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FORMULATION AND PHYSICOCHEMICAL EVALUATIONS OF POLYHERBAL GHRITA

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ABSTRACT

The use of ghee in suitable conditions with appropriate doses of desired preparations will render various benefits due to its potency to nullify toxins and toxic effects of drugs; and its capability to act as a media to dissolve and enhance the efficacy of the active principles in the drugs used. Aim of this study is formulation and Physicochemical evaluation of Polyherbal Ghrita. The Ayurvedic classics have mentioned Rasayana which is described as an herbal or metallic preparation that are health tonics to children, medicines to middle aged and rejuvenators to the elderly. Polyherbal is used as Rasayana since the ancient time especially for children in the management of memory. This research work emphasize practical approach in formulation and physicochemical evaluation of polyherbal ghrita by incorporating the traditional knowledge along with the modern technology in drug manufacture.

KEYWORDS: Polyherbal ghrita, herbal drugs, ghee, Rasayana, traditional knowledge.

INTRODUCTION

History of Ayurveda

The word Ayurveda is derived from the Sanskrit word ‘Ayus’ (all aspects of life from birth to death) and ‘Veda’ (knowledge or science) science of long life.[1] Ayurveda, the most ancient system of traditional medicine of the world, has been practiced in Indian subcontinent since 5000 BC. Ayurveda is a holistic approach towards life, health and disease management through medicinal herbs, minerals, diet, lifestyle and spirituality. Ayurveda was developed through daily experiences and mutual relationship between people and nature and thus not only cure diseases but also prevent disease, maintaining health and promoting longevity. This

holistic system looks at the whole person as a combination of body, mind and soul. Therefore, it is a comprehensive and integral medicinal systems, gift of Indian sages to mankind. Ayurveda is widely respected for its uniqueness and global acceptance as it offers natural ways to treat diseases and promote healthcare.[2]

Several traditional healthcare systems exist in India from centuries and out of all the traditional practices, Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy are the official traditional systems of medicine. These systems are collectively known as Indian Systems of Medicine (ISM), and are currently called as an acronym for Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy which collectively provides healthcare to the vast majority of people of India and neighboring countries.[3]

Principles of Ayurveda

The ayurveda consist of five basic elements (panchamahabhutas) first element is space (aakash), and remaining four elements are earth (prithivi), air (vayu), water (jala), and fire (agni), exist within the space.[4] Both the systems, human (microcosm) and universe (macrocosm) are linked permanently, since both are built from the same elements. Thus human being is a replica of nature and everything which affects human being, also influence the macro-cosm. Hence, the evolution of life and the creation of the universe can be concerned with Ayurveda.[5] Along with these panchamahabhutas, the functional aspect like movement, trans-formation and growth are governed by three biological humors, viz. vata (space and air), pitta (fire and water) and kapha (water and earth), respectively.[6] These three humors usually known as tridhatus regulate every physiological and psychological processes in the living organism. Additionally, oja-Jeebaniya sakti (vital force) developed from tissue metabolism is also essential for healthy functioning of the body. It is believed that after intake of food/diet, it undergoes a process of digestion and ultimately forms two types of products – prasadas (nutritional products) and malas (excretory products).[7] The prasada builds the seven dhatus (tissue) of the body, whereas malas become waste products. Body produces three types of waste products: feces (solid) and urine and sweat (liquid). Both prasada and mala are important and their presence in the right proportion in the body is indispensable for health and well being. Health is considered as balance between body, mind and consciousness along with three humors vata, pitta and kapha.[8]

