International Journal of Pharmaceutical and Clinical Research
www.pharmaceuticaljournal.in
Online ISSN: 2664-7605, Print ISSN: 2664-7591
Received: 12-03-2019, Accepted: 16-04-2019, Published: 30-05-2019
Volume 1, Issue 1, 2019, Page No. 40-50

Qualitative and Quantative estimation of phyto-constituents in *Hemidismus Indicus* roots by using advanced analytical tools

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DOI: https://doi.org/10.33545/26647591.2019.v1.i1a.38

Abstract
The roots of *Hemidismus Indicus* have been used in traditional and indigenous system of medicines thought the humankind for thousands of years. The plant *Hemidismus Indicus* enriched with many phyto-constituents, which are responsible to cure many ailments. The present study was aimed to physiochemical screening and estimation of the bioactive compounds by using advance analytical tools. The study results in the qualitative and quantative estimation of the secondary metabolites; which are industrially important in standardization of the herbal drug products. The heavy metals and residues are the main intimidation in herbal drug formulations. The advance analytical instruments like HPLC and ICPMS are used in the estimation of Aflotoxins and Heavy metals in the study. The validated analytical methods and tools are applied here for the identification and quantifications of the *Hemidismus Indicus* root powder. The same method and assay can be used as quality control parameters in standardization of the plant extracts and herbal drug formulations. The developed HPTLC method is found to be sensitive, specific, precise and accurate for the identification and quantification of isovanillin, rutin and coumarin in *Hemidismus Indicus*. The finger prints derived from the HPTLC can be used as the athentification tool for the *Hemidismus Indicus* roots.

Keywords: Bio- active compounds, HPTLC, HPLC, ICPMS, standardization, quality control, herbal drug and athentification

Introduction
Medicinal plants play an important role in the treatment of many diseases they are the foremost source of medicine around the world. Herbal remedies are the most important source of life saving drugs because of their lower or no side effects. The study of medicinal plants consequently important in new herbal drug development either as pure compounds or standardised plant extracts. The new product development mainly depends upon the reliable methodologies for proper athentification, standardization and quality assurance of herbal drugs. (Ranjit saryam *et al* 2012) [47].
Hemidismus Indicus commonly known as Indian Sarsaparilla belongs to the family Asclepiadaceae found commonly across India.Its called as Sarasaparilla in English, Ananthmool in Hindi and Sarivaa in Sanskrit. According to indigenous medicinal texts of Ayurveda, Unani, Sidda and modern researches the root of *Hemidismus Indicus* possess anti
-Venom anti- inflammatory anti-microbial, anti-pyretic, anti- diabetic, anti-hyperlipidemic, anti-ulcer chemoprotective, renopretective, anti-acne antioxidant and anti-artheritic activity. (Devang J *et al* 2018) [6]. The roots are traditionally used to make decoction and beverages because it's a best cooling and detoxifying herbs. The roots of *Hemidismus Indicus* also shows anticancer potentials against MCF and breast cancer cell lines. (Saraswathi A *et al*. 2013) [62]. the present investigation was aimed at evaluating pharmacognostical features and phyto-chemical analysis for identification and authentication of the plant material. Successful determination of biologically active compounds from plant material depends on the type of solvent used in the extraction procedures. The tests for the identification and screening of bioactive compounds from *Hemidesmus indicus* was achieved by using standard procedures such as spectrophotometry, gravimetry and color reactions etc. The tests for active compounds would be flavonoids, tannins, saponins, polyphenols, steroids, bitters, glycosides terpenoids phenols and alkaloids etc. This study also concerned with the identification of the compounds by HPLC analysis and development of HPTC fingerprint profile. The HPTLC fingerprint of botanically authenticated raw material serves as a primary reference against which unknown material can be authenticated for its chemical identity.

Fig 1: *Hemidesmus indicus* (Linn.) R.Br.

2. Materials and Methods

2.1 Collection of plant material

Hemidesmus indicus (Linn.) R.Br. was collected from Mullayanagiri is the highest peak in Karnataka, India. Mullayyanagiri is located at 13°23′26″N 75°43′18″E/ 13.39056°N 75.72167°E/ 13.39056; 75.72167 in the Chandra Dhrona Hill Ranges of the Western Ghats of Karnataka. (December 2017).The collected plant material was dried in shade and taken for the study maintained and the plant are grown in greenhouse and used for the further studies.

