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EVALUATION OF POLYHERBAL FORMULATION

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ABSTRACT

The present study aimed to evaluate the polyherbal formulation. Application of modern scientific tools and techniques is important for the quality evaluation and standardization of polyherbal formulation. For evaluation of raw materials various parameters are studied as per the World Health Organization's guidelines, Ayurvedic Pharmacopoeia of India and other standard references. The present study investigates the standardization of raw materials which constitutes of polyherbal formulation *Berberis aristata*, *Terminalia chebula*, *Emblica officinalis*, *Terminalia belerica* and *Cyperus rotundus.* To evaluate the raw materials include organoleptic characters, Morphological characters, Microscopic character of every plant and raw materials were subjected to physicochemical studies like ash values, extractive values, phytochemical studies and safety profiles which include heavy metal analysis, microbial load analysis of each powder is identified. Standardized raw materials were subjected to ethanolic extraction and followed by preliminary phytochemical screening and chromatographic analysis for polyherbal formulation were carried out.

KEYWORDS: Polyherbal formulation, Standardisation, Physicochemical parameters, Phytochemical, chromatography.

INTRODUCTION

Herbal Medicine is a major component in all indigenous medicine and common element in Ayurvedic, Siddha, Homeopathy and naturopathic system of medicine. The herbal medicines gain their popularity due to the absence of side effects. Many potent herbs are combined in a poly herbal formulation, its gives an added advantage and higher confidence to the patient.

The concept of standardization and quality control of drug can be found in ancient Ayurvedic texts. In those days, the physician himself identifies, checks the drugs based on habitat, morphology, taste, colour, texture and uses as medicine. But in modern times, these tests and tools are not sufficient to control the quality. Hence, the World Health Assembly (WHA42.43-1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. Assessment of complete and accurate physicochemical value of *Ayurvedic* herbs not only provides scientific basis of its quality but also helps in globalization of *Ayurveda.* Under these circumstances, pharmacognosy, pharmacology and phytochemistry are necessary for authentification of crude drug and to prove therapeutic action as well.[1]

In traditional systems of medicine, many plants have been documented to be useful for various systemic disorders. Many of the Traditional/Indigenous systems of medicine are effective but they suffer from lack of complete standardization which is one of the important challenges posted by the traditional system of medicine. The concept of polyherbal formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential, Hence, the present study was planned to formulate and standardize a polyherbal formulation using plants having known anti diabetic potential. As a part of our research work five herbal drugs were selected for the preparation of polyherbal anti diabetic formulation.[2-7]

MATERIALS AND METHODS

Plant Przofile[8-13]

Table 1: Selected plant and its Traditional uses.

Plant Name	Family	Common Name	Parts Used	Traditional Uses
Berberis aristata	Berberidiaceae	Tree turmeric	Stem	Diarrhoea,diabetes, opthalmic diseases
Terminalia chebula	Combretaceae	Myrobalan	Pericarp of fruit	Diabetes, dysentery, diarrhoea, gout, malaria, pneumonia, Anaemia, typhoid
Emblica officinalis	Euphorbiaceae	Amla	Pericarp of fruit	Diabetes, dysentry, diarrhoea, Anaemia, bronchitis, leprosy, leucorrhoea, inflammation of the eyes, malaria, scurvy, constipation
Terminalia belerica	Combretaceae	Beleric myrobalan	Pericarp of fruit	Diabetes, diarrhoea, Anaemia, asthma, bronchitis, leprosy, cough, cardiac diseases, liver problems, headache, constipation, skin diseases, inflammation , malaria, sore throat
Cyperus rotundus	Cyperaceae	Nut grass	Rhizome	anthelmentic, anti- inflammatory, diabetes, expectorant, jaundice, leprosy, scabies, dysmenorrhoea, dyspepsia, flatulence, ulcer, stomach pain

Collection of the Plant and Authentication

The selected plant materials viz *Berberis aristata (*dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried matured fruit), *Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome) were procured from the authentic suppliers and further authenticated by Dr. K.N. Sunil Kumar R.O. and HOD Pharmacognosy, Central Siddha Research Institute, Govt. of India, Arumbakkam, Chennai - 106 and a voucher specimen were deposited at the Department for future reference.

Processing of Raw Materials

The procured plant materials were cleaned thoroughly. They were then dried under shade for a week or so. Once they were completely dried, they were ground into coarse powder and stored in air tight containers and preserved for the further processing.