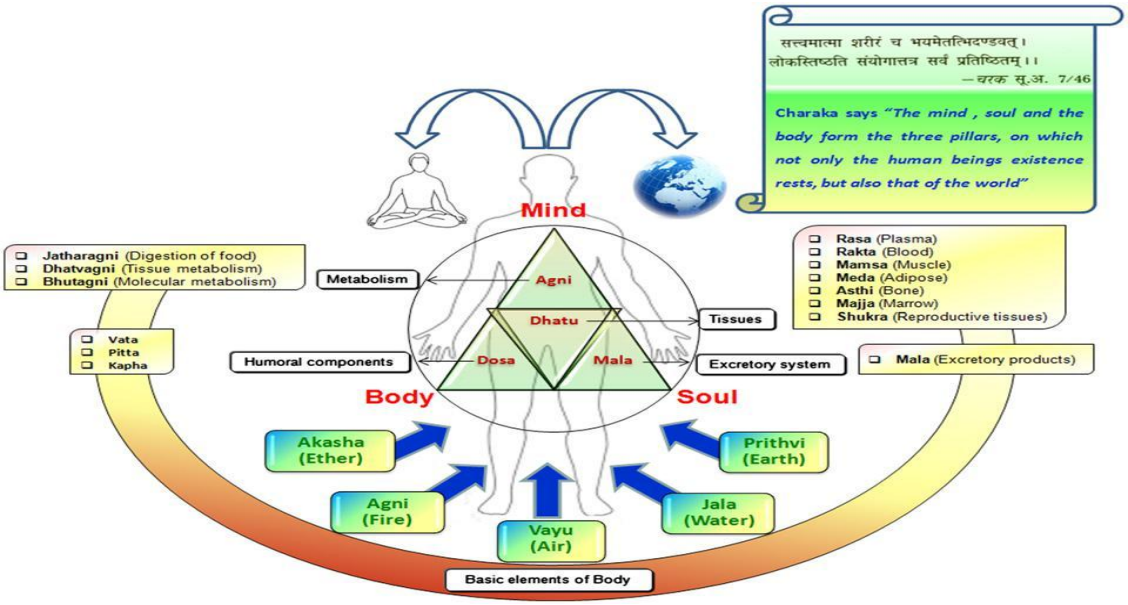


Figure-1: Ayurvedic principle on the basic elements of human physiology

GHRITA

Ghrita /medicated ghee a ayurvedic medicinal preparation. In which the ghee is processed with some herbal decoction and paste of fresh herbs. The choice of decoction and paste of herbs are based on the formula. Ghrita or ghee is a type of fat (sneha dravya) hence the fat-soluble active principle of the ingredients are properly dissolved in ghee and ensures their absorption in body. Also, only ghee is the medium, which cross the blood brain barrier, the drugs indicated for brain nervous system disorder, which processed in ghee and used, acts best which no other dosage form can.[9]

Ghrita is prepared by the kalka and the dravya mentioned in the formula are first mixed together in the vessel. Sneha dravya/ ghee is then added and boiled in mild fire. It is stirred well continuously so that kalka (solid part of the mixture) should not adhere to the vessels, filter it and packed with air tight container.[10,11]

Types of Ghrita

1. Bramhi Ghrita
2. Ashwagandha Ghrita
3. Triphala Ghrita
4. Mahatiktaka Ghrita
5. Maha Triphaladi Ghrita
6. Saraswata Ghrita
7. Phal Ghrita

Figure-2: Different types of Ghrita formulation

PLANT PROFILES

Bramhi

Biological Source: *Centella asiatica* (L)

Family: Apiaceae

Synonyms:

San : Bramhi, jala-Bramhi, Manduki.

Eng : Thyme-leaved Gratiola.

Hind : Brambhi, Safed Kammi.

Mar : Nir-bramhi, Bamba

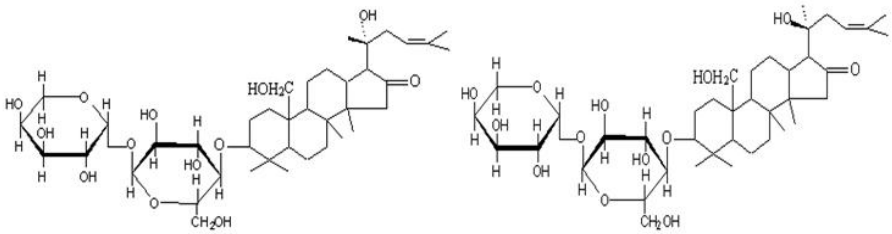
Figure-3: Bramhi

Habitat

- This small creeping plant is found in marshy ground throughout India.

Chemical constituents

- Contain Alkaloids, Brahmine, Herpestine and the mixture of three basis. Contains saponins, monnieris, harsaponin, bacoside A and bacoside B.