2.2. Authentication

The authentication was done by Dr. R Kannan Principal Scientist - R&D Centre, The Himalaya Drug Company, Makali, Bengaluru - 562 162, India, the authenticated material was taken for the experiments.

2.3. Drug Preparation

The collected roots were shade dried for 15 days and powdered in a laboratory grinding mill.The powdered root material was passed through 40# size mesh particle size and stored in a clean dried airtight container at room temperature.

2.4. Qualitative analysis

2.4.1. Preliminary screening for phyto-chemicals

Standard screening tests were carried out by using general accepted standard laboratory techniques. The crude extracts were screened for presence of secondary metabolites such as flavonoids, saponins, tannins, alkaloids and phenols etc.

The preliminary phytochemical screening of *Hemidismus Indicus* root showed the presence of proteins, carbohydrates, saponins bitters tannins and phenolic compounds, flavonoids, amino acids with different chemicals and reagent test. So, different groups of phyto constituents are estimated in the dried root powder. The results are tabulated in the Table.2.

2.5. Quantitative analysis

2.5.1. Estimation of Ash by Gravimetry

2.5.1.1. Total Ash

The ash remaining following ignition of herbal materials is determined by three different methods which measure total ash, acid- insoluble ash and water-soluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both -physiological ash‖, which is derived from the plant tissue itself, and -non-physiological‖ ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Place about 2–4 g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500–600°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes, then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

2.5.1.2. Acid-insoluble

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute Hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70 g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash less filter-paper and wash with hot water until the filtrate is neutral. Transfer the filter-paper containing the insoluble matter to the original crucible, dry on a hotplate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

2.5.1.3. Water-soluble ash

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water. To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water- soluble ash in mg per g of air-dried material. (Quality control methods for herbal plant material 1998 WHO Library Cataloguing-in- Publication Data Organization. ISBN 978 92 4 150073 9.)

2.5.2. Loss on drying

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. This is the thermo Gravimetry is a technique in which the weight of sample is recorded as a function of temperature according to a controlled temperature programme. There are two methods were used for the loss on drying by Oven method and automated Halogen moisture analyser here the halogen bulb is used to determine the loss on drying.

2.5.3. Loss on drying by Oven method

2 g of the test substance was weighed in a stoppered glass weighing bottle dried at 1050C ± 20C.The loaded bottle was placed in the drying chamber for 4 hours and remove the stopper and leave it. Dry the sample to constant weight at 1050C after drying is completed, open the drying chamber, close the bottle promptly and allow it to cool to room temperature in a desiccator before weighing. Weigh the bottle and contents.

2.5.4. Loss on drying by Halogen moisture

2 g of the test substance was used to determine the loss on drying. (The instrument used was Meter MB 45 OHAUS).

2.6. Estimation of Flavonoids by Spectrophotometry

Flavonoids produces yellow colour with the Aluminum chloride reagent. The color produced can be read at 140 nm and the amount of flavonoids can be quantified using Rutin as standard.1 gram of the powdered sample was extracted in methanol at 800 C reflux for 30minutes and filter the extract and taken for the analysis. Sigma Rutin standard of 95% of 1% was prepared in methanol and taken for the analysis. Each 1 ml of sample and standard are taken along with the blank solutions and measured at 410nm in the nm with a double beam UV/Visible spectrophotometer, SHEMADZU 1700 (Japan). After subtracting the corresponding blank values from the sample absorbance values flavonoid content with respect to Rutin and express as% w/w of flavonoid. (Chia –Chi Changi *et al*., 2002; Chang C, Yang M, Wen H, Chern J *et al*., Nadeem Ahmad Siddique *et al*., 2010 and 2002) [10, 8]. European Pharmacopeia PH Eur. monograph 1174).

2.7. Estimation of Tannins by Spectrophotometry

Tannins give greenish blue colour with potassium ferricyanide and ferric chloride reagents. The colour produced can be read at 720 nm and the amount of tannin can be quantified using Tannic acid as standard. The standard tannic acid was used for the analysis, the standard was procured from Sigma of 95% of purity. Approximately 1gm of the powdered sample was taken and extracted for 30minutes in water bath at 100°C. The reading was taken exactly 30 minutes after addition of the reagents, and the optical density read at 720 nm against reagent blank. Reagent blank was prepared by diluting 1 ml of potassium ferricyanide and 1 ml of ferric chloride to 10 ml with purified water. (Augustin Scalbert *et al*., 1989 and Rashmy Nair *et al*., 2015) [3, 48].