Standardisation of Raw Materials[14-16]

Shade dried powdered plant materials of *Berberis aristata (*dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried matured fruit),*Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome) used for the standardization of raw materials.

Organoleptic Evaluation

In this study the following organoleptic characters like physical appearance, taste and odour of plant materials were evaluated.

Powder microscopy[17]

A pinch of the powdered sample was mounted on a microscopic slide with a drop of phloroglucinol and conc. HCl. Characters were observed under microscope.

Physico Chemical Evaluation

The Physicochemical parameters such as loss on drying, ash values, extractive values, heavy metal analysis, microbial load analysis of crude drug were performed according to the official method prescribed.

Phytochemical Studies[18,19]

The Phytochemical parameters such as preliminary Phytochemical screening of powder, Fluorescence analysis of raw materials, preparation of extract and Thin Layer Chromatography of the each extract were performed.

Preliminary Phytochemical screening for raw material

All the plant raw materials were subjected to preliminary Phytochemical screening for the detection of Alkaloid, Flavonoids, Tannin, Saponin, Glycoside, Terpenoids, Steroid, Carbohydrates and Phenolic compounds.[18]

Fluorescence analysis

Raw materials used for the polyherbal capsule preparation were tested for any colour changes under UV light. Samples were tested as such and after treating with organic solvents, alkali and acidic solutions and viewed under ordinary light, short UV (254 nm) and long UV (365 nm) and studied for its fluorescence property.[18,19]

Preparation of Extract

About 200g of coarsely powdered parts of the plant was extracted with Ethanol at 60-70°C. Extract of individual plants were concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of all the extracts were noted.

Formula for Polyherbal formulation

The Polyherbal formulation (per capsule) contained the ethanolic extracts of *Berberis aristata*, *Terminalia*

chebula, *Emblica officinalis*, *Terminalia belerica* and *Cyperus rotundus* in the ratio of 1:3:3:3:2.

Preliminary Phyto-chemical screening

One gram of polyherbal formulation was dissolved in100ml of its own mother solvent to obtain a stock of concentration 1%w/v and tested for the presence of alkaloids, flavanoid, tannin,saponin glycosides, terpenoids, steroid, carbohydrate and phenolic compounds.[18]

Chromatographic analysis[20-22]

Thin layer chromatographic study

The ethanolic extracts of *Berberis aristata*, *Terminalia chebula*, *Emblica officinalis*, *Terminalia belerica* and *Cyperus rotundus* were subjected to thin layer chromatography (TLC) as per conventional method using silica gel 60F254, 5x3 cm (Merck). Different solvent systems ranging from lower to higher polarities were tested for the separation of bioactive components.

In the TLC chamber the solvent system *viz* Butanol: Acetic acid: Aqueous (40:10: 20) were used. After completion of the elution the plates were dried and subjected to visualized under UV chamber and sprayed using different spray reagents.

R_f values determined by using following formula

R_f = Distance travelled by the solute / Distance travelled by the solvent.

High performance thin layer chromatography fingerprint analysis

1mg of Polyherbal extract was dissolved in 1 ml of methanol. A number of solvent systems were tried, for extract. The satisfactory resolution obtained for the Phytochemical constituent was in the solvent butanol-acetic acid-water (4:1:5). 2 µl of standard solution and 2 µl of the test solution (extract) were loaded as 5 mm band length in the 4×10 glass plates. After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x10 cm saturated with previously equilibrated mobile phase for 30 minutes. The chromatographic conditions were previously optimized to active the best resolution and peak shape. The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 420 nm. Quercetin was used as the reference standard. The plates were kept in Photo-documentation chamber and captured the images at White light, UV 254 nm. After scanning the plates the Peak table, Peak display and Peak densitogram were recorded. The retention factor (Rf) was calculated by the WinCats software.

RESULTS AND DISCUSSIONS

The raw materials were authenticated and analysed for their compliance to quality standards as established by WHO guidelines, Pharmacopoeias and standard reference books.

Table 2: Plant Profile.

Plant Name		Image
Berberis aristata **[Dried Stem]**		
Terminalia chebula **[pericarp of matured fruit]**		
Emblica officinalis **[pericarp of matured fruit]**		

Terminalia belerica **[pericarp of matured fruit]**		
Cyperus rotundus **[Dried Rhizome]**		

Organoleptic Evaluation

Organoleptic characters for the raw materials such as colour, odour and taste were given in table 3.