Bacoside- A **Bacoside-B**

Medicinal Uses

- As a brain tonic for improving memory, concentration and learning.
- Nervous deficit due to injury and strok.
- Nervine tonic, mild sedative, mild anticonvulsant, anti-inflammatory.
- Cardiac and nervine tonic: leaves and stalks are diuretic and aperients.

Figure-4: Shankapushpi

Shankapushpi

Biological Source: ***Convolvulus plucaulis***

Family: Convolvulaceae

Synonyms

San : Shankhmalin.

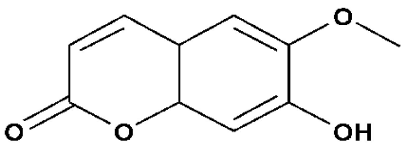
Eng : Morning glory.

Hind : Shankapushpi.

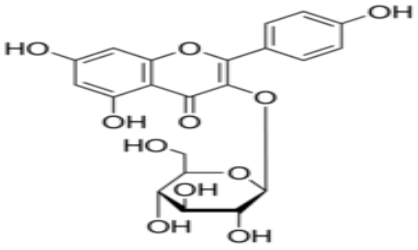
Mar : Sankhvel

Chemical constituents

- Scopoletin
- Kaempferol 3-glucoside
- Kaemferol 3,4 dihydroxycinnamic acid



Scopoletin



Kaempferol 3-glucoside

Medicinal Uses

- Memory enhancer
- Antiulcerogenic

Ashwagandha

Biological Source: *Withania somnifera*

Family: Solanaceae

Synonyms:

Eng : Indian ginseng, Winter Cherry.

Hind : Rasbhari.

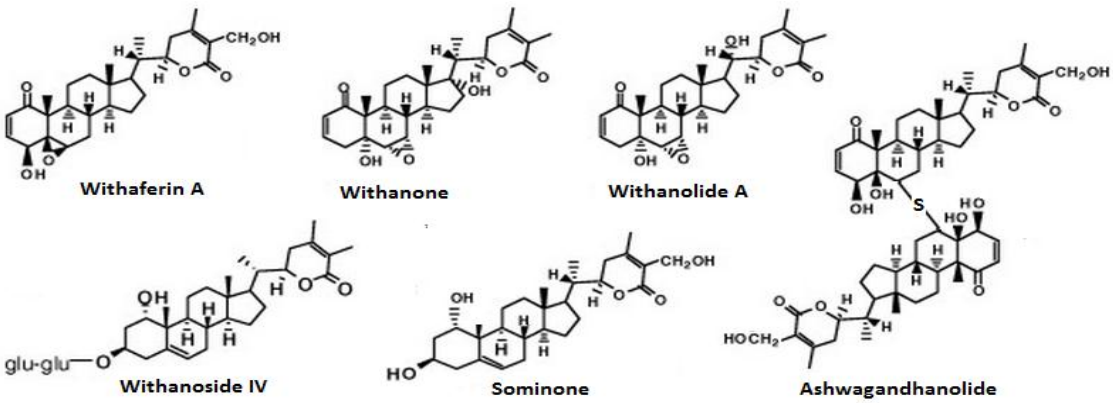
Mar : Ghoda

San : Ashwagandha

Figure-5: Ashwagandha Root

Chemical constituents

- Withanolides A-Y, Withasomniferin-A
- Withaferin-A, Withasomniferols A-C



Medicinal Uses

- Memory enhancement
- Parkinson's disease
- Adaptogenic and Anti-stress activity
- Immunomodulatory activity

Velvet bean

Biological Source: *Mucuna pruriens*

Family: Papilionaceae

Figure -6: Velvet Bean

Synonyms

Eng : Cowhage, cowitch.

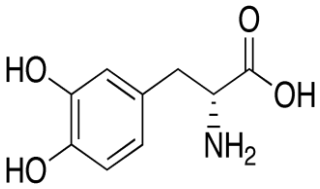
Hind : Kiwanch or Kooch.

Mar : Khaajkuiri.