2.8 Estimation of total polyphenols by spectrophotometry

The estimation of polyphenols was done by using Ciocateu's Phenol reagent reagent, and pyrogallol used as standard. The reaction mixture was measure at at 760 nm with the respective blank, using purified water as the compensation liquid.
(Andressa Blainski *et al*., 2013 and Kalpana P Rathae *et al*., 2013) [1].

2.9. Estimation of Saponins by Gravimetry

5 g of powdered sample was taken for the analysis and extracted with 90% methanol (50 ml) by refluxing for half an hour at 70 - 80oC. Extract the residue few more times by taking 25 ml methanol till the extracts are almost colorless. Combine the methanolic extracts and evaporate the solvent on a water bath followed by drying in oven for 2 hrs. Treat the obtained extract, with 25 ml of petroleum ether chloroform and ethyl alchol. Dissolve

the residue left with 25 ml of 90% methanol and filter using ordinary filter paper. Add the filtrate drop by drop with constant stirring to 125 ml acetone to precipitate saponins. Keep it aside for settling of the precipitate. Decant off the acetone layer completely, and dry the precipitate to constant Weight at 105oC. Calculated the results by subtracting the final weight and initial weight and expressed in percentage.

2.10. Estimation of Bitters by Gravimetry

About 5 g of the finely powdered sample was taken for the extraction, extraction solvent was methanol and reflux it on a water bath at 800C ± 20C for 30 minutes. After the successive extraction the extract was collected and concentrate the combined methanolic extract in to a thick paste. The thick extract was dissolved in hot water (if the extract does not dissolve, add 25 ml of ethyl acetate and sonicate it for 5 minutes).The aqueous extract was then subjected for the ethyacetate extraction by using the separating funnel. The ethyl acetate extract was collected and evaporated in a pre-weighed china dish and dried at 1050C for one hour in oven. The result was calculated by subtracting the final weight and initial weight and expressed in percentage. (Quality control methods for herbal plant material 1998 WHO Library Cataloguing-in- Publication Data Organization. ISBN 978 92 4 150073 9).

2.11. Estimation of Alkaloids by Gravimetry

10 grams of the powdered sample was taken for the analysis with the addition of 200 ml of solvent mixture 6 ml of dilute ammonia was added with the vigorous shaking and kept for 15- 20 hours. The filtrate was extracted with the successive quantities of dilute self-uric acid (35 ml, 25 ml and 20 ml). The acid solution was neutralized with the addition of 10 ml of concentrated ammonia solution and extracted with chloroform in successive quantities (35ml, 25 ml and 20 ml)

The chloroform layer was collected and evaporated on a water bath. The result was calculated by subtracting the final weight and initial weight and expressed in percentage (Quality control methods for herbal plant material 1998 WHO Library Cataloguing-in- Publication Data Organization. ISBN 978 92 4 150073 9).

2.12. Limit test for heavy metals by using ICP-MS (Inductive coupled mass spectroscopy)

The test for heavy metals is designed to determine the content of metallic impurities, the limit for heavy metals is indicated in the individual monograph in terms of parts of per million of substance (by weight). (Shanthi. A *et al*., 2010). The elemental analysis was executed by using PerkinElmer NexION 300 (NexION® 300).

The quantification of heavy metals like arsenic, cadmium, Lead, copper, lithium, molybdenum etc., were determined using ICPMS and the results were expressed in ppm.

2.13. Determination of Aflatoxins HPLC (Supleco Aflatoxin Mix kit-M method)

A reversed-phase HPLC method with fluorescence detector was used for the determination of the aflatoxins B1, B2, G1 and G2.The Supleco Aflatoxin Mix kit-M method was used for the quantification with kit based instructions. The chromatographic conditions were maintained during the analysis tabulated as follows in the Table 1.