Table 3: Organoleptic characters coarse powder of selected plants.

Plant name	Nature	Colour	Odour	Taste
Berberis aristata	Coarse powder	Pale Yellowish brown	Odorless	Bitter
Terminalia chebula	Coarse powder	Dull Yellow	Odorless	Astringent, slightly bitter
Emblica officinalis	Coarse powder	Grey to black	Odourless	Sore and Astringent
Terminalia belerica	Coarse powder	Dark brown to black	None	Astringent
Cyperus rotundus	Coarse powder	Dark black	Pleasant	Pleasant

Microscopical Evaluation

Powder microscopy was done for the chosen plants and observations were given in table 4.

Table 4: Powder microscopy of selected plants.

Plant name	Observation
Berberis aristata **(dried stem)**	Presence of **Non-lignified elements:** Fibres, stone cells, Thick walled fibres, Parenchyma with crystals. **Lignified elements:** Cork cells in oblique view, Pitted vessel fragment, Fibre fragment, Vessel, Tracheid, Sclereid.
Terminalia chebula **(pericarp of fruit)**	Presence of **Non-lignified elements:** Epicarp, Thick walled parenchyma, **Lignified elements:** Different types of sclereids, Fibro-sclereids, Pitted tracheids.
Emblica officinalis **(pericarp of fruit)**	Presence of **Non-lignified elements:** Epicarp, Thick walled parenchyma, Fibre, Parenchyma. **Lignified elements:** Sclereids of different types
Terminalia belerica **(pericarp of fruit)**	Presence of **Non-lignified elements:** Trichome, Epicarp, Parenchyma with tannin, Tracheids, Vessels. **Lignified elements:** Vessels, Sclereids, Fibro-sclereid
Cyperus rotundus **(dried rhizome)**	Presence of **Non-lignified elements:** Cork, Aerenchyma from root, Tracheidal fibres, Vessels. **Lignified elements:** Group of sclereids, Parenchyma.

Physiochemical parameters

Various physicochemical parameters like loss on drying, ash values and extractive values were calculated for the herbal plants used in polyherbal formulation. Table 4,5and 6 gives the evidence of the report of various physiochemical parameters.

Loss on Drying

Loss on drying for the raw materials were done the results obtained and the standard values are given in table 5.

Table 5: Loss on drying.

Plant Name	Loss on drying (% W/W)	Acceptable Limits (W/W %) As Per API
Berberis aristata	2.74±0.59	NMT 8
Terminalia chebula	3.67±0.12	NMT 8
Emblica officinalis	4.61±0.14	NMT 6
Terminalia belerica	4.16±0.07	NMT 5
Cyperus rotundus	4.25±0.01	NMT 5

The value are expressed as mean ± SD, (n=3); NMT-Not more than, API-Ayurveda Pharmacopoeia of India

Determination of Ash Values

Total ash value, acid insoluble ash and water soluble ash values were determined and the values are within the standard limits as prescribed in the Ayurveda Pharmacopoeia of India.

Table 6: Ash values of selected plants.

Plant Name	Total ash (% w/w)		Acid insoluble Ash (% w/w)		Water soluble ash (%w/w)	
	Observed value	Limits (w/w%) as per API	Observed value	Limits (w/w%)as per API	Observed value	Limits (w/w%) as per API
Berberis aristata	4.06±0.12	NMT 14	0.34±0.08	NMT 5	0.78±0.01	NMT 3
Terminalia chebula	2.31±0.02	NMT 5	0.45±0.02	NMT 5	2.31±0.02	NMT 5
Emblica officinalis	5.36±2.23	NMT 7	1.17±0.16	NMT 2	1.76±0.37	NMT 3
Terminalia belerica	5.16±0.04	NMT 7	0.68±0.01	NMT 1	2.74±0.59	NMT 6
Cyperus rotundus	6.18±0.01	NMT 8	2.27±0.04	NMT 4	5.73±0.21	NMT 7

The value are expressed as mean ± SD, (n=3); NMT-Not more than API-Ayurveda Pharmacopoeia of India

Determination of Extractive Values

Water soluble Extractive values, alcohol soluble extractive values and ether soluble extractives values for the raw materials were determined and the results are tabulated in table 7.

Table 7: Extractive values of the selected plants.