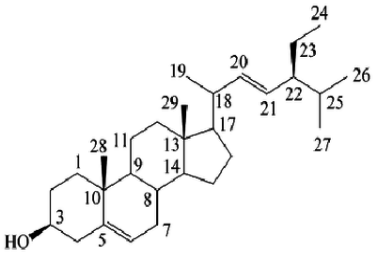
San : Atmagupta or Kapikacchu.

Chemical constituents

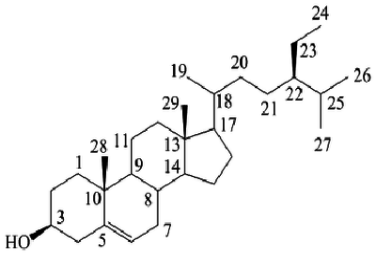
- **Proteins and aminoacids:** L-dopa, methionine, phenylanine, glutamic acid.
- **Alkaloids :** Mucunine, mucunadine, Prurienene & prurieninine.
- **Triterpenes and sterols:** β-Sitosterol & stigmasterol.



L-Dopa



Stigmasterol (1)



β-Sitosterol (2)

Medicinal Uses

- Antiparkinson's activity
- Neuroprotective activity
- Hypoprolactinaemic, hypoglycaemic & antihaemorrhagic activity

Almond

Biological Source: *Prunus dulcis var.*

Family: Rosaceae

Figure-7: Almond

Synonyms

Eng : Sweet almond, almond.

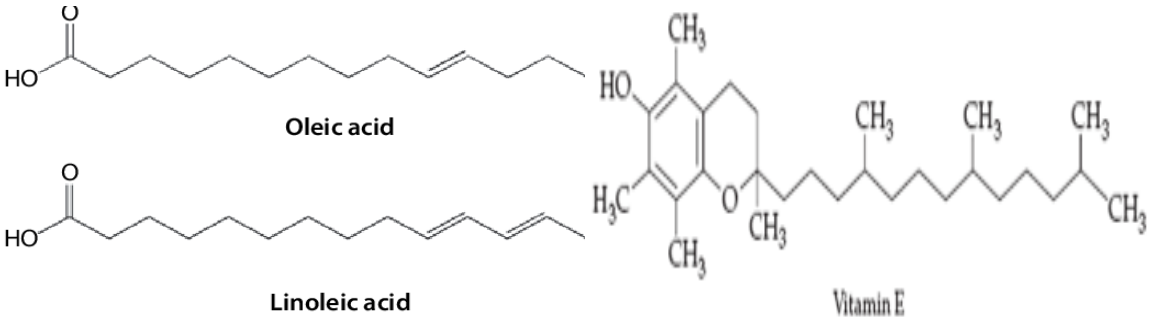
Hind : Baadam.

Mar : Badam.

Chemical constituents

- Linoleic acid, Monounsaturated oleic acid, Saturated fatty acid.

- Vitamin-E



Medicinal Uses

- Alzheimer's disease
- Brest cancer
- Reduce blood sugar

Pistachio

Biological Source: *Pistacia vera (L).*

Family: Anacardiaceae

Figure-8: Pistachio

Synonyms

Eng : Pistace.

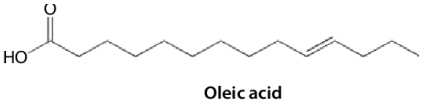
Italian : Pistacchio.

Mar : Pista.

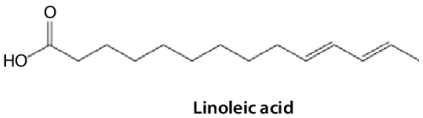
Chemical constituents

- Oleic acid, palmitic acid & Linoleic acid.

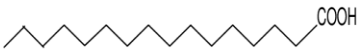
- Arginine, lysine, cystine.
- Vitamin-B6



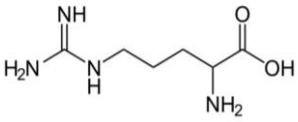
Oleic acid



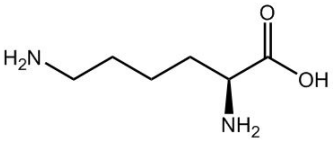
Linoleic acid



Palmitic acid



Arginine



L-lysine

Medicinal Uses

- Neurotrasmitters
- Alzheimer's disease
- Brest cancer
- Antioxident

Walnut

Biological Source: *Juglans regia*

Family: Juglandaceae

Figure-9: Walnut

Synonyms

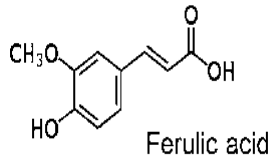
Eng : Walnut.

