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Quality Control Parameters for Polyherbal Formulation

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ABSTRACT

Herbal medicines are formulations of therapeutic herbs to prevent and treat disease and ailments or to promote health and healing. There are various aspects Indian manufactures have to focus on to establish their products in the global markets. Right from the analysis of raw materials and in-process testing to analysis of finished products, system have to be established to stringently monitor every step in the manufacture. Maintaining quality of herbal products includes a strict set of processes within the specified limits laid down by the World Health Organization (W.H.O) to maintain consistency. D-Diabetes S.M.A.R.T powder is an authentic ayurvedic preparation consisting of nineteen herbal medicines namely *Acacia arabica,* Asphaltum**,** *Bombax ceiba*, *Butea monosperma*, *Emblica officinalis***,** *Eugenia jambolana*,*Ficus bengalensis*, *Gymnema sylvestre*,*Holarrhena antidysentrica*, *Momordica charantia*,*Pistacia integerrima*, *Plumbago zeylanica*, *Pongamia glabra*, *Pterocarpus marsupium*, *Santalum album*, *Swertia chirata*, *Terminalia chebula*, *Tribulus terrestris* and *Woodfordia fruticosa*. The herbal medicines *Eugenia jambolana*,*Gymnema sylvestre*, *Momordica charantia* and *Tribulus terrestris*are present in maximum quantity in the formulation and can be chosen as biological standard for studies. World Health Organization has set specific guidelines for assessment of safety, efficacy and quality herbal medicines. The various steps involve are botanical parameter, physicochemical parameters, biological parameters, toxicological parameters and pharmacological parameters.

Keywords: Microscopy, Ash value, Microbial contamination, Chromatographic techniques.

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INTRODUCTION

Medicinal herbs have been known from centuries. They are highly valued all over the world as a rich source of therapeutic agents for prevention of diseases and ailments. There has been increase in the side effects and toxicity caused by synthetic drugs prompting a switch over to herbal medicines. Herbal medicines are being used by nearly about 80% of the world populations for their primary health care. According to W.H.O estimates, the demand for medicinal plants would be US $5 trillion in the year 2050. In 2009, India contributes less than 2% to the global herbal market [1, 2].

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially derived from plants. The general perception that herbal medicines are very safe and free of side effects may always be not true. Herbal medicines have many constituents and some are known to produce toxic reactions, possible mutagenic effects or allergic reactions [1, 3].

There are two kinds of side effects for herbal medicines [3]:

1. Intrinsic to herbs:
    a. Predictable toxicity.
    b. Over-dosage.
    c. Interaction with conventional drugs.
2. Extrinsic to the preparation and are related to several manufacturing problems:
    a. Misidentification of plants.
    b. Lack of standardization.
    c. Failure of good manufacturing practice.
    d. Contamination, substitution and adulteration of plants and incorrect preparation and/or dosage.

In most countries herbal products are launched into market without proper scientific evaluation, and without any mandatory safety and toxicological studies. Consumers can buy herbal products without a prescription and one might not recognize the potential hazard in an inferior products. A well-defined and constant composition of the drug is therefore one of the most important prerequisites for the production of a quality drug. There was a need to follow strict guidelines laid down by World Health Organization for the successful production of quality herbal drug. The source of raw materials, good agricultural practices, manufacturing processes and analysis of finished product are certainly essential steps for ensuring the quality control of herbal medicines. The data collected is documented and recorded. It plays a pivotal role in guaranteeing the quality and stability of herbal preparation[1]. The herbal drugs are available in plenty and therefore,

standardization of these products is very essential/need of the hour. If these are not standardized, it may result in problems with respect to poor quality, improper identification, toxicity and patient's incompliance. Standardized product provides a greater level of reliability which gives patients a feeling of security and also helps in building up compliance[4]. Thus proper standardization and quality control of raw material, in-process material and finished herbal formulation should be conducted[1].

A polyherbal formulation involves usage of two or more herbal drugs in their formulation. The presence of two or more herbal drugs shows synergistic activity which enhances or prolongs the effect of formulation. Synergy is an important concept in herbal pharmacology. Components which are not active by themselves can act to improve the stability, solubility, bioavailability or half-life of the active components. Synergy has pharmacokinetic and pharmacodynamics basis[6].