Plant name	Water soluble extractive (%w/w)		Alcohol soluble extractive (%w/w)		Ether soluble Extractive value
	Observed value	Acceptable Limits (w/w %) as per API	Observed value	Acceptable Limits (w/w %) as per API	Observed value
Berberis aristata	14.29±0.04	NLT 8	9.82±0.17	NLT 6	9.28±0.64
Terminalia chebula	61.94±0.17	NLT 60	42.66±0.16	NLT 40	8.35±0.42
Emblica officinalis	51.63±0.20	NLT 50	42.36±0.22	NLT 40	12.68±0.24
Terminalia belerica	2.61±0.69	NLT 35	14.88±0.28	NLT 8	8.50±0.25
Cyperus rotundus	12.54±0.15	NLT 11	8.08±20	NLT 5	9.25±0.24

The value are expressed as mean ± SD, (n=3); NLT-Not less than API-Ayurveda Pharmacopoeia of India

Analysis of Heavy Metal

Estimation of heavy metals in the raw materials were carried out and the results were recorded and detailed in table 8.

Table 8: Test for heavy metals.

Plant name	OBSERVATION (in ppm)			
	Arsenic (NMT 5)	Lead (NMT 10)	Cadmium (NMT 0.3)	Mercury (NMT 0.5)
Berberis aristata	0.002	0.001	0.024	0.004
Terminalia chebula	0.004	0.003	0.005	0.002
Emblica officinalis	0.002	0.004	0.001	0.001
Terminalia belerica	0.001	0.005	0.04	0.04
Cyperus rotundus	0.005	0.002	0.002	0.004

The estimation of heavy metals in the sample revealed heavy metals are within the prescribed limits. It is safe and does not cause any harm on consumption.

Microbial Load Analysis

Table 9: Microbial load analysis.

Parameters	Berberis aristata	Terminalia chebula	Emblica officinalis	Terminalia belerica	Cyperus rotundus
Total aerobic count (NMT 1000cfu/g)	Nil	Nil	100	100	Nil
Yeast and mould count (NMT 100cfu/g)	Nil	Nil	Nil	Nil	Nil
E.coli (To be absent)	Absent	Absent	Absent	Absent	Absent
Salmonella (To be absent)	Absent	Absent	Absent	Absent	Absent
Pseudomonas (To be absent)	Absent	Absent	Absent	Absent	Absent
Staphylocoucus (To be absent)	Absent	Absent	Absent	Absent	Absent
Shigella (To be absent)	Absent	Absent	Absent	Absent	Absent

From the results, it is shown that the powdered raw materials complies with the WHO standards for Microbial load analysis and hence it is safer to be taken internally.

Priliminary Phytochemical Screening

The chemical test for various phyto constituents in the raw materials were carried out and the results were recorded and detailed in the table 10.

Table 10: Phytochemical analysis.

Phyto-constituents	*Berberis aristata*	*Terminalia chebula*	*Emblica officinalis*	*Terminalia belerica*	*Cyperus rotundus*
Phenolic compounds	_	+	+	+	+
Flavanoids	+	+	+	+	+
Tannins	_	+	+	+	+
Alkaloids	+	+	+	_	+
Steroids	+	+	+	_	_
Glycosides	+	+	+	+	+
Saponins	+	+	_	+	+
Proteins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Terpenoids	+	+	_	+	_

Active constituents, (+) presence and (-) absence

Fluorescence Analysis of Raw Materials

Table 11: Fluorescence analysis.

Sample	Before Treatment			After Treating With 50 % Hcl			After Treating With 50% NaoH		
	Ordinary Light	Short UV	Long UV	Ordinary Light	Short UV	Long UV	Ordinary Light	Short UV	Long UV
Berberis aristata	Light yellow	Black	Black	Brown	Brown	Brown	Black	Light Green	Dark brown
Terminalia chebula	Light yellow	Brown	brown	Light brown	Brownish Green	Dark brown	Light brown	Greenish brown	Dark brown
Emblica	Dark	Light	Black	Light Yellow	Green	Black	Light brown	Green	Green

officinalis	brown	brown							
Terminalia belerica	Dark brown	Brown	Brown	Yellowish Brown	Light green	Light green	Reddish brown	Dark green	Light green
Cyperus rotundus	Black	Black	Light brown	Brown	Brown	Dark brown	Light brown	Dull brown	Light brown

Preparation of Extracts

The shade dried crude dried drugs of *Berberis aristata (*dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried matured fruit), *Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome) were extracted in soxhlet extractor with ethanol. All the extracts were concentrated using rotary vacuum evaporator. The percentage yield was calculated for every extract in terms of dried weight of plant material. The colour and consistency of the concentrated extracts are given in table 12.

