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Documentation, standardization and nutritional value analysis of selected folklore formulation Used for malnutrition control in Chitrakoot region, Satna (M.P.)

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Abstract
India has a rich repository of oral medical knowledge as a traditional home remedies, folk medicine and regional health practices. The isolated area's inhabitants, who are unaffected by contemporary civilizations, rely on plants for their fundamental medical requirements. One such location is Chitrakoot, which is located in the Satna district of Madhya Pradesh. For present study 18 tribal dominant villages were selected and 674 interviews conducted in 86 Intensive field visits were carried out during 2020-2021. The study is focused on documentation of folklore knowledge with special reference to malnutrition control dietary nutritious folk formulation, and their standardization. The study deals with the various parameters such as macroscopy, powder microscopy, physicochemical tests, detection of heavy metals, screening of microbiological limit tests and high performance thin layer chromatography (HPTLC) fingerprints of methanolic extract and nutritional value analysis were performed. Heavy metals (Pb, Cd, As & Hg) tests were performed and found as per the limits/absent of WHO guidelines. Microbiological analysis of pathogenic bacteria, *viz. Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found that absent in formulated sample. Nutritional value analysis of formulated sample was done such as total fat % by wt. 22.63%, Protein (N*5.95) % by wt. 13.56%, Crude fibre % by wt. 3.2%, Total dietary fibre % 4.53%, Carbodydrates by difference, % by wt. 53.00%, Energy value K cals/100mg 469.91, Vitamin D mg/100g 3.39, Sodium mg/100gm 80.20, Calcium mg/100gm 1720.72, Iron mg/100g 1110.48, Potassium mg/100gm 1550. 7 were found respectively. These findings indicate that formulated sample is a very useful traditional herbal formulation used by tribal communities of Chitrakoot region to maintain resistant power and immunity against common ailments as well as malnutrition of the people. In future, it can be used as strength promoter, malnutrition food supplementary product, immune modulator and will definitely enhance the healthcare system not only the rural India but also in urban societies.

Keywords: Malnutrition, healthcare recipe, standardization, HPTLC fingerprinting, nutritional value

Introduction
Malnutrition is a national and international issue. It is the biggest problem of world including India. Malnutrition is a result of eating diet that have too little nutrients or does not supply a healthy amount of nutrients (Facts for life, 2010) [1]. The nutrients include protein, carbohydrates, vitamins, minerals, calories and minerals. A lack of nutrients is called under nutrition or undernourishment Young, E.M. (2012) [2]. Malnutrition word is often used under nutrition (means when body is not getting enough proteins, carbohydrates, calories or micro-nutrients Jones & Bartlett (2011) [3]. If malnutrition, under nutrition occurs during pregnancy or before two years age of children, it may result in permanent problems with physical (a short height, thin body, very poor energy levels, and swollen legs and abdomen) and mental development, Young, E.M. (2012) [2].

India is one of the countries with the greatest knowledge of spirituality, religion, culture, traditional foods, and folk remedies (Chaturvedi *et al*., 2017 & Rajith *et al*., 2010) [4, 5]. It possesses a rich repository of oral medical knowledge that is accessible in the form of local health customs, tribal remedies, and home health practices (Sehgal A B 2015 & Chaturvedi A 2011) [6, 7]. The isolated area's inhabitants, who are unaffected by contemporary civilizations, rely on plants for their fundamental medical requirements. One such location is Chitrakoot, which is located in the Satna district of Madhya Pradesh. It is inhabited by four types of tribal communities like Kol, Gond, Mawasi and Khairwar and others backward and forward communities. Tribal communities developed herbal remedies and nutritious herbal compound formulations which they used to attained good health (Sikarwar *et al*., 2012) [8].

