamalaki churna,* ithas been considered the best of the ayurvedic rejuvenative herbs, because it is *tridosaghna*. Uniquely, it has a natural balance of tastes (sweet, sour, pungent, bitter and astringent) all in one fruit, it stimulates the brain to rebalance the three main components of all physiological functions, the water, fire, and air elements within the body (Bajracharya, 1979). Because of its cooling nature, *amla* is a common ingredient in treatments for a burning sensation anywhere in the body and for many types of inflammation and fever; these are manifestations of *pitta* (fire) agitation (Williamson, 2002).The fruit is a very rich source of vitamin C according to most if not nearly all references, this is probably not the case (Ghosal, Triphati and Chauhan, 1996). It was proposed that superior effect of the mistaken "vitamin C" component is actually the more stable and potent anti-oxidant effect of the tannins that appeared to be the vitamin. Its mineral and vitamin contents include calcium, phosphorous, iron, carotene, thiamine, riboflavin, and niacin. The fruits are rich in tannins, gallic acid and ellagic acid etc. The use of amla as an antioxidant has been examined by a number of authors (Bhattacharya *et al.*, 1999).

The other and main ingredient of nisamalaki churna is *Haridra* (Turmeric) balances all the three *doshas*. Because of its hot potency; it pacifies *kapha* and *vatadoshas*; and because of mild laxative action and bitter taste it pacifies the *pitta dosha*. According to *Ashtanga Samgraha*; *haridra* is the best ayurveda medicine for treatment of all the metabolic disorders like diabetes. It is well known for its complexion enhancing action, anti-bacterial activity and healing qualities. Along with these it also possesses scraping action so help in removing the unwanted fats from the body; corrects metabolism and useful in anemia, diabetes and liver problems. The active constituents of turmeric are the flavonoid curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone, and zingiberone. Other constituents include sugars, proteins, and resins. The best-researched active constituent is curcumin, which comprises 0.3-5.4 percent of raw turmeric (Leung, 1980).

MATERIAL AND METHODS

Physico-chemical studies like total ash, acid insoluble ash, water and alcohol soluble extract, loss on drying at 105 ^{o}C and successive extraction values by soxhelet extraction method were carried out as per WHO guide lines (WHO, 1998). Powder microscopy and preliminary phytochemical tests were performed as per the standard methods (Harbone, 1978).

Plant Material
Nisamalaki churna is poly herbal formulation, it consisting of 2 ingredients viz, Haridra (*Curcuma longa*) and Amalaki (*Emblicaofficinalis*) (Table 1).

Table 1: Ingredients of *Nisamalaki* churna.

Botanical name of the Ingredient	Sanskrit name	Part used	Quantity (per 100 gm)
Curcuma longa Linn.	Haridra	Root	50 gm
Emblicaofficinalis Gaertn.	Amalaki	Fruit	50 gm

Preparation of Formulation (Batch I)
The drugs used in the preparation were procured from the SMP unit and cleaned and dried in shade and powdered separately. Each powder weighed in gms and sieved by using 22 No. Mesh according to the formulation and mixed uniformly and mixed well (Vaidya Yoga Ratnavali, IMPCOPS).

Standard sample (Batch II)
Standard market sample (Batch No APT 227) procured from IMPCOPS, Chennai, India.

Standardization Parameters
The various standardization parameters studied were organoleptic properties, physicochemical investigations, fluorescence analysis, & preliminary phytochemical analysis, powder microscopic analysis, determination of pH, moisture content and TLC studies.

Organoleptic Evaluation
The organoleptic characters of the samples were evaluated based on the method described by Siddiqui and Hakim *et al.* (1995). Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste and texture etc.

Determination of pH
10% solution of polyherbal formulation was prepared in distilled water and pH was determined using pH meter MICROPRO Lab Mate digital pH meter.

Determination of Moisture Content
Moisture content was determined by loss on drying (LOD) method (Mukherjee, 2002). 3 gm of the weighed quantity of the drug was taken and kept in oven at 105 ^{o}C till a constant weight was obtained. Amount of moisture present in the sample was calculated as reference to the air dried drug.

Physicochemical Investigations

Physico-chemical investigations of formulations were carried outwere thedetermination of extractive values and ash values (Indian Pharmacopoeia, 1996).

Preliminary Phytochemical Analysis

Preliminary qualitative phytochemical analysis of all the extracts was carried out by employing standard conventional protocols (Trease and Evans, 1978; Sazada *et al.*, 2009 and Kokate, Purohit and Gokhale, 2006).