The mechanism underlying synergistic therapeutic actives of polyherbal medicines are [5]:

i. Different agents may regulate either the same or different target in various pathways, and therefore cooperate in an agonistic, synergistic way.
ii. Regulate the enzymes and transporters that are involved in hepatic and intestinal metabolism to improve oral drug bioavailability.
iii. Overcome the drug resistance mechanism of microbial and cancer cells.
iv. Eliminate the adverse effects and enhance pharmacological potency of agents by "processing" or by drug-drug interaction.

The dose required in polyherbal is minimum as compared to single herb to show multiple effects.

Polyherbal helps in treatment of disease or disorder along with preventing or curing complications or problems related to it. The usage of polyherbal formulation shows inhibition of side effects of one herb by other in the preparation [6.]

India needs to explore the medicinally important plants. This can be achieved only if herbal products are evaluated and analyzed. India can emerge as the major and leading country in production of standardized therapeutically effective ayurvedic formulation [7].

Figure1: D-Diabetes Polyherbal Formulation

Table 1: A schematic representation of W.H.O guidelines for Quality control and Standardization of herbal medicines:

Quality control and Standardization of Herbal Medicines	Botanical Parameters	Macroscopic: appearance, color, odour, taste Microscopic: powder study
	Physical Parameters	Moisture content, extractive value, ash value
	Biological Parameters	Microbiological contamination, Toxicological studies: heavy metal, pesticide residue
	Chemical Parameter	Phytochemical screening, Chromatographic techniques
	Pharmacological activity	

Table 2: D-Diabetes S.M.A.R.T powder is an ayurvedic preparation manufactured by Shree Maruti Herbal, Mumbai to be sold in various countries consisting of nineteen herbal medicines

Herbals	Parts	Quantity Present (each 50gm contains)
Acacia arabica	Bark	3
Asphaltum	Mineral pitch	1
Bombax ceiba	Leaves	1
Butea monosperma	Gum	1
Emblica officinalis	Dried fruit	3
Eugenia jambolana	Fruit	5
Ficus bengalensis	Bark	3
Gymnema sylvestre	Leaves	5
Holarrhena antidysentrica	Bark	2
Momordica charantia	Fruit	5
Pistacia integerrima	Leaf galls	3
Plumbago zeylanica	Roots	1
Pongamia glabra	Seed	1
Pterocarpus marsupium	Heartwood	1
Santalum album	Heartwood	1
Swertia chirata	Whole plant	3
Terminalia chebula	Dried fruits	3
Tribulus terrestris	Whole plant	5
Woodfordia fruticosa	Whole plant	3

MATERIALS AND METHODS

BOTANICAL PARAMETERS FOR POLYHERBAL FORMULATION

Organoleptic evaluation

Organoleptic evaluation of polyherbal was carried for appearance, color, odour and taste [6].

Microscopical evaluation

The plant drugs are generally used in powdered or comminuted form where the macromorphorlogy is destroyed, so that the evaluation of the microscopically cell characters is essential [6].

PHYSICOCHEMICAL PARAMETERS FOR POLYHERBAL FORMULATION

PHYSICAL CHARACTERISTICS:

Bulk and tapped density [8]

The previously air dried polyherbal powder was weighed (15g) and carefully added to a graduating cylinder with the aid of funnel without any losses.The initial volume was noted and the polyherbal powder was then tapped 50 times in bulk density apparatus until no further reduction in volume was noted.The initial volume gave the bulk density value and after tapping gave the value of tapped density.

Carr's index and Hausner ratio [8]

Carr's index has been used as in indirect method of quantifying powder flowability from bulk density; this method was developed by Carr. The percentage compressibility of a powder is a direct measure of the potential powder arch, bridge strength and stability, and was calculated according to the following equation. Carr's index (% compressibility) = $100 \times (1-D_b/D_t)$. Where D_b= Bulk density, D_t= Tapped density.

Hausner ratio has been also used as indirect method of quantifying powder flowability from bulk density. Hausner ratio = D_t/D_b.Where D_b= Bulk density, D_t= Tapped density.

Extractive values

5g of air dried polyherbal was macerated with 100ml of water, hydroalcohol and ethanol in a conical flask 24 hours and shaken frequently. 25ml of filtrate was evaporated to an evaporating dish, dry at 105°C and weigh. Calculate the percentage of water, hydroalcohol and ethanol soluble extractive values with reference to air dried drug [9].

Ash Values

Total ash value

2g of air dried material was accurately weighed, in a previously ignited and tarred crucible. The material was spread in an even layer and ignited in Bunsen burner until it is white, indicating the absence of carbon. Cooled in desiccator and weighed. The percentage of total ash with reference to air dried material was calculated [9, 10].

