



PHARMACEUTICAL STANDARDIZATION OF *CHITRAKADI GHRITA*

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ABSTRACT

Ghrita (ghee) preparation is superior among all the *Sneha dravyas* because of its power to assimilate effectively the properties of the substances. *Chitrakadi Ghrita*, a polyherbal formulation is suggested in the management of *Vandhyatva* (Infertility) caused by *Yonidosha* mentioned by *Acarya Sushruta* in *Sushruta Samhita*. Physico-chemical analysis of the coarse powder made out of the crude drugs and final preparation i.e., *Chitrakadi Ghrita* was carried out in the Indian Institute of Chemical Technology (IICT), Hyderabad, A.P. Qualitative analysis was done to identify the compounds Triterpnoids, Steroids, Flavonoids, Phenolic compounds, Carbohydrates got positive results in raw herbal powder and *Chitrakadi ghritam*. *Chitrakadi ghritam* shows Acid value was 4.78, Saponification value 280.5, Refractive Index value 1.53, Iodine value 28.7, Specific gravity was 0.9, Ester Value 275.72, Bulk density value 1.60, Peroxide value 9.22. Scanning Electron Microscope (SEM) with Energy Dispersive X-ray analysis (EDX) shows no heavy metals in *Chitrakadi Ghrita*. TLC was carried out both ChG and HPChG after organizing appropriate solvent system in which TLC examination revealed that it is a complex mixture of 10 to 12 compounds having close Rf values respectively. The present study provided to demonstrate applicability of the methods to standardization of *Chitrakadi ghrita*, the analysis report shows the values are within the limits, and there is no harmful metals found. The normal limit of Microbial contamination was estimated. It does not contain any harmful microbes such as *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. *Chitrakadi ghrita* can be given internally to the patients for the management of *Vandhyatva* (Infertility).

Keywords: Standardization, *Chitrakadi ghrita*, Physical parameters, TLC, Scanning Electron Microscope (SEM) with Energy Dispersive X-ray analysis (EDX).

INTRODUCTION

In the Ayurvedic Pharmaceutics, *Sneha dravyas* (fat soluble) and water soluble active principles are extracted in a suitable fat media, termed as *Sneha kalpana*. Broadly two main types of *Sneha kalpana* are described into the classics i.e. *Taila* and *Ghrita kalpana*^[1].

Ghrita, *Taila*, *Vasa*, and *Majja* are mainly four *Sneha dravyas* as described in *Ayurvedic* classics, amongst them ghee is best because of its power to assimilate effectively the properties of other substances when added to it^[2]. In the eight kinds of ghee from eight different animal milk and ghee made from cow's milk is said to be superior among them^[3].

In *Bhavaprakasha Goghrita* properties mentioned as *Rasayana*, good for the eye, stimulate digestion, supports glow and beauty, enhances memory and stamina, promotes longevity and protects body from various diseases and considered the best among all types of ghee^[4].

Sneha siddha (fat soluble) drugs have better pharmacokinetic action (ADME- absorption, distribution, metabolism, and excretion) in comparison to other dosage forms because of the lipid nature of the biomembranes, as lipid soluble substances readily permeate into the cells^[5]. Present drug *Chitrakadi ghrita* mentioned *Acarya Sushruta* in

Sushruta samhita, uttarantra in the management of female infertility with *Yoni Dosh*^[6].

A clinical study of Effect of *Chitrakadi Ghritam* in the management of *Vandhyatva* (Infertility) with *Yonidosha*^[7] study was also conducted in 50 female patients suffering from infertility in the OPD and IPD of Dr. B.R.K.R.Govt. Ayurvedic College and Hospital, Hyderabad and observed highly significant result by statistical values.

Physicochemical analysis, TLC, Scanning Electron Microscope (SEM) with Energy Dispersive X-ray analysis (EDX) to *Chitrakadi Ghrita* was conducted in the Indian Institute of Chemical Technology (IICT), Hyderabad.