Fluorescence Analysis and TLC Studies

Fluorescence characters of powdered plant material with different chemical reagents were determined under ordinary and ultraviolet light (Chase and Pratt, 1949). 1 mg of the poly herbal sample was taken in a glass slide and treated with various reagents for the presence of their fluorescence characters under ultra-violet lamp. TLC studies have been carried out according to Igon Stahl *et al.*, (1969).

Powder Microscopic Analysis

For powder microscopic analysis, about 1 to 3 pinch of the sample was warmed with 2 to 4 drops of chloralhydrate solution, water and little safranin stain and observed under the microscope to identify the diagnostic features of the compound formulation for the presence of different fragments of tissues in curna. It was also observed by putting a pinch of phloroglucinol, 2 drops of concentrate dihydrochloric acid and few drops of alcohol for the presence of lignin containing tissues (Trease and Evans, 1978).

RESULTS AND DISCUSSION

Organoleptic evaluation was used for identification of sensory characteristics powder like colour, odour (smell), touch and taste. The taste of prepared sample (batch I) is bitter dominates than sour, whereas standard sample's (batch II) sour taste dominates then bitter (Table 2).

Table 2: Organoleptic characters.

Entry	Batch-I	Batch-II
Colour	Yellow	Mustered yellow
Odour	Characteristic	No smell
Touch	Smooth	Hard
Taste	Bitter and sour	Sour and bitter

Microscopic Characters of Batch I & II

The polyherbal powder was treated with chloral hydrate, water and glycerin and microscopical examination was carried out for the presence of following different fragments of tissues. The batch-I (Figure 1(a-s)) has the correlation and all the characteristic features are comparable with the standard formulation (batch-II) in powder microscopy (Figure 2(a-s)).

The following important identifying characters were observed in microscopic examination Figure 1 (a-s) of prepared nisamalaki churna (batch–I). Different fragments of tissues.

- Different fragments of tissues like xylem vessels with helical type
- Fragments of Cork cells
- Parenchymatous cells
- Starch grains
- Reticulate xylem vessel
- Elongated xylem fiber
- Fragments spiral vessel
- Stone cells
- Epicarp cells
- Xylem vessel
- Yellow content
- Groups of stone cells.
- Epidermal cells Surface view
- Calcium oxalate crystal
- Reticulate xylem vessel
- Elongated fiber

The following important identifying characters were observed in microscopic examination Figure 2 (a-s) of nisamalaki churna tablet (batch–II). The tablet was crushed in to powder form and treated with 2-3 drops of Chloralhydrate solution, water and observed under the microscope following fragments of different tissues were observed.

- Groups of fibers
- Different fragments of tissues with stone cells, starch grains, thin walled parenchymatous cells, fibers
- Stone cells
- Thin walled parenchymatous cells
- Single fiber
- Thin walled parenchymatous cells and branched fibers
- Thin walled parenchymatous cells
- Groups of xylem fibers
- Epicarp cells surface view
- Cork cells
- Reticulate xylem vessel
- Calcium oxalate crystal
- Yellow content of tannin
- Different fragments of tissues like reticulate vessel, starch grains and parenchymatous cells
- Different fragments of tissues like fibers, starch grains, Parenchymatous cells
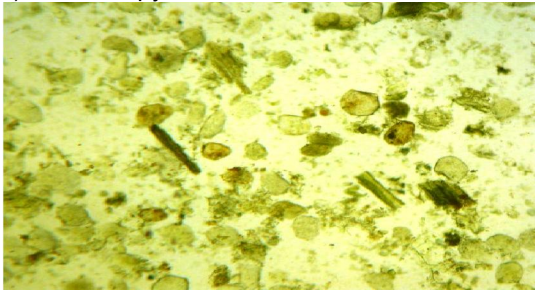- Elongated fiber
- Groups of Stone cells