Acid insoluble ash value:

The ash obtained was boiled for 5minutes in 25ml of hydrochloric acid. The insoluble matter was collected on an ashless filter paper, washed with hot water until the filtrate is neutral and ignited to constant weight. Cooled in desiccator and weighed. The percentage of acid insoluble ash with reference to air dried material was calculated [9, 10].

Water soluble ash value:

The ash obtained was boiled for 5 minutes in 25ml of distilled water. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited in a crucible for 15 minutes on a Bunsen burner. The weight of this residue from the weight of total ash was subtracted. The percentage of water soluble ash with reference to air dried material was calculated [9, 10].

Loss on drying

3g of air dried sample was placed on a tarred flat previously weighed crucible. The sample was dried in an oven at 100-105°C. Cooled in desiccator and weighed. Dry until two consecutive weights do not differ by more than 5mg. The percentage loss of weight with reference to air dried material was calculated [10].

pH value of extract

pH value of freshly prepared 1%w/v and 10%w/v of extract in distilled water was determined potentiometrically by using simple glass electrode digital Elico Li 120 pH meter.

Crude fiber determination by Dutch method

2g of powder was weighed in a beaker; 50ml of 10% nitric acid was added. It was heated to boil with constant stirring. The mixture was strained through cotton cloth on Bucher funnel and washed with hot water. The residue was transferred to a beaker and 50ml of 2.5% of sodium hydroxide was added. It was heated to boil with constant stirring. The mixture was again strained through cotton cloth and washed with hot water. The residue was dried and weighed and further incinerated and weighed. The difference between the weight of the dried residue and that of the incinerated residue represent the weight of crude fiber. It is expressed as percentage of the original weight of the material [9]

BIOLOGICAL PARAMETER FOR POLYHERBAL FORMULATION

MICROBIAL LIMIT TEST

Total viable count:

1g of powder was pretreated with 10ml of buffered sodium chloride solution pH 7 in a sterilized test tube for revivification.

For bacterial count, 0.1ml of pretreated material was spread on a sterilized nutrient agar petri plates. At least 2 dishes was prepared using same dilution, inverted and incubated at 30-35°C for 48 hours. Sample was diluted to obtain an expected colony count of NMT 300. The numbers of colonies formed were counted. For fungal count, 0.1ml of pretreated material was spread on a sterilized sabouraud dextrose agar petri plates. At least 2 dishes was prepared using same dilution, inverted and incubated at 20-25°C for 48 hours. Sample was diluted to obtain an expected colony count of NMT 100.The numbers of colonies formed were counted [10].

Test for *Escherichia coli*:

1g of powder was pretreated with 10ml of sterilized lactose broth in a sterilized test tube for revivification at 30-37°C. 0.1ml of pretreatment material was added to 10ml of sterilized MacConkey broth and incubated for 24 hours at 43-45°C. 0.1ml of sample was pipetted out and then subculture on a sterilized MacConkey agar media and further incubated for 24 hours at 43-45°C. The petri plates were checked for presence or absence of growth of red, generally non-mucoid colonies of gram negative rods indicating the presence of *E. coli* [10].

Test for *Salmonella* species:

1g of powder was pretreated with 10ml of sterilized lactose broth in a sterilized test tube for revivification at 30-37°C. 0.1ml of pretreated material was added to 10ml of sterilized tetrathionet bile brilliant green broth and incubated for 24 hours at 43-45°C. 0.1ml of sample was pipetted out and then subculture on a sterilized xylose lysine deoxycholate agar media and further incubated for 48 hours at 35-37°C. The petri plates were checked for presence or absence of well-developed red with or without black centered colonies indicate the presence of *Salmonella* species [10].

Test for *Pseudomonas aeruginosa*:

1g of powder was pretreated with 10ml of sterilized lactose broth in a sterilized test tube for revivification at 30-37°C. 0.1ml of pretreated material was added to 10ml of sterilized cetrimide broth and incubated for 24 hours at 35-37°C. 0.1ml of sample pipetted out was and then subculture on a sterilized cetrimide agar media and further incubated for 48 hours at 35-37°C. The petri plates were checked for presence or absence of gram negative rods with a usually greenish fluorescence indicate the presence of *P. aeruginosa* [10].