2.13.1. Chromatographic conditions

Table1: Chromatographic conditions

Column	C18 thermo, 250 x 4.6 mm particle size - 5µ or equivalent
Mobile phase	Purified water: Methanol: Acetonitrile (63: 22: 15) v/v/v Add 119 mg of potassium bromide and 350 µl of dilute nitric acid (4M nitric acid) per liter of mobile phase.
Flow rate	1 ml/minute
Run time	40 minutes
Column Temperature	27 ± 20C
Detector	Fluorescence detector Excitation – 365 nm Emission – 435 nm
Post column	Photochemical reactor (PHRED)
Derivatization	1. Reactor unit with one 254 nm low pressure mercury UV bulb

Table 2: Chromatographic conditions

	(Minimum 8 W)
	Polished support plate
	Knitted reactor coil; polytetrafluoroethylene tubing knitted tightly around the UV bulb, length – 25 m, internal diameter
	0.25 mm, nominal void volume 1.25 ml; 4. exposure time - 2 minutes;
Elution order	G2, G1, B2 and B1
Retention time	G2 About 13 minutes
	G1 About 16 minutes B2 About 19 minutes B1 About 23 minutes

2.13.2. Chromatographic procedure

Weighed about 25 g of sample and 5 g of sodium chloride into a blender jar with 100 ml of extraction solvent. Blend the mixture at high speed for 1 minute and pipette 10 ml of filtrate into 50 ml graduated cylinder. Add 40

ml of purified water to the cylinder and mix. Filter the contents of the graduated cylinder through a glass fiber filter into a 150 ml beaker. This filtrate will be used for the aflatest column. Supelco Aflatoxin Mix kit-M standard was used for the analysis. After the stabilization of the instrument with the above mentioned mobile phase, inject 20 µl of standard solution and sample solution into the column of the HPLC instrument and record the chromatogram for about 40 minutes. The result was calculated based on the area under the curve of peak in the sample solution corresponding to standard, expressed in ppb. The Agilent HPLC 1200 series instrument with Ezchrome Elite software was used for the analysis. (Supelco Aflatoxin Mix kit-M kit methodGhali. R *et al*., 2009 Noreen Mahoney *et al*., 2010).

2.14. HPTLC Characterization of *Hemidesmus indicus* L R. Br

For the characterization of the *Hemidismus Indicus* all solvents and reagents used during chromatographic studies were of HPLC grade, supplied by Merck (Germany). Isovanillin Rutin and Coumarin was procured from Sigma-Aldrich, USA. The purity of standard was 99%.The complete HPTLC characterization *Hemidismus Indicus* A CAMAG TLC system comprising of a Linomat-5 applicator and CAMAG TLC scanner and single pan balance of Shimadzu model was used, for the study. The stationary phase was used here was
Pre coated thin layer silica plate 60 F254, 10 x 10 cm, E-Merck. The mobile phase was Butanol: Acetic acid: Water (4: 1: 1) spotted with concentration of 10 µl.
2g of finely powdered sample was taken in a in a 250 ml flat bottomed flask with 10 ml of methanol and reflux in water bath at 800C ± 20C for 30 minutes. The filtered solution was spotted and developed in the Camag development tank (10 x 10 cm).The developed plate was dipped in the derivati zing reagent vanillin sulphuric acid reagent and heat the plate at 1100C ± 50C for about 10 minutes in oven and visualize under white light. The image of the plate to be captured under UV 254 nm and 366 nm before dipping and at white light after dipping

3. Results

The preliminary qualitative phytochemical screening of *Hemidismus Indicus* root showed the presence of proteins, carbohydrates, saponins bitters tannins and phenolic compounds, flavonoids, amino acids with different chemicals and reagent test. So, different groups of phyto constituents are estimated in the dried root powder.

Table 3: Qalitative analysis of *Hemidismus Indicus*

Test	Sample Hydro alcoholic extract of 65:35	Reagent	Observation	Remarks
Flavonoids	2ml of extracted sample	Alkaline reagent	Yellow colour to colorless	Positive
Phenolic compounds	2ml of extracted sample	Alcoholic Ferric chloride	Bluish green	Positive
Amino acids	2ml of extracted sample	Ninhydrin reagent	Violet colour	Positive
Proteins	2ml of extracted sample	Conc. nitric acid and 40% Na OH	Orange colour	Positive
Carbohydrates	2ml of extracted sample	Barcode's reagent	Reddish brown PPT	Positive
Reducing sugars	2ml of extracted sample	Benedict's solution	Red precipitate	Positive
Terpenoids	2ml of extracted sample	Chloroform and Con. sulphuric acid	Reddish brown	Positive
Lignin	2ml of extracted sample	Safranine solution Pink colour	Pink colour	Positive