Therefore there were no significant differences in the two batches of samples with respect to powder microscopic studies
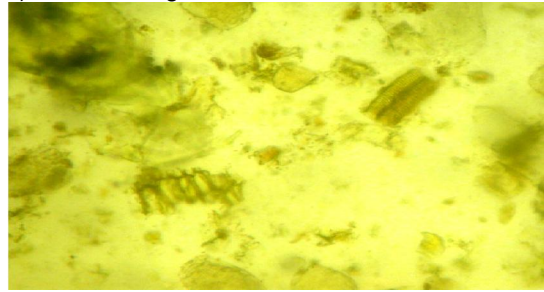
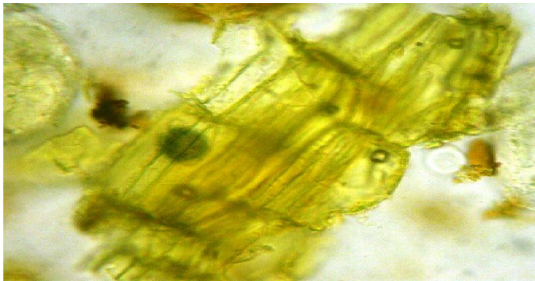a) Macroscopy of the Nisamalakichurna
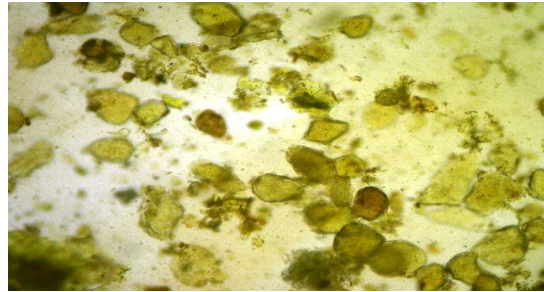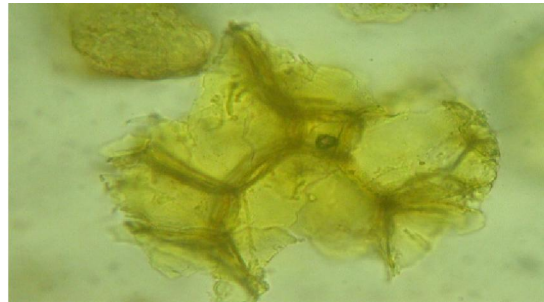
b) Different fragments of tissues.10x X 10x

c) Different fragments of tissues.10x X 10 x

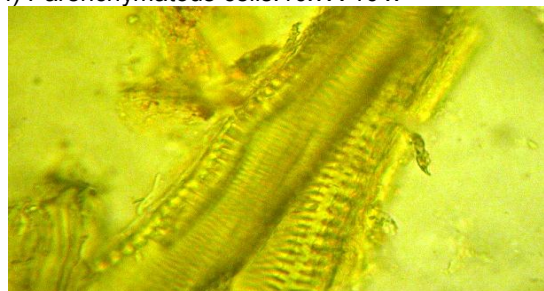d) Fragments of tissues like xylem vessels and
helical vessels.10x X 10 x

e) Fragments of Cork cells.10x X 10 x
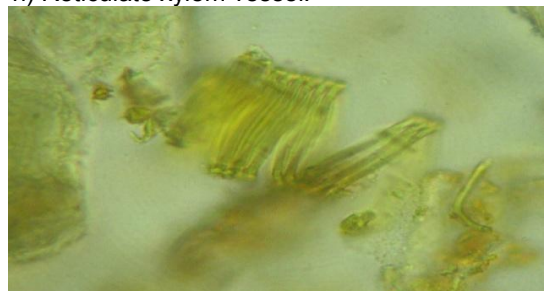
f) Parenchymatous cells.10x X 10 x
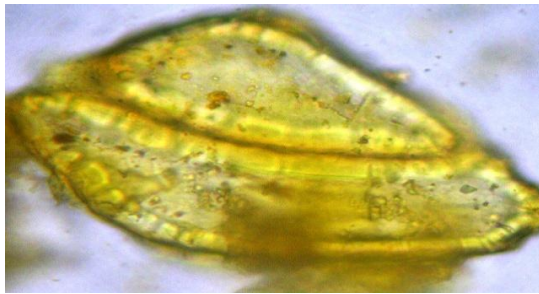
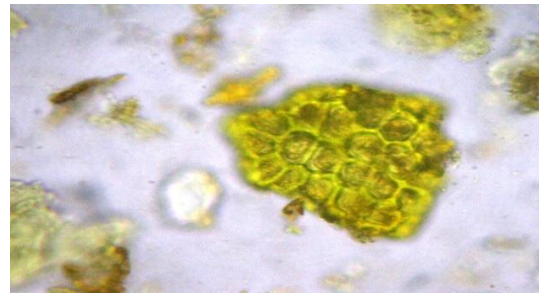g) Starch grains10Xx40X

h) Reticulate xylem vessel.