It has been noticed that rural and tribal groups commonly manufacture their own herbal nutritional component formulations locally. Tribal and rural people including women and children eat the folklore formulations as a breakfast (with milk/without milk) prepared mainly from 7 plant ingredients (*viz*., Mahua (flowers) *Madhuca longifolia* (Koen.) family- Sapotaceae; Til (seeds)- *Sesamum indicum* Linn., family- Pedaliaceae; Alasi (seeds)- *Linum usitatissimum* Linn., family- Linaceae; Chirounji (seeds)- *Buchanania lanzan* Spreng., family- Anacardiaceae; Bidarikand (rhizome)- *Pueraria tuberose* (Roxb.ex Willd.) DC., family- Fabaceae; Ashwagandha (root)- *Withania somnifera* (Linn.) Dunal, family- Solanaceae; Safed Musali (root)- *Chlorophytum borivlianum (*Roxb.), *family*- Liliaceae). The folk formulations are complete nutritional recipes, with rich contents of essential nutrients such as protein, carbohydrates, vitamins crude fibres, dietary fibres and minerals etc. Therefore, an ethno-medicinal survey was conducted among the tribal and folk communities of Chitrakoot region for the documentation of folklore healthcare formulations associated with malnutrition control folk formulation, preparation and standardization. For standardization various parameters were included as per recommended by Ministry of AYUSH or WHO guideline *viz*. macroscopy, powder microscopy study, physicochemical tests, screening of bio -chemicals, heavy metals test, microbiological limit tests, development of high performance thin layer chromatography (HPTLC) and nutritional value analysis.

Materials and Methods

Survey and documentation of folk knowledge

An ethno-medicinal survey was carried out in 18 tribal dominated villages, *viz*. Amiliya, Barua, Bhargawan, Bundelapur, Chandai, Devlaha, Tagi, Hiraundi, Kailashpur, Kathauta, Koldari, Parewa, Patna, Patni, Pindra, Piparitola, Raiya and Turra. The detailed information on nutritious formulation regarding the mode of preparation, ingredients, name and ratio of ingredients, parts used, mode of administration, doses and duration, benefits and age groups etc., has been recorded with the help of standard questionnaires. 674 interview conducted in 86 Intensive field visits were carried out during 2020-2021, covering almost all the seasons of the year, The 45-80 years old knowledgeable men and women, experienced traditional healers and local vaidyas who have actively engaged in ethno-medicinal practices, were interviewed. The data was double-checked during various field visits to the same or different places (Sikarwar *et al*., 2012) [8].

Collection of samples and formulations preparation

The ingredients of nutritious formulation are Mahua (flowers) *Madhuca longifolia* (Koen.) family- Sapotaceae; Til (seeds)- *Sesamum indicum* Linn., family- Pedaliaceae; Alasi (seeds)- *Linum usitatissimum* Linn., family- Linaceae; Chirounji (seeds)- *Buchanania lanzan* Spreng., family- Anacardiaceae; Bidarikand (rhizome)- *Pueraria tuberose* (Roxb.ex Willd.) DC., family- Fabaceae; Ashwagandha (root)- *Withania somnifera* (Linn.) Dunal, family- Solanaceae and Safed Musali (root)- *Chlorophytum borivlianum (*Roxb.), family- Liliaceae), were collected from the tribal villages and forests. Samples were identified and authenticated as per standard procedure (Kokate, 2005) [10] by Dr. Manoj Kumar Tripathi, botanist, Arogyadham, DRI, Chitrakoot**.** Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for further studies. The formulation was prepared by using 7 ingredients. Formulation composition is given in (Table 1). For formulation of sample; seven ingredients Mahua flowers, Til seeds, Alsi seeds, Chirounji seeds, Ashwagandha roots, Safed Musali roots and Bidarikand rhizomes were taken separately as per defined quantity given in (Table-1). Ingredients are properly cleaned. While Mahua flowers, Til seeds, and Alsi seeds roasted separately in a clay pot pan till become free from moisture and prepared granulated powder separately. Weighed each ingredient separately and mix together in specific quantity to obtain a homogenous blend and prepared the sample in the form of granular powder. The powder sample was stored in air-tight container for further analysis (Anonymous 1991) [9].

Macroscopic study

Macroscopic or organoleptic characters of herbal compound formulation like colour, odour and taste were evaluated (Kokate *et al*., 2005) [10].

Preparation of slides for powder microscopic study

About 2 g of powder sample washed thoroughly with potable water, poured out the water without loss of material. Mounted a small portion in glycerin were used to all characters of the samples separately, small quantity of samples cleared by heating with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mounted in glycerin, another small quantity of samples stained with sudan red solution and mounted with glycerin, all mounted slides were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope separately (Anonymous 2010) [11].