Test for *Staphylococcus aureus*:

1g of powder was pretreated with 10ml of sterilized lactose broth in a sterilized test tube for revivification at 30-37°C. 0.1ml of pretreated material was added to 10ml of sterilized soyabean casein broth and incubated for 24 hours at 35-37°C. 0.1ml of sample was pipetted out and then subculture for on a sterilized Vogel Johnson agar media and further incubated for 48 hours at 35-37°C. The petri plates were checked for presence or absence of black colonies of gram positive cocci surrounded by clear zones indicate the presence of *S. aureus*[10].

TOXICOLOGICAL STUDIES FOR POLYHERBAL FORMULATION

Qualitative heavy metal determination

Previously weighed 10g of polyherbal powder in tarred silica crucible and heated to remove moisture. The powder was incinerated in Bunsen burner to remove organic material. After incineration, crucible was cooled in a desiccator. The residue was boiled in 10ml of dilute

hydrochloric acid and filtered. The filtrate was then neutralized by using ammonia and was further analyzed [11, 12].

Qualitative pesticide residue determination

10g of sample, sodium sulphide and 100ml of n-hexane was refluxed in a round bottom flask for 1 hour and filtered. 50ml of filtrate was taken in a separating funnel and extracted with 25ml of acetonitrile. Then acetonitrile layer was mixed with 500ml of demineralized water and 2.5ml of saturated sodium sulphide. The filtrate was again shaken in separating funnel with n-hexane layer and evaporated on water bath [11].

CHEMICAL PARAMETER FOR POLYHERBAL FORMULATION

Qualitative phytochemical screening of phytoconstituents were carried out on aqueous, hydroalcohol and ethanol extract of polyherbal formulation for the presence of carbohydrates, proteins, alkaloids, glycosides, saponins, phytosterols, phenols, tannins and flavonoids [9].

Chromatographic techniques

Thin layer chromatography

The D-Diabetes polyherbal formulation contains *Eugenia jambolana* (Jamun), *Gymnema sylvestre* (Gurmar), *Momordica charantia* (Karela) and *Tribulus terrestris*(Gokaru)which are present in maximum quantity. Therefore, polyherbal extract was compared with three major raw materials extracts such as Karela fruit, Gurmar leaves and Jamun seed. Methanol extract of polyherbal powder and Karela fruit powder was prepared by macerating 1g in 10ml of methanol AR and then filtered through filter paper. Ethanol extract of Gurmar leaves and Jamun seed powder was prepared by macerating 0.5g in 5ml of ethanol and then filtered through filter paper. The solvent system for polyherbal formulation - chloroform: n-hexane: toluene: ethyl acetate: glacial acetic acid (4.0: 1.3: 1.0: 0.9: 0.1), Karela - benzene: methanol (8.0: 2.0), Gurmar - toluene: ethyl acetate: formic acid (9.6: 0.2: 0.1), Jamun - n-butanol: water (1.0: 1.0).

High performance liquid chromatography

Qualitative assessment was done by HPLC on the basis of retention time. Methanol extract of sample was compared with methanol extract of Karela fruit as a standard.

Methanol extracts of polyherbal powder and *Momordica charantia* were prepared by macerating 1g in 10ml of methanol AR and then filtered through 0.45µ filter paper. Instrument used for analysis is Agilent 1200 and software-EZ Chrome elite.

HPLC chromatographic conditions [13]:

Stationary Phase : Purosphere RP18 Column, Particle size 5µ

Mobile Phase : Acetonitrile: Water (86:14)

Wavelength : 210nm

Flow Rate : 0.5 ml/min

Injection Volume : 20µl

Retention Time : 10.34 min

RESULTS AND DISSCUSSION

BOTANICAL PARAMETERS FOR POLYHERBAL FORMULATION

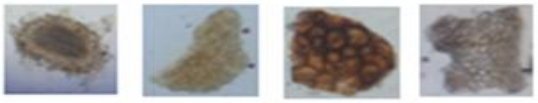
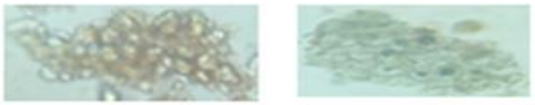
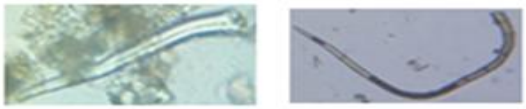
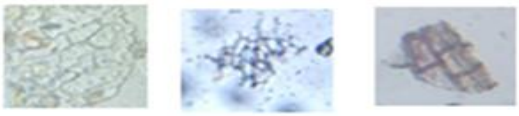
Organoleptic evaluation

Table 3: Organoleptic evaluation for polyherbal formulation

Parameters	Description
Appearance	Rough
Color	Light Brown
Odour	Not specific
Taste	Bitter
Form	Powder

Microscopical evaluation

The images shown in the diagram below are captured from pharmacognosy lab.