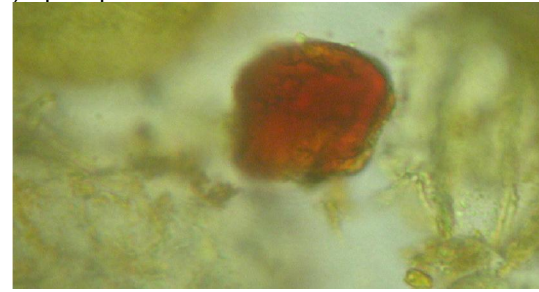i) Elongated xylem fiber. 10Xx40X

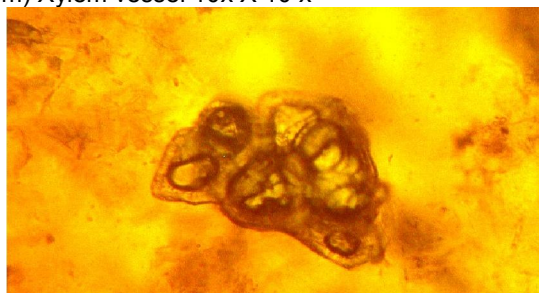j) Fragments spiral vessel 10x X 40x

k) Stone cells10x X 40 x

l) Epicarp cells 10x X 10 x

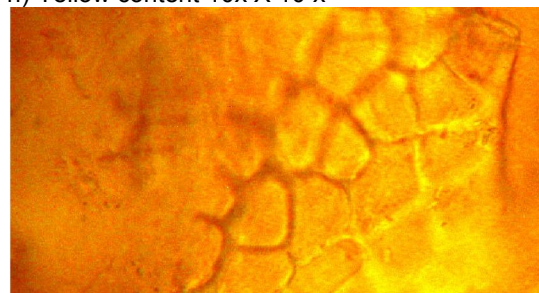m) Xylem vessel 10x X 10 x

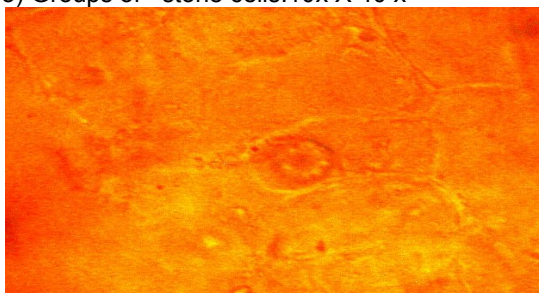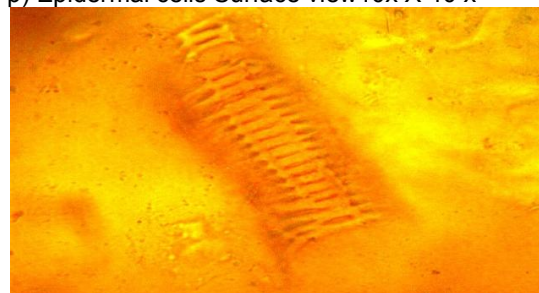n) Yellow content 10x X 10 x

o) Groups of   stone cells.10x X 40 x

p) Epidermal cells Surface view10x X 40 x

q)Calcium oxalate crystal10x X 40 x
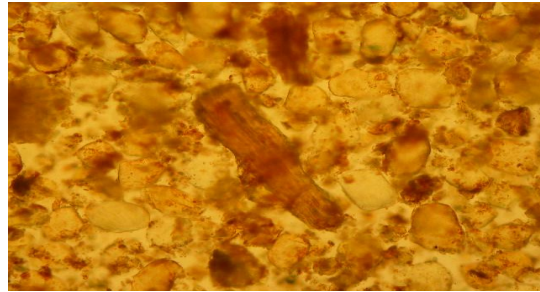

r) Reticulate xylem vessel. 10x X 40 x

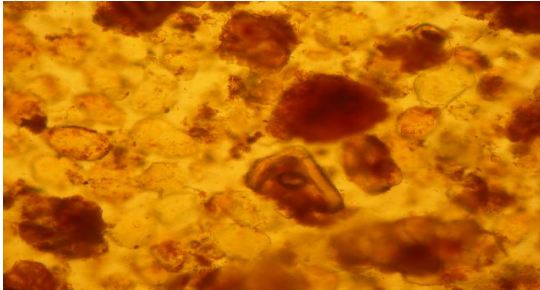
s)Elongatedfiber10x X 40 x

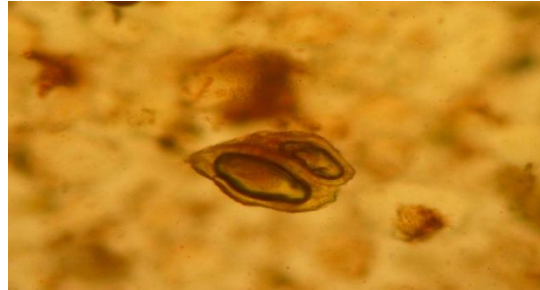**Figure 1(a-s):** Powder microscopy of Batch-I (nisamalaki churna).