Physico-chemical tests

Physico-chemical parameters such as moisture content (loss on drying at 105 °C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and pH (10 %) aqueous solution were studied (Anonymous 2008 & Tripathi *et al*., 2015) [12, 13].

Preliminary phyto-chemical analysis

Preliminary phyto-chemical tests of formulated sample was conducted on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, carbohydrates, flavonoids, protein, resins, saponin, tannin and steroids (Anonymous 2008 & Venugopal *et al*., 2015) [12, 14].

Screening of heavy metals test

Heavy metals are toxic and generally occur through earth in plants. Mainly four types of heavy metals harmful for us they are Pb, Cd, As and Hg. These heavy metals detected through Atomic Absorption Spectrophotometer as per standard method (Anonymous 2010) [11].

Nutritional Value Analysis

Nutritional value analysis have been performed formulated sample through various parameters such as total calories, total corbohydrate, total fat, protein, vitamin C, dietary fiber, Iron (as Fe), Calcium (as Ca), vitamin A, Sodium (as Na) and Potassium (as K) (IS:14433, 2007, Anonymous 2009 & 2016) [15, 16-17].

High performance thin layer chromatography (HPTLC) fingerprint profile

For High performance thin layer chromatography, 2 gm of powdered sample was extracted with 100 ml of methanol

overnight, filtered and concentrated. Ferulic acid, Kempherol and Caffeic acid standard markers were used for identification of Ferulic acid, Kempherol and Caffeic acid active phyto - constituents in formulated sample. For preparation of standard markers working solutions, 10mg of Ferulic acid, Kempherol and Caffeic acid were dissolved in a 10 ml volumetric flask and made up the volume with methanol separately. Then transferred 1 ml from stock solution to a 10 ml volumetric flask and made up the volume with methanol separately. From the solution, prepared standard solutions by transferring aliquots (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml) corresponding to (1, 2, 3, 4, 5 and 6 ug/ml) of stock solution to 10ml volumetric flasks and made up the volume in each case to 10 ml with methanol. It was applied by spotting extracted samples on pre-coated silica-gel aluminium plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The sample and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of *toluene: ethyl acetate*: *formic acid* (7:2.5: 0.5 v/v). Linear ascending development was carried out in 20x20cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, thin layer chromatography plate was dried with the help of Hot Air Oven instrument. The peak area for sample and standards were recorded with camera photo documentation system Camag Reprostar 3. Visualization of spots were made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at 254nm and 366nm after deivatization with Win cat software and R_f values noted (Chaudhary *et al*., 2014 & Anonymous 2009) [18, 16].

Microbiological limit tests

Microbial limit tests for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. The tests were carried out to determine the microbial load in the formulated sample (Anonymous 2009 & 2010) [16, 11]. Enumeration of *Staphylococcus aureus*/gm, *Salmonella* sp./gm, *Pseudomonas aeruginosa*/gm, and *Escherichia coli*, determination of total microbial count (TBC) and determination of Yeast & Mould. The microbiological tests were determined using specified agar media and enrichment media from Himedia, Pvt. Ltd. Mumbai.

Results

Macroscopic characters

A brownish black colour of formulated sample with the characteristic odour and a light sweetish taste.

Powder microscopic characters

Formulated sample ingredients are showed distinguished anatomical characters such as Mahua flower showed- epidermal cells of petals with striated cuticle, covering trichomes, similarly Til seeds- Til seeds- parenchyma tissue with aleurone grains, fragment of cotyledon, Alsi seeds - fragment of pigment cells layer in surface view, fragment of sclerenchymatous cells in surface view, Chiroungi seeds - various types of sclereids, pigment cells of testa in surface view, Bidarikand rhizome - different shape & sized pipette like crystals, articulated fibre, Ashwagandha root- Cortical parenchymatous cells and medullary rays cells containing simple starch grains that are up to 30µ, mular shaped, mostly single, occasionally groups of two or three with slit like or stellate hilum, and Musali root- Fragments of beaded or pitted thick walled cells of epiblema in surface view with prismatic crystals of calcium oxalate, and a few root hairs (Fig.1a-1n).