Characters	Diagrams	Plants
Epidermis		*Pongamia glabra, Bombax ceiba*
Cork cells		*Holarrhena antidysentrica, Plumbago zeylanica*
Cortex cells		*Plumbago zeylanica, Acacia arabica*
Unicellular and multicellular covering trichomes		*Gymnema zylvestre, Bombax ceiba*
Endosperm		*Eugenia jambolana*
Parenchymatous cells		*Swertia chirata*

Characters	Diagrams	Plants
Medullary ray		*Plumbago zeylanica, Acacia arabica*
Fibers		*Tribulus terrestris, Terminalia chebula, Acacia arabica*
Spiral, pitted and reticulate xylem vessel		*Tribulus terrestris, Pterocarpus marsupium, Santalum album, Bombax ceiba*
Phloem fiber		*Plumbago zeylanica*
Sclerides		*Emblica officinalis, Tribulus terrestris, Pistacia integerrima*
Acicular crystal		*Swertia chirata*
Prismatic crystals		*Holarrhena antidysentrica, Momordica charantia, Ficus bengalensis*
Sieve plate		*Gymnema zylvestre*
Brown matter		*Emblica officinalis*
Starch grains		*Plumbago zeylanica, Tribulus terrestris, Eugenia jambolana, Gymnema zylvestre*

Figure 2: Microscopical characters for polyherbal formulation

PHYSICOCHEMICAL PARAMETERS FOR POLYHERBAL FORMULATION

Physical characteristics: Bulk density, tapped density, Carr's index and Hausner ratio

The bulk density and tapped density for polyherbal formulation was found to be 0.3094 ± 0.0031g/ml and 0.4948 ± 0.0093g/ml respectively. The formulation had less density indicating high bulky formulation.

The Carr's index and Hausner ratio for polyherbal formulation was found to be 37.4474 ± 1.7891% and 1.5994 ± 0.045% respectively. These indicate that the formulation had poor flowability.

Extractive values:

The aqueous, hydroalcohol and ethanol extractive value for polyherbal formulation was found to be 20.5888 ± 0.4851 %w/w, 18.0891 ± 1.026 %w/w and 25.9953 ± 0.5935 %w/w respectively.

The ethanol extractive value was found to be higher followed by aqueous and hydroalcohol extractive value which indicates the extract has high ethanol soluble compounds.

Ash values:

The total ash value, acid insoluble ash and for polyherbal formulation was found to be 10.8087 ± 0.1245 %w/w, 2.0887 ± 0.3349 % w/w and 5.1298 ± 0.9821%w/w respectively. The total ash value limit is not more than 15%w/w. A higher ash value indicates adulteration of silica, soil, dust and such inorganic substances. The ash value of the powder is within the limits specified by W.H.O, so the powder passes the limits for these types of contaminants.

Loss on drying:

The loss on drying for polyherbal formulation was found to be 3.2099 ± 0.4279% w/w. The limit for loss on drying is not more than 5%w/w. Moisture is a major factor responsible for deterioration of herbal formulation and also further decreases the flow properties of the powder. Low moisture content shows better stability and flowability of the formulation. The loss on drying for polyherbal formulation is within limit.

pH value of extract:

The pH values of 1%w/w and 10%w/w for polyherbal extract was found to be 5.54 and 5.35 respectively indicating the suitability for human use as it is weakly acidic.

Crude fiber determination

The crude fiber content for polyherbal formulation was found to be 10.7953 ± 0.5457 %w/w.

Biological parameter for polyherbal formulation: Microbial limit test

The total viable aerobic count and total fungal count was found to be 4.5×10^5 CFU/ g and 80×10^1 CFU/ g respectively. They were within W.H.O limits. The pathogenic microbes such as *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were found to be absent.

Table 4: Microbial limit test for polyherbal formulation

Tests	Inference	Limit (W.H.O)
Total viable aerobic count	4.5×10^5 CFU/ g	10^5 CFU/ g
Total fungal count	80×10^1 CFU/ g	10^3 CFU/ g
Escherichia coli	*Escherichia coli* is absent	10^1 CFU/ g
Salmonella species	*Salmonella* species is absent	Absent
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* is absent	Absent
Staphylococcus aureus	*Staphylococcus aureus* is absent	Absent

TOXICOLOGICAL STUDIES FOR POLYHERBAL FORMULATION

Qualitative heavy metal determination

The qualitative analysis of heavy metals such as arsenic, lead, mercury and cadmium was found to be absent in the polyherbal formulation.