Hind : Akharot.

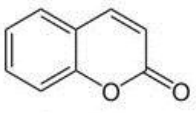
Mar : Akroda.

Chemical constituents

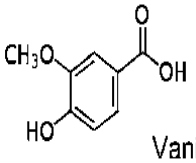
- Phenolic compound: Ferulic acid, Vanillic acid, Coumaric acid and Myricetin.
- Omega3fatty acid.



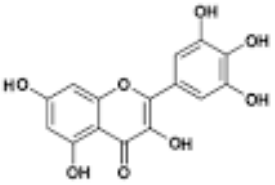
Ferulic acid



Coumarin



Vanillic acid



Myricetin

Medicinal Uses

- Improve memory
- Alzheimer's disease
- Brest cancer
- Antioxidant

Authentification of Herbal Drugs

Figure-10: Bramhi

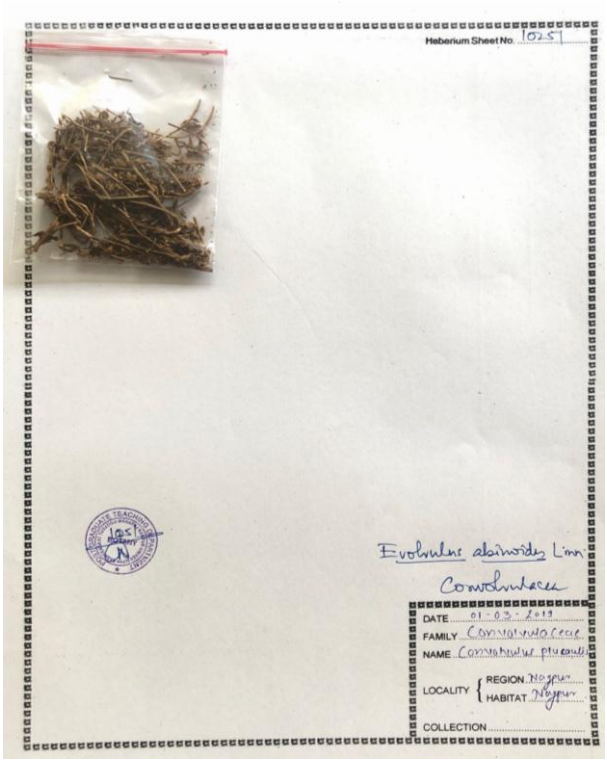


Figure-11: Velvet Bean

EXTRACTION

Maceration process

Preparation of Alcoholic extract

Accuratly weight the dried crud drug and macerate with 250 ml 90% pure ethanol in maceration chamber.[12] After 72hrs the alcoholic extract was filter and evaporate the solvent to get the concentrate extract, keep in air tight container protected from light and moisture.[13]

Figure-13: Maceration

Figure-14: Rotary Shaker

Figure-15: Alcoholic Extract

Figure-16: Concentrated Extract

Figure-17: Extract Packed in container

Fig. Extraction of Crude Drug by Maceration Method

FORMULATION OF POLYHERBAL GHRITA

Sr.N0	Content	Quantity
1	Bramhi	2gm
2	Ashwagandha	2gm
3	Shankapushpi	2gm
4	Velvet bean	2gm
5	Almond	1gm
6	Pistachio	1gm
7	Walnut	1gm
8	Ghee Base	89ml