Physico-chemical analysis

The physico-chemical analysis such as Loss on drying on 105 °C, water such as extractive values, alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean (n=3) ± standard deviation in w/w. Formulation LOD was found 6.1% w/w, total ash value 11.34% w/w, acid insoluble ash value 1.2% w/w, alcohol soluble extractive value 50.26% w/w and water soluble extractive value 52.03%w/w.

Nutritional value analysis

Nutritional value analysis *viz*. total calories, total corbohydrate, total fat, protein, Vitamin C, dietary fiber, Iron (as Fe), Calcium (as Ca), Vitamin A, Sodium (as Na) and Potassium (as K) of four samples of Laata were done separately. Nutritional value analysis results are given in (Table 2).

Preliminary phyto-chemical investigation

Qualitative phyto-chemical tests were performed in water and ethanol extracts. Alkaloids, carbohydrates, flavonoids, protein, resin and soponin were present.

Heavy metals tests

Heavy metal elements (Pb, Cd, As and Hg) test were performed and found under limits as per WHO guideline *viz*. for Lead 10ppm, Cadmium 0.3ppm, Arsenic 03ppm and Mercury 01ppm and results were found Pb 0.5430 ppm, Cd 0.04539 ppm, As0.5631ppb and Hg 0.5672ppb.

Microbiological limit tests

Microbiological analysis of pathogenic bacteria, *viz*. *Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found that absent in formulation while total microbial plate count (TPC) was found 60cfu/g and yeast & moulds found 80 cfu/g. Microbiological profile of the formulation was found satisfactory under prescribed limits in WHO guidelines / Ayurvedic Pharmacopoeia of India such as for *Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* limits absent, where for total microbial plate count (TPC) 10^5 cfu/g and for yeast & moulds 10^3 cfu/g.

HPTLC (high performance thin layer chromatography) finger prints profile

High Performance Thin- Layer Chromatography of the test solutions of sample A was carried out on Silica Gel 60 F_{254} precoated plates (0.2 mm thickness; from Merck India Limited Mumbai). A TLC applicator from Camag Linomat-5 (Camag Switzerland 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. Applied 10 µl each of the test solutions as 8 mm bands and develop the plate in a solvent system *to*luene*: ethyl acetate* (7: 3) to a distance of 9 cm. Dry the developed plate in air and examined under ultraviolet light, at 254 nm and at 366 nm before derivatization. Derivatized the plate using 5%

Methanolic-sulphuric acid reagent and heating at 105 °C till the bands are clearly visible and examined the plate under 254nm and 366nm.HPTLC fingerprint profile of the test solution is depicted in (Fig. 2A, 2B & 2C) indicates the presence of different types of phytochemicals. Development of fingerprint profile would serve as a reference standard of the authentic sample. The TLC plate was examined under 254nm, 366nm, after derivatization 366nm. The R_f values and colours of the bands obtained were recorded. It shows major spots and the R_f values and colours of the bands obtained were recorded. Major spots R_f values with colour were recorded before derivatization at 254nm and after derivatization at 366nm. Major spots of R_f values at 254nm before derivatization are 0.58 black, with Ferulic acid, 0.60 black with Kempherol, 0.40 black with caffeic acid standard and after derivatization at 366nm major spots R_f values are 0.40 sky blue, with ceffeic acid acid standard, 0.55 sky blue colour with Ferulic acid standard, and 0.60 yellow with kempherol standard.

In the present study, a systematic survey and documentation of traditional knowledge with special reference to a nutritious herbal compound formulation used by tribal and local people for malnutrition control in Chitrakoot region was carried out in 18 tribal (Kol, Gond, Mawasi & Khairwar) dominated villages. A total 674 interviews were conducted in 86 intensive field visits during 2020-2021, covering almost all the seasons of the year. The 45-80 years old traditional healers, local vaidyas, especially experienced women who have been actively engaged in preparation of traditional recipes were interviewed (Tripathi *et al.* 2015) [13]. The traditional herbal formulation described in the study involves total 7 plant species belonging to 6 different families.

As per pharmacognostic study of the formulation was tested for relevant macroscopically, macroscopically, physical and chemical parameters. The formulation was subjected to various analytical techniques. Powder microscopic test were performed and established the distinguished anatomical characters for formulated sample, results are depicted as (Fig.2a-2n), and these specific anatomical characters may be helpful for identification of ingredients in the formulation.