a) Nishamalaki tablets sample

b) Groups of fibers 10x X10x

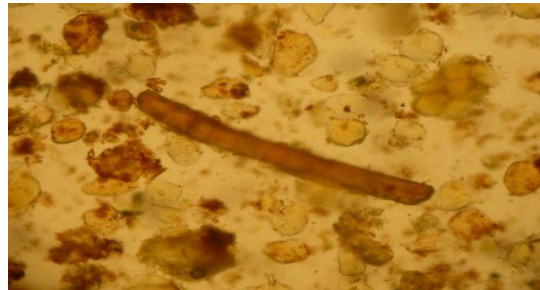c) Different fragments of tissues with stone cells, starch grains, thin walled parenchymatous cells, fibers. 10x X10x

d) Stone cells 10x X10x
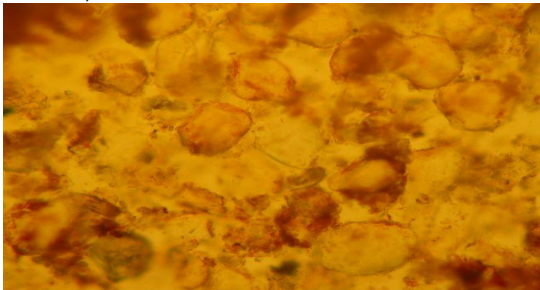
e) Thin walled parenchymatous cells. 10x X10x

f) Single fiber. 10x X10x

g) Thin walled parenchymatous cells and branched fibers. 10x X10x
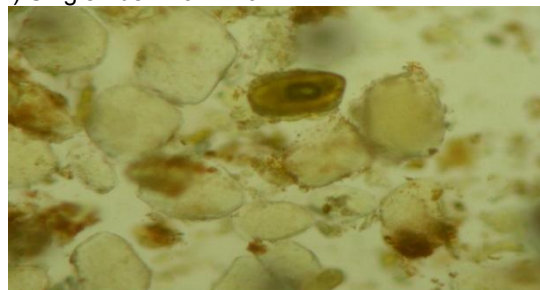
h) Thin walled parenchymatous cells.10xX10x
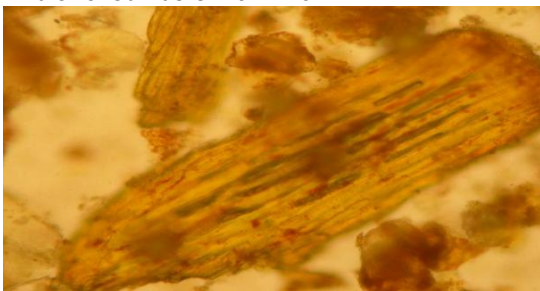
i) Groups of xylem fibers. 10Xx10X
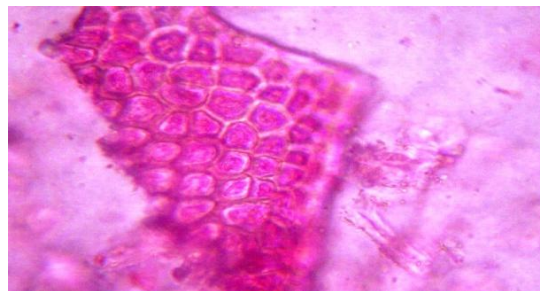
j) Epicarp cells surface view.
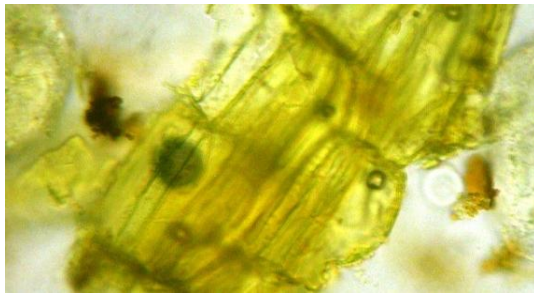
k) Cork cells