AIMS AND OBJECTIVES

- 1) Pharmacognostical study of individual components of *Chitrakadi Ghrita*.
- 2) Physico-chemical analysis of *Chitrakadi Ghrita*.

MATERIALS AND METHODS

- A. Collection and authentication of raw drugs
- B. Pharmaceutical preparation of *Chitrakadi Ghrita*
- C. Standardization of *Chitrakadi Ghrita*

A. Collection and authentication of raw drugs

The authentic ingredients were procured from the local market of Hyderabad, Andhra Pradesh and were thoroughly checked and botanically identified by the experts in the Dr. B.R.K.R.Govt. Ayurvedic College and Hospital, Hyderabad.

B. Pharmaceutical preparation of *Chitrakadi Ghrita*

i. *Ghrita Murchana*

Before preparation of *Ghrita, Murchana* was performed as per the authentic text.^[8] *Aama dosha* and *Durgandha* from raw *Ghrita* can be removed and *Ghrita* pure and is ready absorb of medicinal properties in it from the drugs with which it is processed. The resultant *Ghrita* attains good odour, colour and is easily absorbed and assimilated.

ii. Method of preparation of *Chitrakadi Ghrita*

Paste was prepared with each one *Karsha* (10gm) of *Chitramula, Sariba, Bala, Kalanusarrikaa, Draaksha, Vishaala, Pippali, Chitrapala, Yashtimadhu, Haritaki* and *Amalaki*. One *Aadhaka* (2.56kg) of ghee one *Drona* of milk

(10.24kg), one *Drona* of water (10.24kg) was added. Ghee should be prepared according to *Ghrtapaaka vidhi* according to *Sneha kalpanaa* procedure as per *Sharangadhara samhita madhyama khanda*^[9]. Then it should be filtered at room temperature. Add one *Prastha* (640gm) of sugar, one *Prastha* of (640gm) *Tavaaksheeri* powder and mixed thoroughly and stored.

OBSERVATIONS

1. *Sneha kalka* attains perfect wick shaped when rolled between thumb and index fingers.
2. No sound was produced when *Kalka* is put into fire.
3. Disappearance of bubbles in final stage of *ghrita paka* observed.
4. Colour, odour and taste of the ingredients were appreciable.

Table 1: Ingredients of *Chitrakadi Ghrita*

S.No.	Name	Botanical name	Part
1.	<i>Chitramula</i>	<i>Plumbago Zeylanicum</i> Linn.	10gm
2.	<i>Sariba</i>	<i>Hemidesmus indicus</i> R. Br	10gm
3.	<i>Bala</i>	<i>Sida Cordifolia</i> Linn.	10gm
4.	<i>Krishna sariva</i>	<i>Ichnocarpus frutescens</i> R.Br.	10gm
5.	<i>Draaksha</i>	<i>Vitis vinifera</i> Linn	10gm
6.	<i>Vishaala</i>	<i>Citrullus Coloegnthis</i> st kard	10gm
7.	<i>Pippali</i>	<i>Piper Longun</i> Linn.	10gm
8.	<i>Chitrapala</i>	<i>Trichosanthes brateata</i>	10gm
9.	<i>Yashtimadhu</i>	<i>Glycyrrhiza glabra</i> Linn.	10gm
10.	<i>Haritaki</i>	<i>Terminalia Chibula</i> Ritz.	10gm
11.	<i>Amalaki</i>	<i>Emblica officinalis</i> Gaertn.	10gm
12.	<i>Kshira</i>	Milk	10.24kg
13.	<i>Go Ghrita</i>	Cow's ghee	2.56kg
14.	<i>Jala</i>	Water	10.24 lts
15.	<i>Sugar</i>		640gm
16.	<i>Tavakshira</i>		640gm

C. Standardization of Chitrakadi Ghrita

Evaluation of Physicochemical Parameters

Pharmacognasy implies particular knowledge of methods of identification and evaluation of drugs. For the identification of compounds in Poly herbal formulation of Herbal Powder for Chitrakadi ghritam (HPChG) and Chitrakadi ghritam (ChG) by colour reactions TLC, Acid value, Saponification value, Refractive index value, Iodine value, Ester value and bulk density, heavy metals analysis, Microbial contamination, Test for specific Pathogen tests were conducted as per the references^[10-13], at Indian Institute of Chemical Technology (IICT), Hyderabad. The results are furnished in the following tables.