Table 5: Heavy metal qualitative test for polyherbal formulation

Tests	Observation	Inference
For Arsenic		
Silver nitrate solution	No yellow precipitate of silver arsenite observed	Arsenic is absent
Copper sulphate solution	No red precipitate of cuprous oxide observed	Arsenic is absent
Nitric acid and excess of ammonium molybdate solution	No crystalline yellow precipitate observed	Arsenic is absent
For Lead		
Hydrochloric acid	No white precipitate observed	Lead is absent
Hydrogen sulphide gas	No black precipitate observed	Lead is absent
Potassium iodide solution	No yellow precipitate observed	Lead is absent
For Mercury		
Potassium iodide solution	No scarlet precipitate observed	Mercury is absent
Sodium hydroxide solution	No yellow precipitate observed	Mercury is absent
For Cadmium		
Ammonium hydroxide	No white precipitate of calcium hydroxide observed	Cadmium is absent
Potassium ferricyanide solution	No white precipitate of cadmium ferricyanide observed	Cadmium is absent

Qualitative pesticide residue determination

Table 6: Pesticide residue qualitative test for polyherbal formulation

Pesticides	Color Test	Inference
Organochlorine	Isopropyl alcohol	Dichloropropane is absent
Organophosphates	10% Potassium hydroxide in ethanol	Organophosphate is absent
Carbamates	Ethanol + 1 drop of furfural + 1 drop of hydrochloric acid	Amide group is absent

(The sensitivity of color test is 0.05ppm)

CHEMICAL PARAMETER FOR POLYHERBAL FORMULATION

Qualitative phytochemical screening of phytoconstituents:

The polyherbal formulation contains phytoconstituents like carbohydrates, alkaloids, glycosides, phytosterols, phenols, tannins and flavonoids.

Chromatographic techniques:

Thin layer chromatography (TLC)

For polyherbal drug, the pronounced quenching was found at R_f value 0.47 (254nm). Two red spots at R_f value 0.85, 0.13 (366nm) and three blue spots at R_f value 0.75, 0.37, 0.22 (366nm) were found. After spraying with Liebermann Burchard reagent and Vanillin Sulphuric Acid reagent two grey spots at R_f value 0.75 and 0.47 and violet color at R_f value 0.47 respectively were found.

For sample and karela extract, the pronounced quenching was found at R_f value 0.57 (254nm). Blue spot was found at R_f value 0.87(366nm). After spraying with Liebermann Burchard (LB) reagent, purple spot at R_f value 0.28was found. For 366nm sample and gurmar extract, At R_f value 0.34 (white spot), 0.28 (pink spot) and 0.15 (pink spot) was observed for ethanol extract of gurmar. For 254nm sample and jamun extract, the quenching was found at R_f value 0.35 and 0.5 for ethanol extract of jamun.