l) Reticulate xylem vessel.
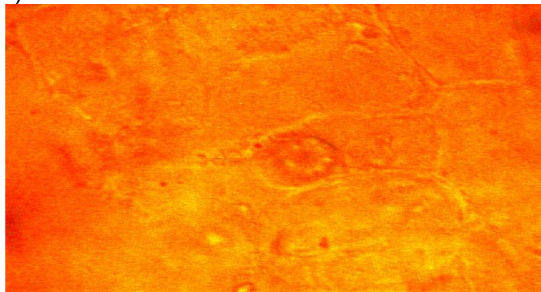
m) Calcium oxalate crystal. 10x X10x
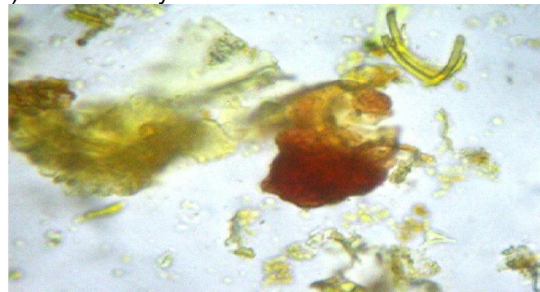
n) Yellow content of tannin.
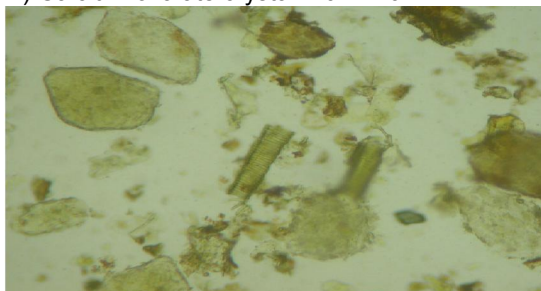
o) Fragments of tissues like reticulate vessel, starch grains and parenchymatous cells. 10xX 40x
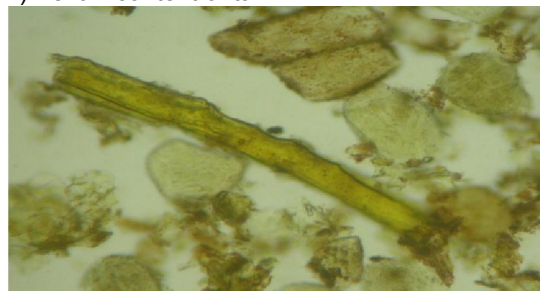
p) Fragments of tissues like fibers, starch grains, Parenchymatous cells.
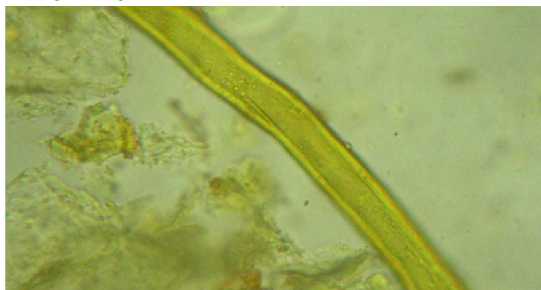
q) Elongated fiber.10x X 40x
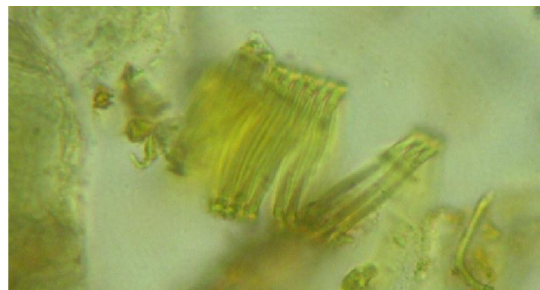
r) Spiral xylem vessel10x X 40x

s) Groups of stone cells.10x X 40x

Figure 2 (a-s): Powder microscopy of standard sample Batch-II (Tablet).

Physicochemical & Preliminary Phytochemical Studies

The physicochemical studies were carried out for both the batches (prepared and standard market sample) and the observations of prepared one are comparable with the standard market sample, and are given in table 3. The test for percentage of moisture content (loss on drying) determines both water and volatile matter. Total ash measures the amount of materials remaining after ignition. Acid insoluble ash measures the amount of silica present especially sand and siliceous matter. Extractive

values were examined which are useful for evaluation of nature of chemical constituents present in drug (Table 3).