Identification of compounds in Poly herbal formulation of Herbal Powder for Chitrakadi ghritam (HPChG) and Chitrakadi ghritam (ChG) by colour reactions. Procedures to carry out some colour reactions were discussed in this section.

1. Detection of triterpenoids and steroids (Liebermann -Burchard test)

Play of colors changing from green to red colour develops when compound (10 mg) dissolved in chloroform (2 ml) is treated with acetic anhydride (few drops) and con. H₂SO₄ (2 drops). It is positive test for triterpenoids and steroids.

2. Detection of steroids (Salkowshi test)

Red colour develops when the compound (10mg) dissolved in chloroform (2 mL) is treated with conc. H₂SO₄ (2 drops). It is a positive test for steroids only. No red colour obtained, indicating the absence of steroids.

3. Detection of flavonoids (Shinoda test)

Pink colour develops when compounds (10gm) dissolved in methanol (2 mL) is treated with magnesium powder (30 mg) and conc. HCl (1 mL). It is positive test for flavonoids.

4. Detection of phenolic compounds (Ferric chloride test)

Green or brown colour develops when compounds (10 mg) dissolved in methanol (2 mL) is treated with neutral ferric chloride (2 drops). This test is positive for phenolic compounds.

5. Detection of carbohydrates (Molish test)

Compounds (10mg) was dissolved in methanol and then mixed with 2 drops of

concentrated solution of α -naphthaol in alcohol. Then allow few drops of concentrated H₂SO₄ to flow down the side of the inclined test tube. A red ring appears at the junction of the liquids. The colour quickly changes on standing or shaking and the solution becomes a dark purple slowly indicating the presence of carbohydrates.

6. Acid value

This determination we have carried out on the fat or oil extracted from the sample by continuous extraction with ether. The acid value of oil or fat is defined as the number of milligram of potassium hydroxide require to neutralize the free acid in one gm of the sample.

Method: Mix 25 ml ether with 25 ml alcohol (95%) and one ml of 1% phenolphthalein solution and neutralize with N/10 potassium (or sodium) hydroxide shaking constant constantly unit a pink color which persists for fifteen seconds is obtained

Acid value = $\frac{\text{No of ml of N/10 alkali used} \times 4.61}{\text{Weight of sample in gms.}}$

7. Saponification value

Hydrolysis of an ester (oil, fat) by an alkali to form soap and alcohol is called saponification. The terms saponification value or saponification number indicate a measure of amount of alkali required to saponify a definite quantity of fat and its is usually express as number milligram of potassium hydroxide required to saponify one gram of fat. The saponification equivalent (SE) is the number of gram of fat that can be saponified by one mole of potassium hydroxide.

The product of SV and SE is thus equal to 56.108 (mol wt of KOH expressed as mg). The neutralization value (NV) and neutralization equivalent are corresponding terms applied to fatty acids. The value (EV) is the difference between the saponification value and acid value.

SV is a very valuable characteristic of oils and is useful in the estimation of composition of mixture. The common vegetable, animal and marine oils as well as fats exhibit SV's ranging from 160 to 264. For example SV of rapeseed oil about 170-180 indicates the presence of glycerides of long changing fatty acids while a value of 225- 264 for coconut oil is indicative of short chain acids.