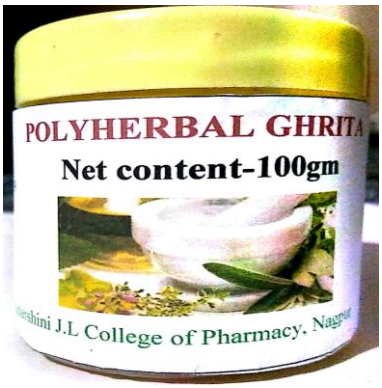


Figure-18: Polyherbal Ghrita

Formulation of Polyherbal Ghrita, 250mL beaker containg 89ml of pure ghee are boil to evaporate the moisture in ghee, cool and add the required quantity of all crude extract at moderate fire with continue sterring at 30min. After that filter with muslin cloth and packed with wide mouth air tight container.[14,15]

PHYSICOCHEMICAL EVALUATION

Organoleptic characters

Organoleptic characters like colour, odour and taste of the ghrita were documented.[16]

Refractive index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Noted the reading.[17] Distilled water has a refractive index of 1.3325 at 25˚C. The difference between the reading and 1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.[18]

Specific gravity

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room Temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Rancidity test

1ml of melted fat was mixed with 1ml of conc. HCL and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

Determination of Acid value

Weighed 10g of sample in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 + 25ml) previously neutralised by the addition of 1 ml of Phenolphthalein solution and titrated against 0.1N potassium hydroxide solution. End point was the appearance of pale pink colour which persists for 15sec. Repeated the experiment twice to get concordant values.[19]

Acid value= 5.61 n/w

Determination of Saponification value

About 2g of the substance was weighed in tared 250 ml round bottom flask. 25ml of the alcoholic solution of KOH was added and a reflux condenser was attached. Kept it for boiling on water bath for 1hr, the contents of the flask was rotated frequently. The flask was cooled and 1ml phenolphthalein solution was added and excess of alkali titrated with 0.5N HCl. The number of ml (a) required was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml required (b) was noted. The experiment was repeated twice to get concordant values.[20]

Saponification value= 28.05(b-a)/w

Iodine value

The sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4, 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17^0 C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

Iodine value=1.269(b-a)/w

Melting point

10gms of ghrita was taken in a 25ml beaker which was immersed in a preheated water bath the thermometer was dipped meanwhile in the beaker; the ghrita was stirred with glass rod so that uniform dissolution was obtained, the temperature at which the uniform dissolution of ghrita into a clear liquid was noted as melting point.[21]

UV: Prepare stock solution of formulation. 1gm in 100 mL of Acetone and absorbance are note at 319nm.[22]

High Performance Thin Layer Chromatography (HPTLC)

Preparation of sample solution

The 1gm of Ghrita sample was dissolve in 10 mL of Acetone and used for High Performance Thin Layer Chromatography (HPTLC) identification.

Chromatographic conditions

1. Stationary phase: Silica gel GF 254 precoated TLC plates.
2. Mobile phase: Dichloromethan: Methanol: Water (4.5/1.0/0.1v/v/v).
3. Sample volume : 5µl
4. Sample for HPTLC: Ghrita dissolve in Acetone.
5. Spray reagent: Vaniline-sulfuric acid.

Procedure

Before spotting, the plates were prewashed with methanol. Sample solutions were applied to the plates as sharp bands by means of Camag Linomat V sample applicator. The spots were dried in a current of air. The mobile phase (20 ml) was poured into a twin trough glass chamber whole assembly was left to equilibrate for 30 min and the plate was placed in the chamber. The plate was then developed until the solvent front had travelled at a distance of 80 mm above the base of plate. The plate was then removed from chamber and dried in a current of air. Detection and quantification was performed with Camag TLC scanner 3 at a wavelength of 254 and 366 nm.[23,24]

RESULTS

Table 1: Organoleptic Characteristics of Polyherbal Ghrita.

Sr.No	Observation	Polyherbal Ghrita
1	Colour	Dark Brown
2	Odour	Characteristics
3	Taste	Astringent
4	Texture	Smooth
5	PH	7.0

Table 2: Physicochemical Evaluation of Polyherbal Ghrita.