Table 12: Percentage yield of various extracts.

Plant name	Method of extraction	Physical nature	Colour	Yield %w/w
Berberis aristata	Continuous hot percolation	Semi solid	Dark brown	**5.29**
Terminalia chebula				**22.05**
Emblica officinalis				**16.9**
Terminalia belerica				**16.16**
Cyperus rotundus				**8.75**

Phytochemical Analysis

The chemical tests for various Phytoconstituents in the polyherbal formulation were carried out and the results were recorded and detailed in table 13.

Table 13: Phytochemical analysis of Polyherbal formulation.

Phyto-constituents	Observation
Phenolic compounds	+
Flavanoids	+
Tannins	+
Alkaloids	+
Steroids	+
Glycosides	+
Saponins	+
Proteins	+
Carbohydrates	+
Terpenoids	+

Active constituents, (+) presence

Chromatographic Analysis

Thin layer Chromatographic Analysis

The chromatographic analysis for each individual ethanolic extract and polyherbal formulation was performed and the results were given in the Table 14.

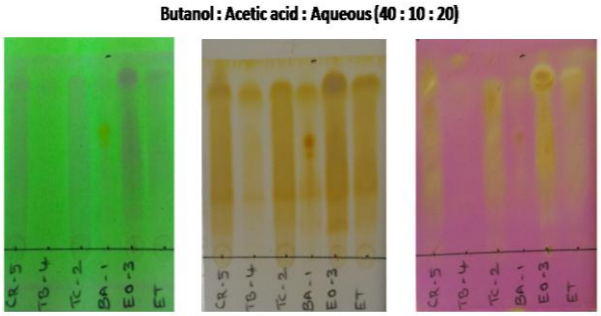

Fig 1: Thin layer chromatography of individual extract and PHF.

CR- *Cyperus rotundus,* TB- *Terminalia belerica,* TC- *Terminalia chebula,*
BA - *Berberis aristata,* EO - *Emblica officinalis,* ET - Poly herbal formulation

Table 14: Thin Layer Chromatography Rf values of the extracts.

Extract	Solvent system	Number of spots	Distance travelled by the solute (cm)	Distance travelled by the solvent (cm)	Rf value (cm)
Cyperus rotundus	Butanol: Acetic acid: Aqueous (40: 10: 20)	4	2.8; 2.5; 1.4; 0.5	3	0.93; 0.83; 0.46; 0.16
Terminalia belerica		3	2.5; 1.9; 0.9	3	0.83; 0.63; 0.3
Terminalia chebula		4	2.6; 2.2; 0.9; 0.6	3	0.86; 0.73; 0.3; 0.2
Berberis aristata		6	2.6; 1.8; 1.5; 1.2; 1.0; 0.5	3	0.86; 0.6; 0.5; 0.4; 0.33; 0.16
Emblica officinalis		6	2.6; 2.5; 2.0; 1.5; 1.0; 0.5	3	0.86; 0.83; 0.66; 0.5; 0.33; 0.16
ET (Polyherbal formulation)		6	2.6; 2.4; 2.2; 1.4; 1.0; 0.5	3	0.86; 0.8; 0.73; 0.46; 0.33; 0.16

High Performance Thin Layer Chromatography

HPTLC was performed for the ethanolic extract of polyherbal formulation**.** The chromatographic conditions were carried was detailed in materials of this study. There were six peaks observed with different R_f values.

Mobile Phase: Butanol-Acetic Acid-Water (4:1:5).

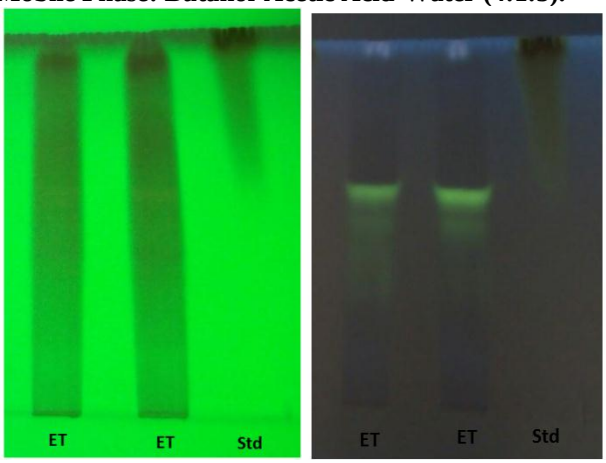

Fig. 2: ET- polyherbal extract powder, Std – Flavanoid.