The results of physicochemical analysis are expressed as mean (n=3)+- standard deviation. The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards, the extractive values, alcohol soluble and water soluble indicates the amount of active constituents in given amount of plant material when extracted with respective solvent. Qualitative phyto-chemical analysis was performed in water and ethanol extracts. Alkaloids, carbohydrates, flavonoids, protein, resin and soponin were present in the sample which could make the formulation useful for potential and preventive healthcare needs. Microbiological analysis of pathogenic bacteria, *viz*. *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found absent in the sample. Microbiological profile of the sample was found satisfactory under prescribed limits such as for *Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* limits absent, where for total microbial plate count (TPC) 10^5cfu/g and for yeast & moulds 10^3cfu/g as per WHO guidelines, these results indicates that samples are free from harmful bacteria. Similarly detection of heavy metals (Pb, Cd, As & Hg) tests were performed and found under limits/absent as per WHO guidelines (Tripathi *et al.* 2015) [13].

Nutritional value analysis of formulated sample was done such as total fat % by wt. 22.63%, Protein (N*5.95) % by wt. 13.56%, Crude fibre % by wt. 3.2%, Total dietary fibre % 4.53%, Carbodydrates by difference, % by wt. 53.00%, Energy value K cals/100 mg 469.91, Vitamin D mg/100g 3.39, Sodium mg/100gm 80.20, Calcium mg/100 gm 1720.72, Iron mg/100g 1110.48, Potassium mg/100 gm 1550.7 were found respectively. These findings are indicated that the sample is very good traditional herbal nutritious recipe used by tribal and folk communities of Chitrakoot region to maintain a good health. These practices if integrated with modern healthcare system could elevate the health status of thousands of rural people as well as urban people. This study may also helpful in the preparation of formulation and their quality evaluation.

Table 1: Formulation Composition of malnutrition control sample

S. No	Ingredients name	Botanical name	Family	Part used	Quantity
1	Mahua	*Madhuca longifolia* (Koen.)	Sapotaceae	Flower	3part
2	Til	*Sesamum indicum* Linn.	Pedaliaceae	Seed	2part
3	Alasi	*Linum usitatissimum* Linn.	Linaceae	Seed	1part
4	Chirounji	*Buchanania lanzan* Spreng.	Anacardiaceae	Seed	1part
5	Bidarikand	*Pueraria tuberose* (Roxb.ex Willd.) DC.	Fabaceae	Rhizome	1part
6	Ashwagandha	*Withania somnifera* (Linn.) Dunal	Solanaceae	Root	1part
7	Safed Musali	*Chlorophytum borivlianum (*Roxb.)	Liliaceae	Root	1part

Table 2: Nutritional value analysis of malnutrition control sample

S. No	Parameters	Unit of measurement	Result
1	Total calories	Kcal/100 g	469.91
2	Total corbohydrate	g/100 g	53.00
3	Total fat	g/100 g	22.63
4	Protein	g/100 g	13.56
5	Vitamin C	mg/100 g	3.39
6	Dietary fiber	g/100 g	4.53
7	Iron (as Fe)	mg/100 g	10.48
8	Calcium (as Ca)	mg/100 g	17.72
9	Vitamin A	IU/100 gm	BLQ (10.0)
10	Sodium (as Na)	mg/100 g	80.20
11	Potassium (as K)	mg/100 g	155.70