Sr.No	Test	Result
1	Acid Value	0.398±0.02
2	Saponification Value	191.36±0.14
3	Iodine Value	61.23±0.21
4	Ester Value	225.126±0.03
5	Peroxide Value	9.6±0.245
6	Refractive index	1.3625±0.0007
7	Specific gravity	0.9125±0.012
8	Rancidity	Negative
9	Melting point	38°c

UV

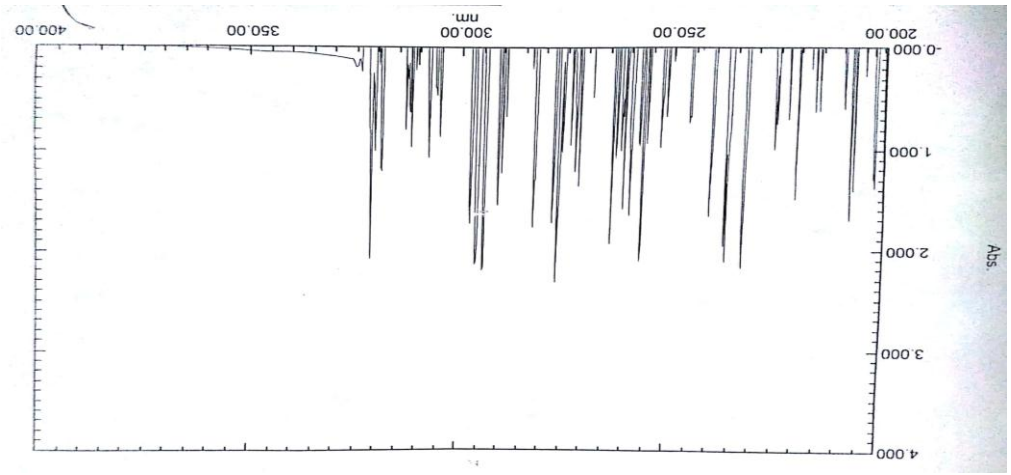
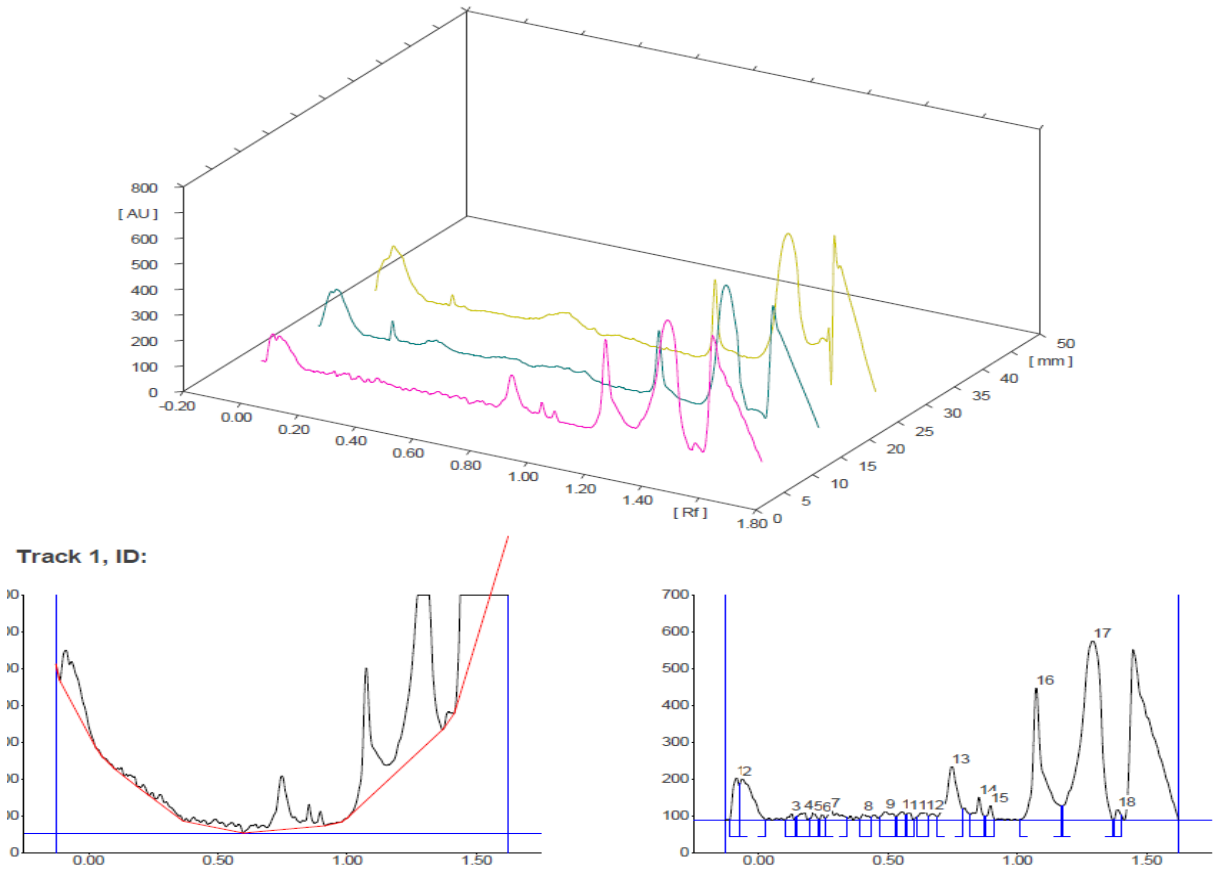