Hptlc Finger Printing Of the Polyherbal Extract

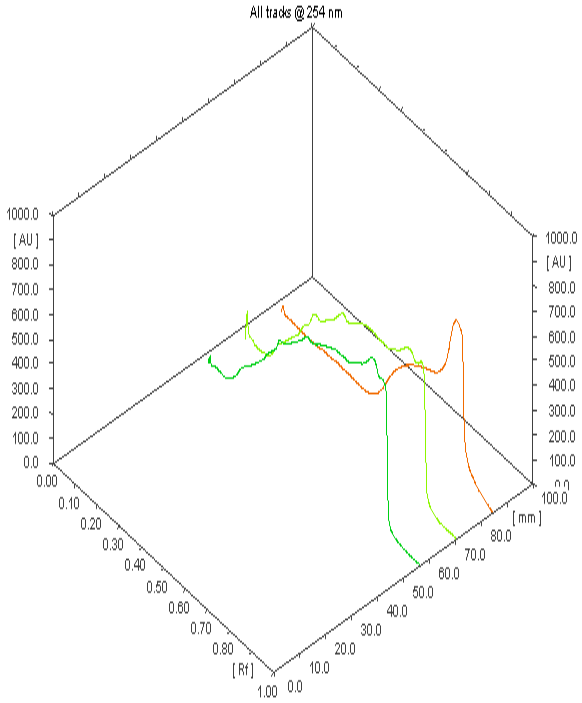

Fig. 3: HPTLC of finger print data.

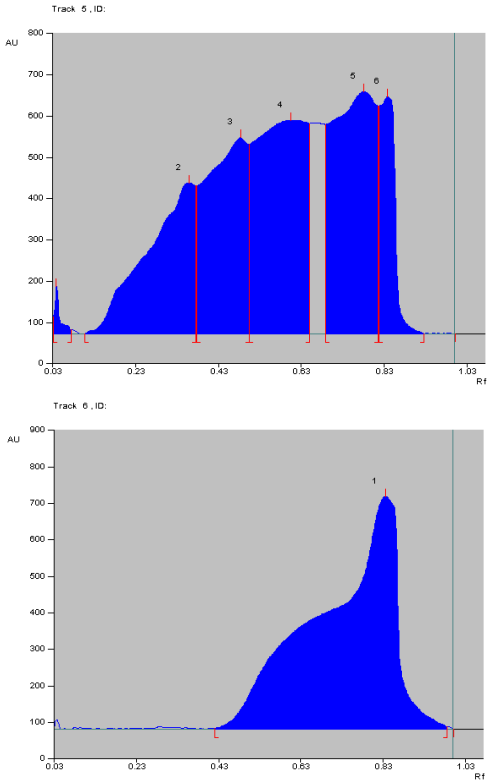

Fig. 4: Chromatographic finger print analysis of formulation and standard.

Table 15: HPTLC finger printing spectral values.

Peak	Rf value	Assigned substance
1	0.05	Unknown
2	0.31	Unknown
3	0.48	Unknown
4	0.59	Unknown
5	0.78	Unknown
6	0.97	**Standard**

The HPTLC finger printing of the polyherbal ethanolic extracts showed the above tabulated R_f values with corresponding peak area.

HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents of multicomponent sample and also a diagnostic method to find out the adulterants to check purity.

CONCLUSION

In the present investigation the raw materials of polyherbal formulation were standardised as per Ayurvedic Pharmacopoeia of India and other standard references. The raw materials were identified by observing its Macroscopical and Microscopical characters. Physicochemical Standardisation and Phytochemical analyses were carried out for raw materials and compared with that standard limits. Preliminary Phytochemical screening, TLC and HPTLC for the ethanolic extracts were also carried out in order to find out the chemical constituents present in the drugs.

From the present investigation, it can be concluded that the raw materials standardised were in accordance with the standards laid down in Ayurvedic Pharmacopoeia of India. Further studies are focussed on Preparation, Standardisation and Evaluation of Capsule for anti diabetic activity.

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