8. Refractive Index^[14]

The refractive index (n) of a substance with reference to air is the ratio of the sine of the

angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Unless otherwise prescribed, the refractive index is measured at 25^o(±0.5) with reference to the wavelength of the D line of sodium (=589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

9. Determination of Iodine Value^[15]

The Iodine value of a substance is the weight of iodine absorbed by 100 part by weight of the substance,

Place the substance accurately weighed, in dry iodine flask, add 10 ml of carbon tetrachloride, and dissolve. Add 20 ml of iodine monochloride solution, insert the stopper, previously moistened with solution of potassium iodine and allow to stand in a dark place at a temperature of about 17^o or thirty minutes. Add 15 ml of solution of potassium iodine and 100 ml water; shake, and titrate with 0.1 N sodium thiosulphate, using solution of starch as indicator. Note the number of ml required (a). At the same time carry out the

operation in exactly the same manner, but without the substance being tested, and note the number of ml of 0.1 N sodium thiosulphate required (b).

Calculate the iodine value from the formula:

$$(b-a) \times 0.01269 \times 100$$

$$\text{Iodine value} = \frac{\text{-----}}{W}$$

Where 'W' is the weight in gm. of the substance taken.

10. Specific gravity^[16]

The specific gravity of a liquid is the weight of a given volume of the liquid at 25^o (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighings being taken in air.

11. Bulk density

Physical characteristics like bulk density were determined for ChG formulation. The term bulk density refers to method used to indicate a packing of particles or granules. The equation for determining bulk density (D_b) is $D_b = M/V_b$ where M is the mass of particles and V_b is the total volume of packing. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical

tapping device (Jolting Volumeter) that has a specially cut rotating can. Hundred gm of weighed formulation powder was taken and carefully added to cylinder with the aid of a funnel. The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

Where, D_f = Tapped density and D_o = Bulk density.

12. Determination of Peroxide Value^[17]

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

Unless otherwise specified in the individual monograph, weigh 5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression

$$\text{Peroxide value} = 10 (a - b)/w$$

Where w = weight, in g, of the substance.

13. Thin Layer Chromatography

Thin layer chromatography was accepted as a separation method of the analytical laboratory after suitable sorbents became available for self-preparation of thin layer plates. This development took place at the beginning of the 1960's. Traditionally, analytical TLC has found application in the detection and monitoring of compounds through a separation process.

Separations by TLC is effected by the application of the mixture or extract as a spot or thin line to a sorbent that has been applied to a backing plate. Analytical TLC plates

(thickness 0.1-0.2 mm) are commercially available, e.g. the commonest analytical silica gel plate is the 20 X 20 cm, plastic or aluminum baked Kiessl gel 60 F₂₅₄ plate, which has a 0.2 cm thickness of silica sorbent.

$$R_f = \frac{\text{Solvent front distance from origin}}{\text{Distance from origin}}$$

Compounds distance from Origin (mid point)

a. Identification of Herbal Powder for Chitrakadi ghritam (HPChG) Drug by TLC

Sample	:	(HPChG) Drug
Solvent system	:	Chloroform: Ethyl acetate: Methanol 5 : 60 : 35
Sample preparation	:	Powder sample extracted with methanol. The Solution concentrated and the residue dissolved in methanol.
Detection	:	Anisaldehyde sulfuric acid reagent.
Results	:	TLC examination revealed that it is a complex mixture of more than 12 compounds having close R _f values

b. Identification of Chitrakadi ghritam (ChG) Drug by TLC

Sample	:	Chitrakadi ghritam (ChG)
Solvent system	:	Chloroform: Ethyl acetate: Methanol 5 : 60 : 35
Sample preparation	:	Powder sample extracted with methanol. The Solution concentrated and the residue dissolved in methanol.
Detection	:	Anisaldehyde sulfuric acid reagent.
Results	:	TLC examination revealed that it is a complex

TLC Results

TLC Solvent system

Chloroform: Ethyl acetate: Methanol

5 : 60 : 35

TLC Identification of HPChG and ChG

1. Herbal powder Chitrakadi ghritam (HPChG) drug by TLC examination revealed that it was a complex mixture more than 12 compounds having close R_f values.
2. Identification of Chitrakadi ghritam (ChG) drug by TLC examination revealed that it was a complex mixture 10 compounds having close R_f values.