Figure-19: UV Spectrum of Polyherbal Ghrita formulation

HPTLC



winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.11	1.7	-0.09	114.5	7.39	-0.07	93.3	1279.4	3.79	unknown *
2	-0.07	99.2	-0.06	111.8	7.21	0.03	0.0	2382.5	7.07	unknown *
3	0.10	0.6	0.13	17.9	1.15	0.14	8.0	135.2	0.40	unknown *
4	0.15	9.6	0.18	20.8	1.34	0.20	4.7	243.8	0.72	unknown *
5	0.20	4.2	0.22	18.5	1.19	0.23	1.0	137.3	0.41	unknown *
6	0.23	0.0	0.25	15.2	0.98	0.26	4.4	77.0	0.23	unknown *
7	0.26	5.2	0.28	26.2	1.69	0.34	6.6	472.0	1.40	unknown *
8	0.39	2.4	0.41	15.9	1.03	0.44	6.2	179.7	0.53	unknown *
9	0.47	8.8	0.49	23.1	1.49	0.53	8.0	410.5	1.22	unknown *
10	0.54	8.9	0.56	22.4	1.44	0.57	13.6	252.7	0.75	unknown *
11	0.57	14.0	0.58	20.4	1.32	0.61	2.9	165.5	0.49	unknown *
12	0.61	7.8	0.65	20.7	1.34	0.66	10.3	295.2	0.88	unknown *
13	0.69	8.4	0.75	145.8	9.40	0.79	29.6	2763.7	8.20	unknown *
14	0.82	14.4	0.86	61.3	3.95	0.88	9.5	612.7	1.82	unknown *
15	0.88	9.7	0.90	40.3	2.60	0.92	1.2	292.8	0.87	unknown *
16	1.01	2.1	1.08	360.1	23.23	1.17	38.1	6491.3	19.25	unknown *
17	1.18	39.2	1.29	486.5	31.38	1.37	0.1	17304.6	51.33	unknown *
18	1.38	0.8	1.39	28.8	1.86	1.40	12.8	217.2	0.64	unknown *

Figure-20: HPTLC fingerprinting profile of Polyherbal Ghrita Under UV 254nm

CONCLUSION

The advent of modern technology in pharmaceutical sector has increased the efficacy of Ayurvedic formulations. Then also, more practical approach should be incorporated in the field manufacture of Ayurvedic medicine. As Ayurveda is foremost among the traditional health practices in the world, traditional inspired practical approach should be made in

preparing prime quality preparations. Pharmacognostical studies confirmed the ingredients present in the Polyherbal Ghrita. This study reveals the authentification of individual raw drugs of Ghrita and is cross verified. The pitted vessels, tannins, prismatic crystal, fibers are observed in the ingredients. All the physiochemical parameters, acid value, saponification value, iodine value, ester value, peroxide value, refractive index, specific gravity, melting point are analyzed. According to UV spectrum, various peaks of different wavelength shows the presence of different types of drug constituents in Polyherbal Ghrita. In HPTLC Shows the one spot was detected in Rf value 0.69, which indicate the presence of bacoside in Polyherbal Ghrita.

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