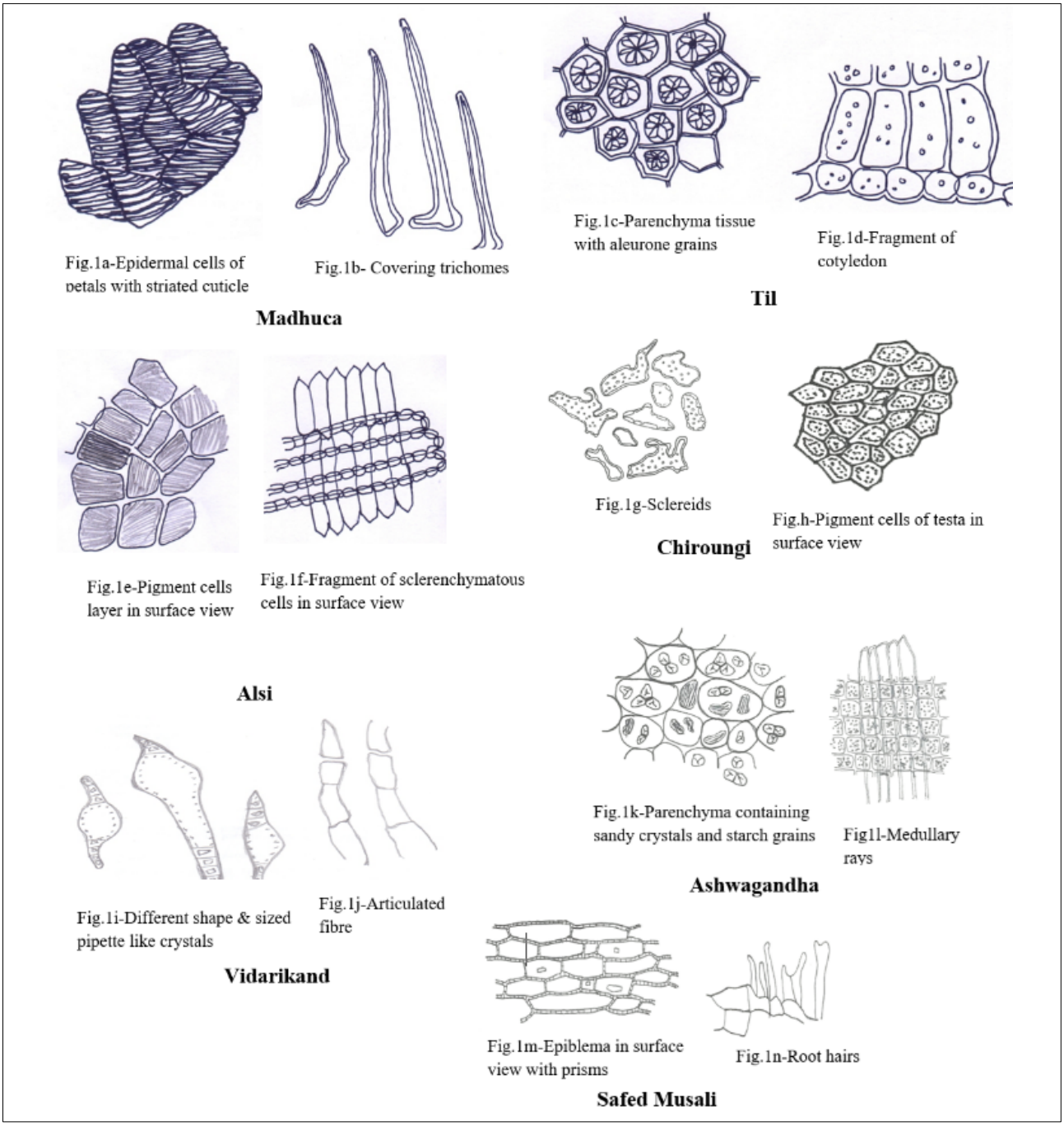

Plate 1: Powder Microscopic characters of malnutrition control sample

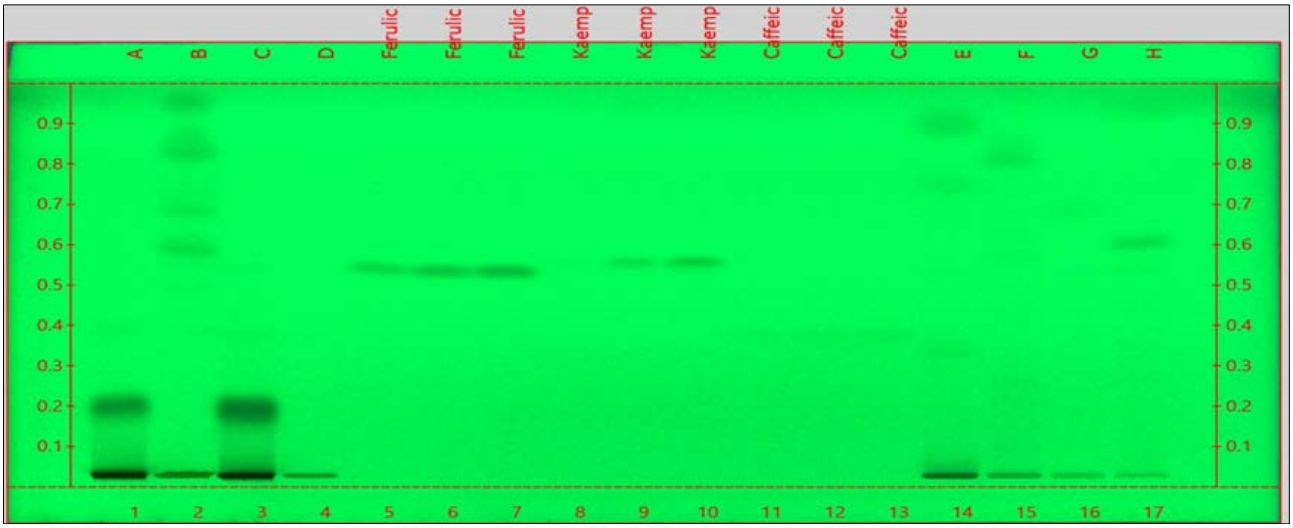

Fig 2a: HPTLC fingerprints profile at 254nm before derivatization

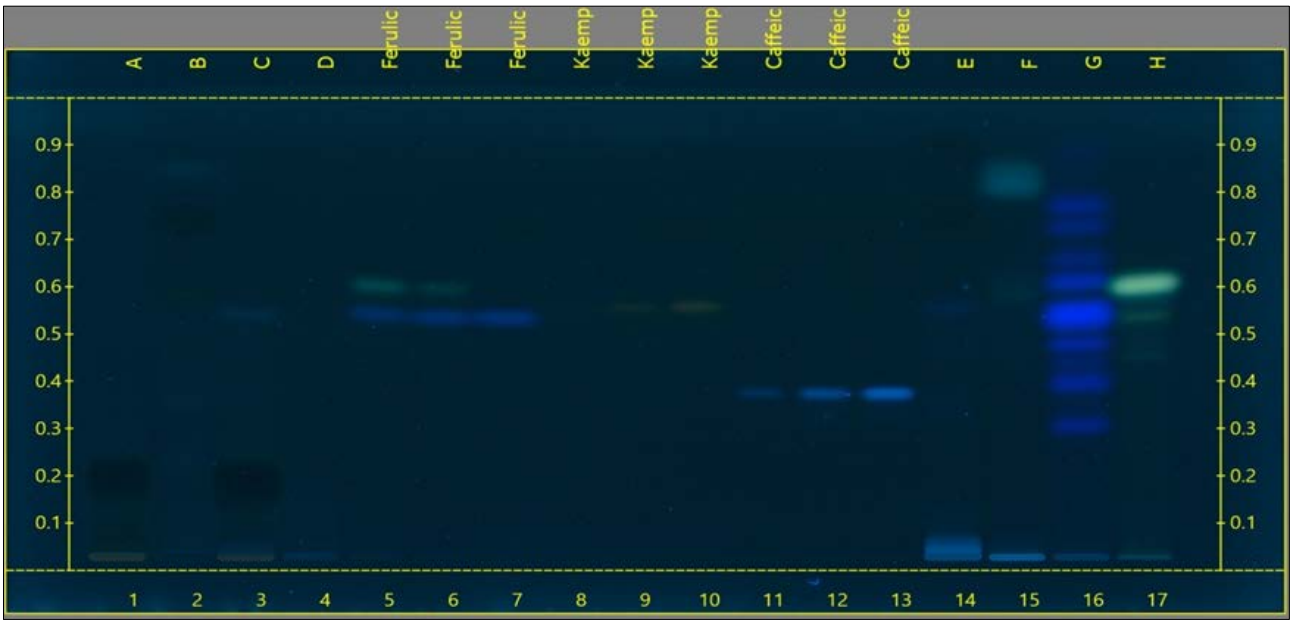

Fig 2b: HPTLC fingerprints profile at 366nm after derivatization