14. Scanning Electron Microscope (SEM)

The Scanning Electron Microscope (SEM) is a microscope that uses electrons rather than light to form an image. There are many advantages to using the SEM instead of a light microscope. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. Preparation of the samples is

relatively easy since most SEMs only require the sample to be conductive. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most heavily used instruments in research areas today. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample of ChG data given below.

Energy Dispersive X-ray analysis

EDX Analysis stands for Energy Dispersive X-ray analysis. It is sometimes

referred to also as EDS or EDAX analysis. It is a technique used for identifying the elemental composition of the specimen, or an area of interest thereof. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM), and can not operate on its own without the latter.

During EDX Analysis, the specimen is bombarded with an electron beam inside the scanning electron microscope. The bombarding electrons collide with the specimen atoms' own electrons, knocking some of them off in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. To be able to do so, however, the transferring outer electron must give up some of its energy by emitting an X-ray.

The amount of energy released by the transferring electron depends on which shell it is transferring from, as well as which shell it is transferring to. Furthermore, the atom of every element releases X-rays with unique amounts of energy during the transferring process. Thus, by measuring the amounts of energy present in the X-rays being released by a specimen during electron beam bombardment, the identity of the atom from which the X-ray was emitted can be established.

The EDX spectrum is just a plot of how frequently an X-ray is received for each energy

level. An EDX spectrum normally displays peaks corresponding to the energy levels for which the most X-rays had been received. Each of these peaks are unique to an atom, and therefore corresponds to a single element. The higher a peak in a spectrum, the more concentrated the element is in the specimen.

An EDX spectrum plot not only identifies the element corresponding to each of its peaks, but the type of X-ray to which it corresponds as well.

Estimation of Microbial Contamination

Using petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the preparation and about 15 ml of plate count agar M 091 (HIMEDIA) medium at not more than 45 C. Alternatively spread the preparation on the surface of the solidified medium in a petri dish of the same diameter. If necessary, dilute the preparation so that a colony count of not more than 300 may be expected. Prepare at least two such petri dishes using the same dilution and incubate at 35 2 C for 72 hours. Count the numbers of colonies that are formed. Calculate the results using the plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

The result shows in Table 4, in which the pathogens are within the limits as per mentioned in Ayurvedic Pharmacopeia.^[18]

RESULTS AND DISCUSSIONS

Table 1: Element containing in the Chitrakadi Ghrita

Elements	Spectra type	Element%	Atomic %
C K	ED	33.93	42.81
O K	ED	53.09	50.28
Si K	ED	12.29	6.63
S K	ED	0.16	0.08
K K	ED	0.53	0.20
Total		100.00	100.00

Table 2: Showing colour reactions of HPChG and ChG

S.No.	Test name	HPChG	ChG
1.	Triterpenoids	Positive	Positive
2.	Steroids	Negative	Positive
3.	Flavonoids	Positive	Positive
4.	Phenolic compounds	Positive	Negative
5.	Carbohydrates	Positive	Positive

Table 3: Physicochemical parameters

S.No.	Test	Result
1.	Physical feature	
2.	Description	
3.	Colour	Pale yellow
4.	Odour	Characteristic
5.	Acid value	4.78
6.	Saponification value	280.5
7.	Refractive index value	1.53
8.	Iodine value	28.7
9.	Specific gravity at room temperature	0.9
10.	Ester value	275.72
11.	Bulk density	1.60
12.	Peroxide value	9.22

Table 4: Microbiological Analysis

S.No.	Pathogens	Results
1.	Total microbial plate count (TPC)	2000 CFU/gm
2.	Total Moulds & Yeast	11 CFU/gm
3.	Escherichia coli	Absent
4.	Salmonella spp.	Absent
5.	Pseudomonas aeruginosa	Absent
6.	Staphylococcus aureus	Absent

Qualitative tests for different active principles of *Chitrakadi Ghrita* were done with qualitative tests to reveal the presence of Triterpenoids, Steroids, Flavonoids, Carbohydrates. Positive test for triterpenoids and steroids in both.

Detection of steroids (Salkowshi test) found negative for powder and positive for *Ghrita*, positive test for both to the flavonoids.