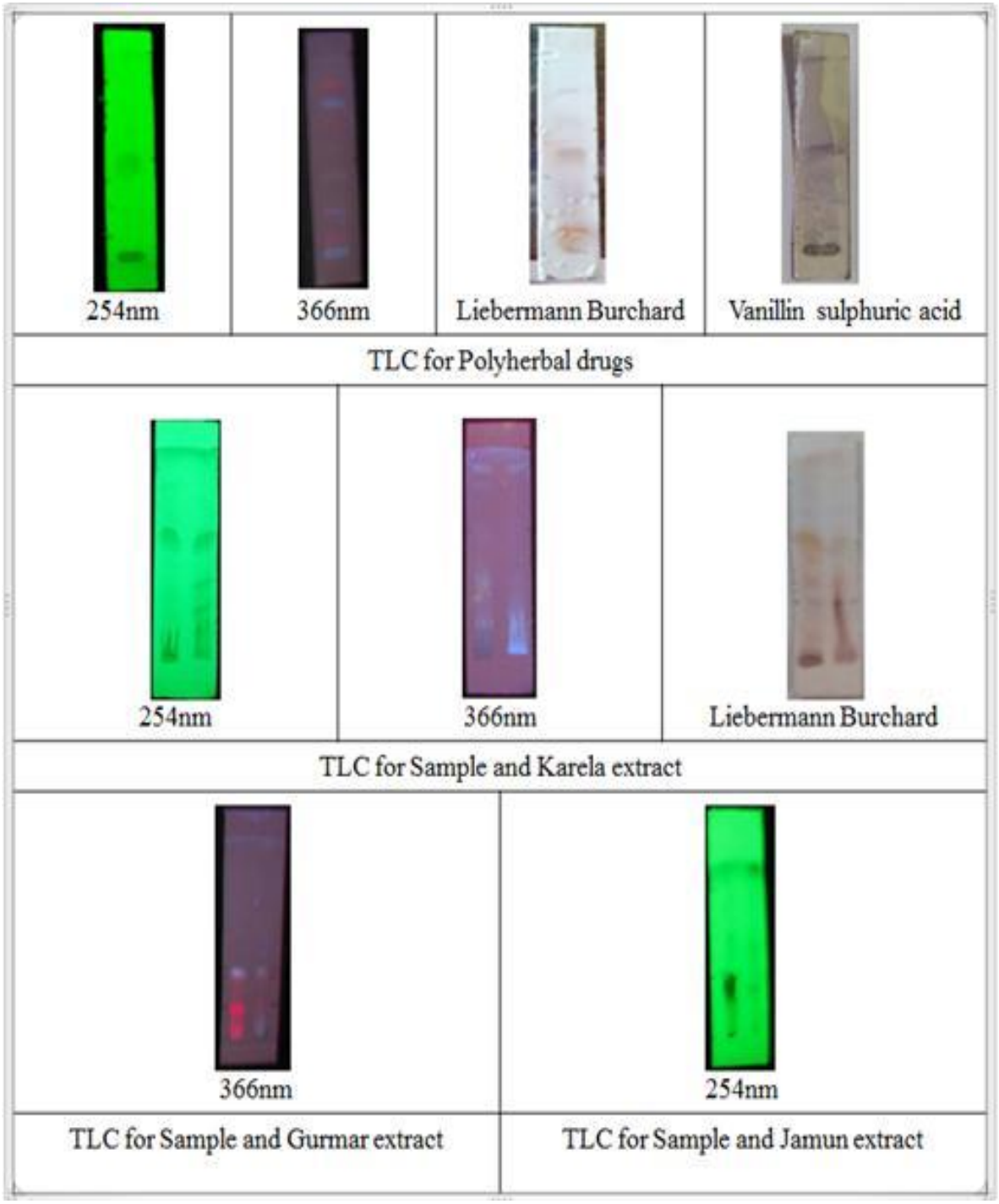


Figure 3: Thin layer chromatography

High performance liquid chromatography (HPLC)

The polyherbal formulation was evaluated by high performance liquid chromatography by using *Momordica charantia* extract as standard and the retention time for standard and sample was found to be 10.84 and 10.813 minutes. The percentage purity for standard and sample was found to be 71.66% and 74.28%.