- The total ash content in the root found to be 3.81% w/w Water-soluble ash was 2.90% w/w Acid-insoluble 0.56% w/w.
- Loss on drying was found to be less than 5% in both the methods (Loss on drying by Oven method 3.56% w/w and Loss on drying by Halogen moisture 4.46%w/w).
- The total tannin contents in the dried whole plant powder was found 1.131% w/w and total flavonoids content in root was found to be 0.1w/w% and in leaves it was around 0.5%w/w.
- The total saponin content of the *Hemidesmus indicus* root was found to be about 0.76% w/w. The total bitter content in the dried root powder was found 3.80% w/w, the alkaloidal content in the dried whole plant powder drug was found 0.11% and 0.056% in root powder by gravimetric method.
- The result obtained from the HPTLC studies showed the presence of the active constituent 2-hydroxy-4-methoxybenzoic acid, Rutin, and Coumarin in the methanolic extract. The Rf values obtained were 0.57, 0.87, 0.88, and 0.91,(Fig.2.Fig.3. and Fig.4) the HPTLC 2D
- densitometric values states that the root part contains many active constituent, many peaks were detected at 254,366 nanometer (Fig.2 and Fig. and white light (Fig.4). After the derivatization in day light rutin peak was observed very clearly at the Rf of 0.57. In day light (after spraying), Fig.4 one major dark brown spot at an Rf 0.2 to 0.3 was observed and some greenish brown coloured spots were observed between 0.35 to 0.6 Rf and one thick violet band was observed near Rf
- of 0.9. At 254 nm Fig.2 (before spraying). Very light quenching spots are visible at Rf 0.2 to 0.9. At 366 nm Fig.3 (before spraying) fluorescence spots are visible. Shown in the Figures and densitometers. Fig.5, Fig.6, and Fig.7.This study provides a means of identification of 2-hydroxy-4-methoxybenzoic acid, Rutin, and Coumarin. The present work can be helpful to herbal industry as an important standardization parameter of *Hemidismus Indicus* roots, and especially its hydro alcoholic formulations and extracts, since H. indicus roots are a part of several Ayurvedic and marketed herbal products, as they are indicated in a broad spectrum of diseases. This work can be specifically useful for authentication of raw material of the roots and

in detection of adulteration, which will ultimately benefit the people who consume *Hemidismus Indicus* root formulations.

- The heavy metals results was found to be within the regulatory limit shown in thefig.10 arsenic was 0.3ppm, cadmium 0.04ppm, mercury 0.014ppm, lead 1.3ppm, lithium 0.9ppm and molybdenum was 0.015ppm.The results are tabulated in the fig.10.ICPMS was used for the quantification of heavy metals. ICPMS is the highly efficient and sensitive tool for the quantification.
- No aflatoxins observed from the collected plant materials the sample was analyzed with respect to the aflatoxins like Aflatoxin G2, G1 and Aflatoxin B2, B1.The HPLC method (Mix kit-M method) with fluorescence detector was used for the determination of the aflatoxins. The standard chromatogram was shown in the Figure 8. The sample chromatogram of both leaf and root are shown in Figure 9 and Figure 10. The different mobile phases are used for the optimization; consisting of acetonitrile: methanol: water (8:27:65, 17:0:83, 17:13:70, 8:20:72 and 10:20:70) were tried and finalized with the Purified water: Methanol: Acetonitrile (63: 22: 15) v/v/v.

3.1. Figures and graphical representation

HPTLC 0.14Chromatogram of *Hemidismus Indicus*

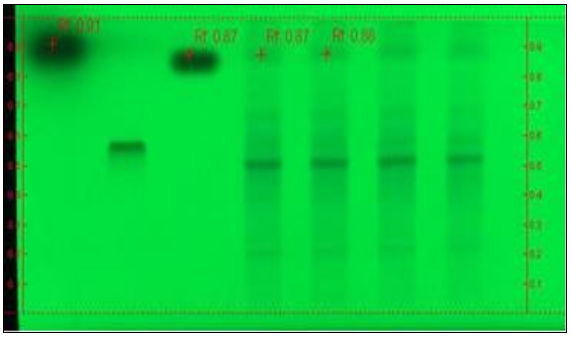


Fig 2: at 254 nm

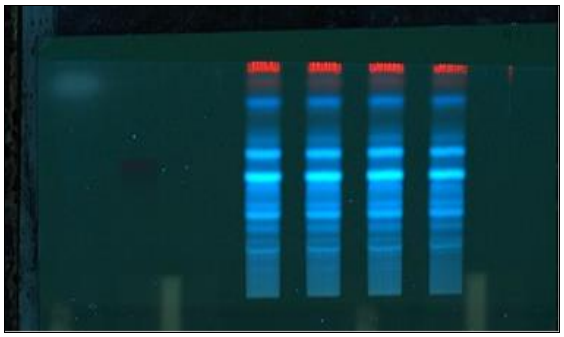


Fig 3: at 366 nm.