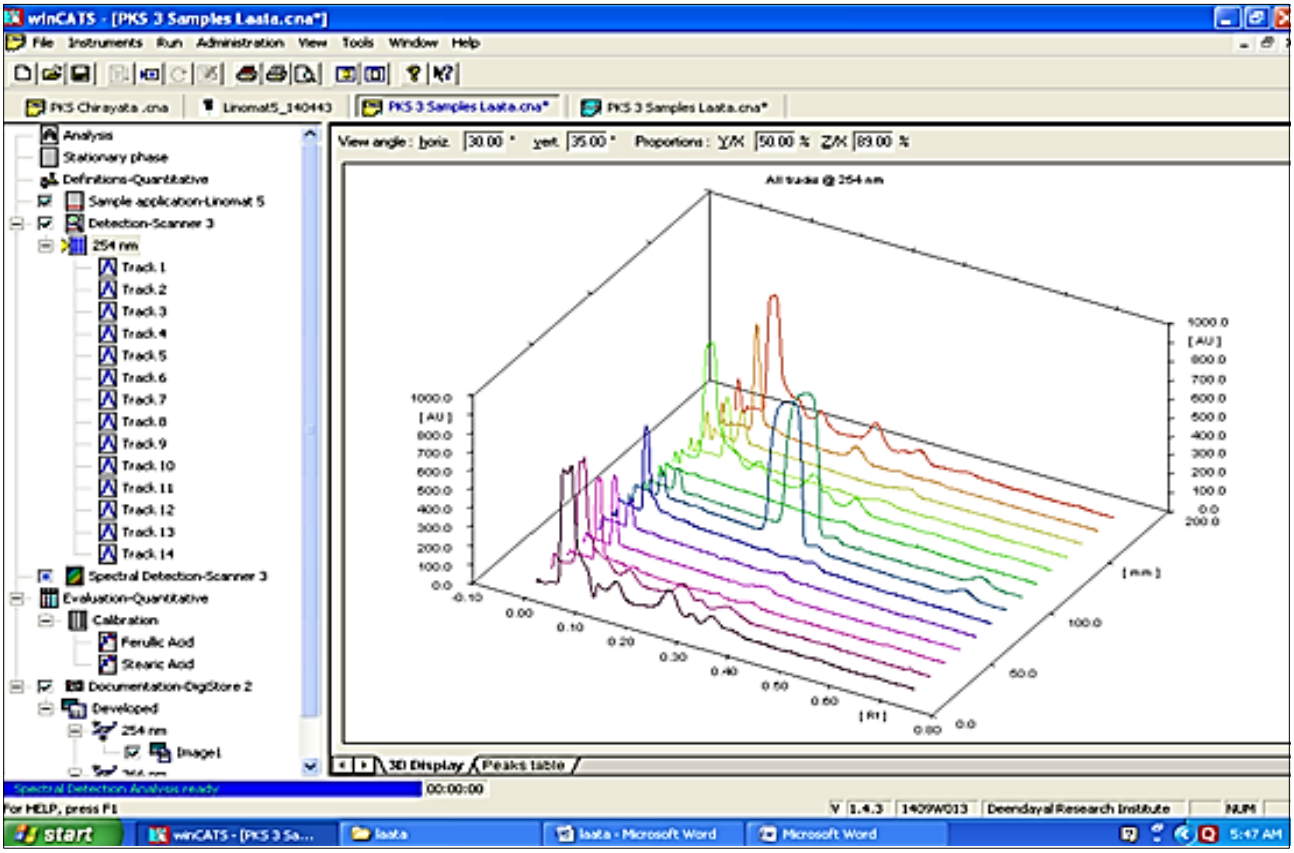

Fig 2c: 3D densitometry graph
Plate 2: HPTLC fingerprints profile of malnutrition control sample

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Conflict of interest
Authors declare no conflict of interest.

Abbreviations: Track1 Mahua, track 2 formulation, track 3 til, track 4 alasi, track 5-7 ferulic acid standard, track 8-10 10 kempherol standard, track 11-13 caffeic acid standard, track14 chiroungi, track 15-Bidarikand, track 16 Ashwagandha, track 17 Safed Musali

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