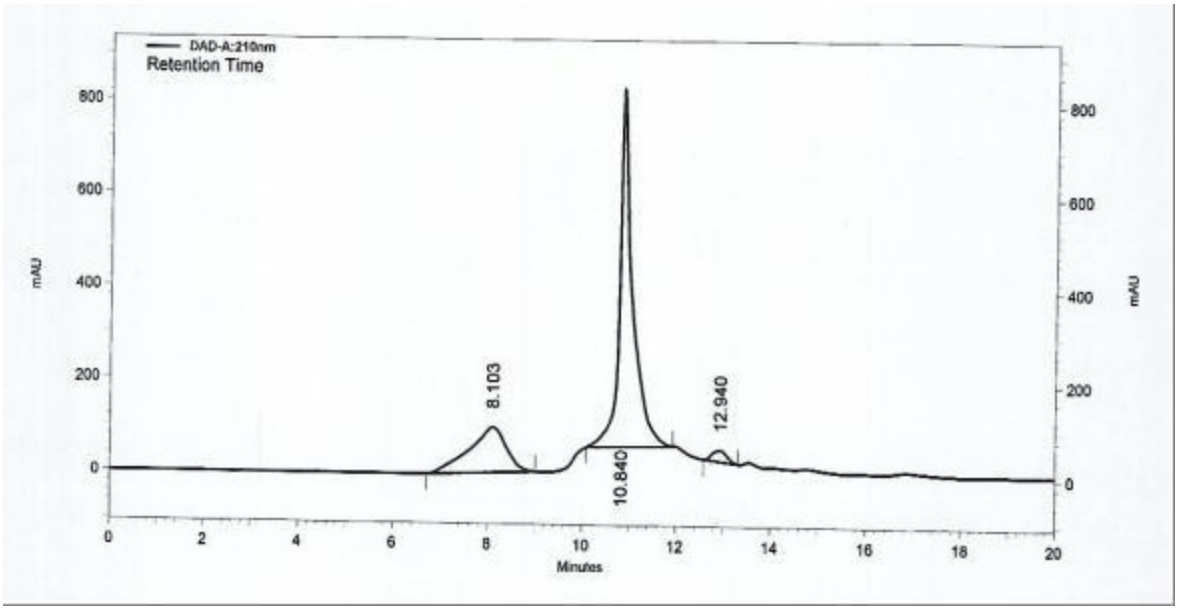


Figure 4: HPLC for standard *Momordica charantia* extract

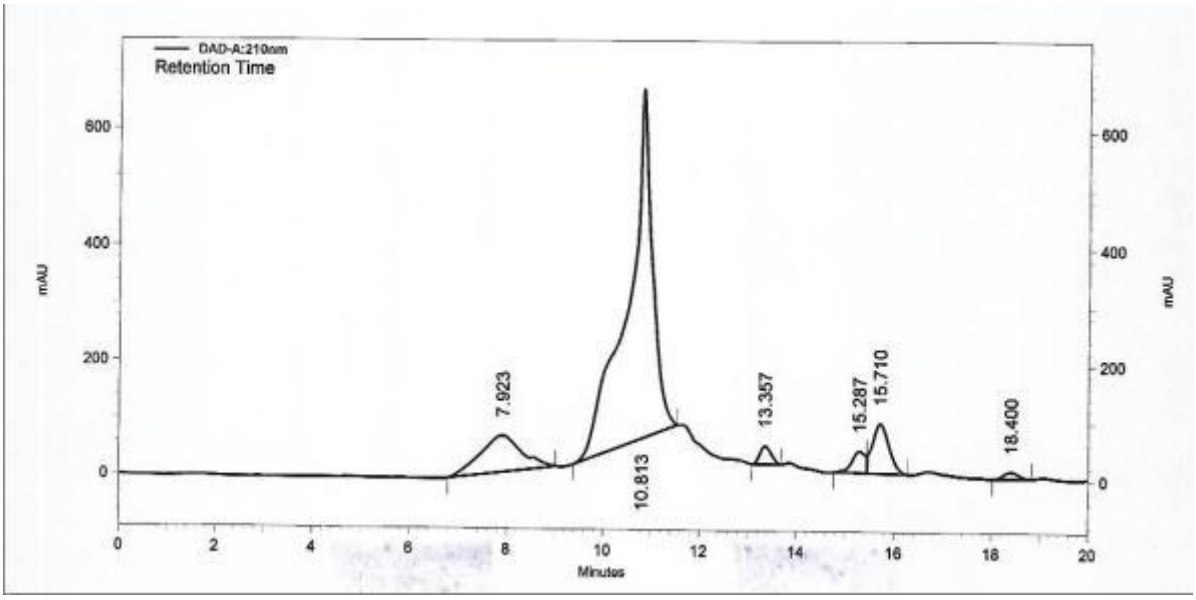


Figure 5: HPLC for sample

CONCLUSION

The present work was carried out for quality control parameters of marketed D-Diabetes polyherbal formulation. The organoleptic, microscopic, physicochemical was evaluated for D-

Diabetes polyherbal formulation. The safety of polyherbal formulation was evaluated for its microbial count, pathogenic microbes, heavy metal and pesticide residue and found to be free from such contaminants and also within specified limits of WHO guidelines. Phytochemical evaluation shows presence of constituents in extract. TLC fingerprinting serve as an important parameter to ascertain the quality and purity of the different plant materials present in this polyherbal formulation. The TLC fingerprinting was also done to compare it with three major raw materials i.e. Karela, Gurmar and Jamun. These chromatograms also showed matching bands thus proving the presence of the raw materials. This can be taken up further for HPTLC method of standardization. HPLC was carried out with of Karela extract as standard along with the sample extract to show the presence of Karela in the powder; it can be further quantified using markers which can be also used for standardization of this formulation. Further studies on isolation and characterization of the specific constituent are needed to validate our results. Hence, these parameters may be considered as a tool for assistance to the scientific organization and manufacturers in developing standards.

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