Detection of phenolic compounds (Ferric chloride test) found negative for *Ghrita* and positive for powder, detection of carbohydrates (Molish test) positive test for both.

The text sample saponification value of CHG drug showing 280.5 it's indicating of short chain fatty acid in the drug.

Most Ayurvedic preparations are made with ghee. Digestion, absorption and delivery to a target organ system is crucial in obtaining the maximum benefit from any formulation. This is facilitated by ghee. Since active ingredients are mixed with ghee, they are easily digested and absorbed. Lipophilic action of ghee facilitates transportation to a target organ and final delivery, inside the cell, because cell membrane also contains lipid. This lipophilic nature

facilitates entry of the formulation into the cell and its delivery to the mitochondria, microsome and nuclear membrane. [19]

CONCLUSION

This study was focused on qualitative estimation of Flavonoids, Triterpenoids, Steroids, Flavonoids got positive results which are herbal constituents of *Chitrakadi ghrita* by using chemical analysis. TLC examination also revealed that it is a complex mixture of 10 to 12 principal compounds having close Rf values respectively. Preliminary phytochemical studies, Heavy metal analysis shows values are within the limits and there is no harmful metals found. The normal limit of Microbial contamination was estimated. It does not contain any harmful microbes such as *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*. *Chitrakadi ghrita* can be given internally to the patients for the management of *Vandhyatva* (Infertility). The analytical data generated here may be considered as the standard parameters for this formulation.

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PHOTOGRAPHS



Chitrakadi Herbal Powder

Figure 1: *Chitrakadi Herbal Powder*



Chitrakadi Ghrita

Figure 2: *Chitrakadi Ghrita*

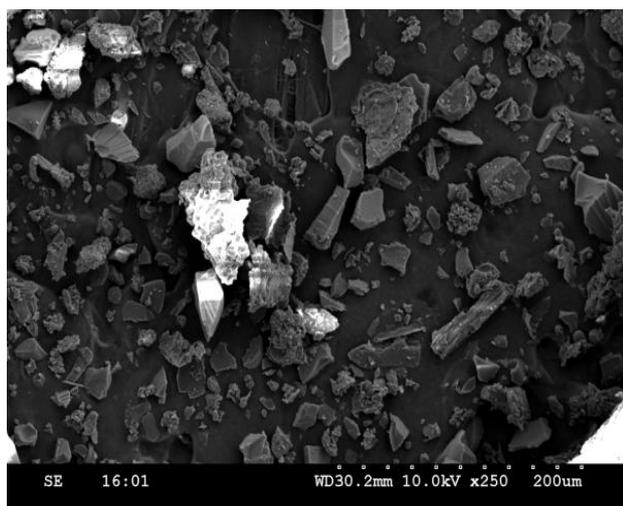


Figure 3: *Chitrakadi Herbal Powder SEM*

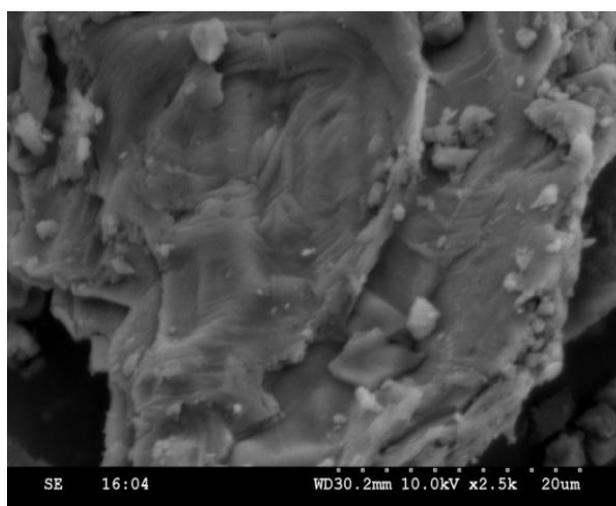


Figure 4: *Chitrakadi Ghrita SEM*



Figure 5: *TLC of Chitrakadi Ghrita*