Preliminary phytochemical screening of compound formulation was identified through qualitative chemical analysis indicated the presence of alkaloids, carbohydrates, flavonoids, terpenoids, resins, saponins, steroid and tannins etc., of both the batches (Table 4). The physicochemical constituents in both the samples were comparable.

Table 3: Physicochemical Parameters.

| Sl.No.	Test	Result Batch-I	Batch-II
1	%Loss on drying at 105° c	10.38	8.16
2	%Total- ash	5.53	5.1
3	%Acid- insoluble ash	0.425	0.41
4	%Water- soluble extractive	18.6	19.8
5	%Alcohol- soluble extractive	10.84	10.82
6	pH (10% aqueous solution)	2.91	3.01
7	Successive extraction %Petroleum ether 60-80° C %Chloroform % Ethyl alcohol	1.42 2.38 28.25	0.88 1.21 13.0
8	TLC	Table 4 and Fig. 3 (a-c)	Table 4 and Fig. 3 (a-c)

Table 4: Preliminary Phytochemical constituents of Nishamalki churna.

Sl.No	Phytochemical Constituent	Solvents Extract	Batch I	Batch II
1	Crabohydrates	Aqueous	+	+
2	Tannins	Aqueous/alcoholic	+	+
3	Phenols	Alcoholic	+	+
4	Steroids	Chloroform	+	+
5	Triterpenoids	Petether	+	+
6	Racins	Alcoholic	+	+
7	Flavonoids	Alcoholic	+	+
8	Alkaloids	Aqueous/alcoholic	-	-
9	Acids	Aqueous	+	+
10	Saponins	Aqueous	+	+
11	Starch	Aqueous/	+	+

Fluorescence Analysis

The fluorescence behavior of the powdered drug of both batches I & II (prepared sample and standard sample) in different solutions towards ordinary light and Ultra Violet light (both long 365 nm and short 254nm wave lengths) were observed and both the batches are exhibited in same color to different chemical reagents (Table 5).

Thin Layer Chromatographic Studies (TLC)

TLC studies were carried out by observing comparatively with standard market sample extracts of petroleum-ether, chloroform and ethanol solvents under successive extraction through soxhlet apparatus. TLC plates were observed in various mobile phase for all three different extracts of both batches by keeping two tracks (left track is sample prepared and right track is standard sample) on one plate to develop chromatogram and correlate.TLC of
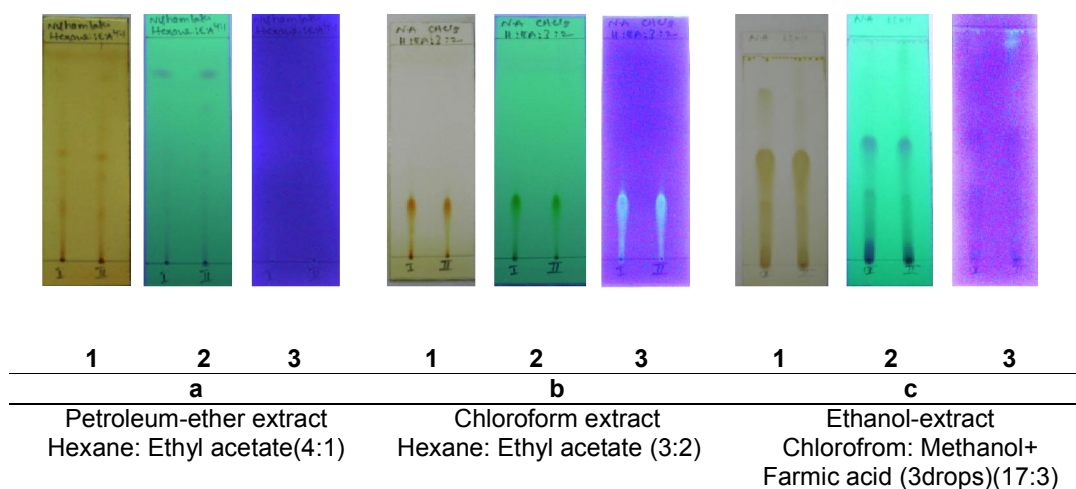
petroleum-ether extract of both the batches were observed in mobile phase hexane: ethyl acetate(4:1), and showed 5 spots with R_f (0.02 to 0.47) Figure 3 (a) (entry a, Table 6); TLC of chloroform extract of both batches were observed in mobile phase hexane: ethyl acetate (3:2) with one spot R_f (0.22) Figure 3 (b) (entry b, Table 6); TLC of ethanol-extract extract of both the batches were observed in mobile phase chlorofrom: methanol + farmic acid (3drops) (17:3) with four spots R_f (0.03 to 0.82) Figure 3 (c) (entry c, Table 6). TLC of samples belonging to both batches with petroleum-ether extract observed in mobile phase hexane:

ethyl acetate (4:1) revealed that the classically prepared nisha amalaki sample had a compound corresponding to Rf value 0.47 whereas the market sample consisted of a compound corresponding to Rf value: 0.46. Similarly when TLC of ethanol-extract extract of both the batches were observed in mobile phase chlorofrom: methanol + farmic acid (3drops) (17:3) the classically prepared nisha amalaki sample had compounds corresponding to Rf value 0.27 and 0.82 whereas, the market sample consisted of compounds corresponding to Rf values: 0.26 and 0.80. Further studies with HPTLC will help decipher exact nature of these compounds.

Table 5: Fluorescence studies of batch-I and II.

SI No.	Sample + Reagent	Ordinary light	UV Long wave 365 nm	U.V. Short wave 254 nm
1	Powder as such	Yellow	Green	Yellowish green
2	Powder + Water	Yellow	Fluorescent green	Fluorescent green
3	Powder +1N. HCl	Yellow	Green	Fluorescent green
4	Powder +1N. NaOH	Yellowish red	Blue	Brown
5	Powder + 1N NaOH in MeOH	Yellowish red	Yellowish green	Greenish brown
6	Powder + 50% KOH	Yellowish red	Blue	Brown
7	Powder + 50% H2SO4	Pinkish red	Black	Black
8	Powder + Con. H2SO4	Reddish black	Black	Black
9	Powder + 50% HNO3	Yellow	Black	Fluorescent green
10	Powder + Con. HNO3	Reddish yellow	Black	Fluorescent green
11	Powder + Acetic acid	Yellowish brown	Yellow	Greenish yellow
12	Powder +Iodine water	Reddish brown	Black	Greenish yellow

	1	2	3	1	2	3	1	2	3
		a			b			c	
	Petroleum-ether extract Hexane: Ethyl acetate(4:1)			Chloroform extract Hexane: Ethyl acetate (3:2)			Ethanol-extract Chlorofrom: Methanol+ Farmic acid (3drops)(17:3)		

Figure 3 (a-c): TLC Plates 1, 2, 3 are observed under Iodine vapor, short and long wave UV respectively.

Table 6: TLC studies.

Entry	Extractives	Adsorbent	Solvent System	Viewing Medium	Rf. Values [Iodine-Medium] Batch-I	Batch-II
a	Petroleum-ether 60-80°C	Silica gel 60 F $_{254}$ pre coated sheets	Hexane: Ethyl acetate (4:1)	Iodine vapour	0.02,0.06, 0.23 0.37, 0.47	0.02, 0.06, 0.23, 0.37, 0.46
b	Chloroform	Silica gel 60 F $_{254}$ pre coated sheets	Hexane: Ethyl acetate (3:2)	Iodine vapour	0.22	0.22
c	Ethanol	Silica gel 60 F $_{254}$ pre coated sheets	Chlorofrom: Methanol+ Farmic acid (3drops) (17:3)	Iodine vapour	0.03,0.27, 0.50, 0.82	0.03, 0.26, 0.50, 0.80

CONCLUSION

Ayurvedic antidiabetic herbal formulation 'nisamalaki churna' was correlated with the standard IMPCOPS nisamalaki tablet with respect to all standardization parameters viz., organoleptic properties, physicochemical investigations, fluorescence analysis, and preliminary phytochemical analysis, powder microscopic analysis, determination of p^{H}, moisture content and TLC studies.

Likewise comparative microscopical studies on the poly herbal formulation nishamalaki churna and standard IMPCOPS nishamalaki tablet was carried out and revealed that thepresence of all most all identifying microscopical characters of the individual drugs namely amalaki, haridra and the standard IMPCOPS tablet. The TLC studies revealed that the R_f values of the prepared nisamlaki churna correlated with that of the standard nisamalki tablet. It can be concluded that this type of study is of great importance in characterization of powdered individual drugs and helps in establishing the pharmacopoeial standards. The study also establishes the fact that the Nisha Amalaki formulation prepared classically in Karnataka state of India is comparable to that of the market sample prepared by IMCOPS Chennai in Tamil Nadu state of India.

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