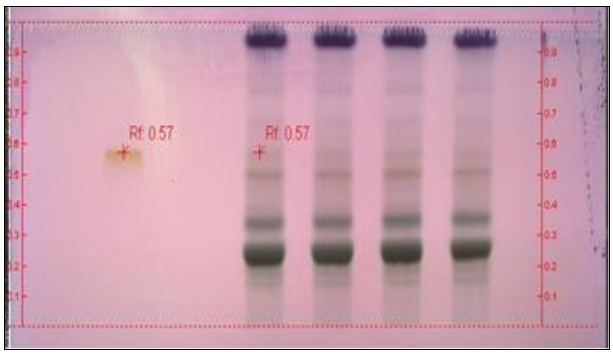


Fig 4: White light (Derivatizing agent 1% Vanillin Solution and 10% H2SO4 in methanol).

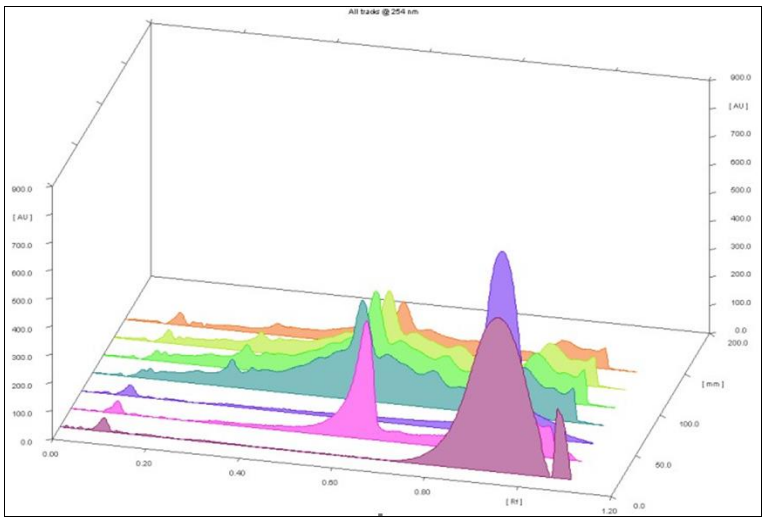


Fig 5: HPTLC 2D densitometric superimposable chromatogram of methanol extract at 254nm (Butyl Acetic acid: water 4:1:1).

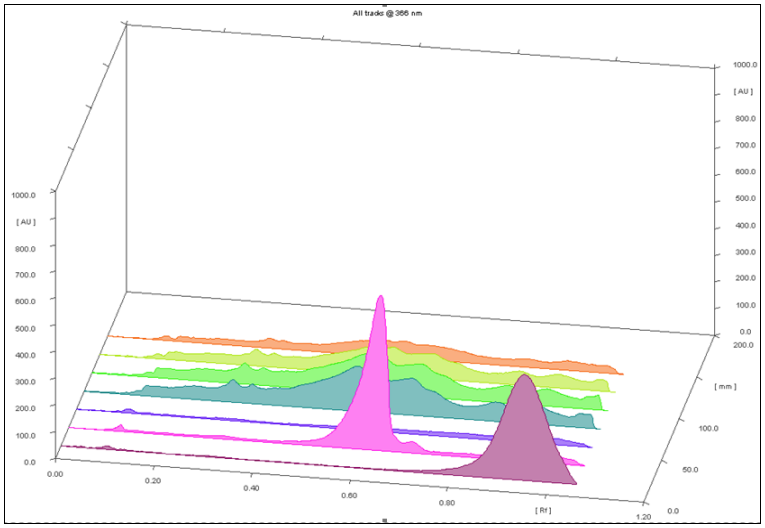


Fig 6: HPTLC 2D Densitometric superimposable chromatogram of methanol extract at 366nm (Butyl Acetic acid: water 4:1:1).

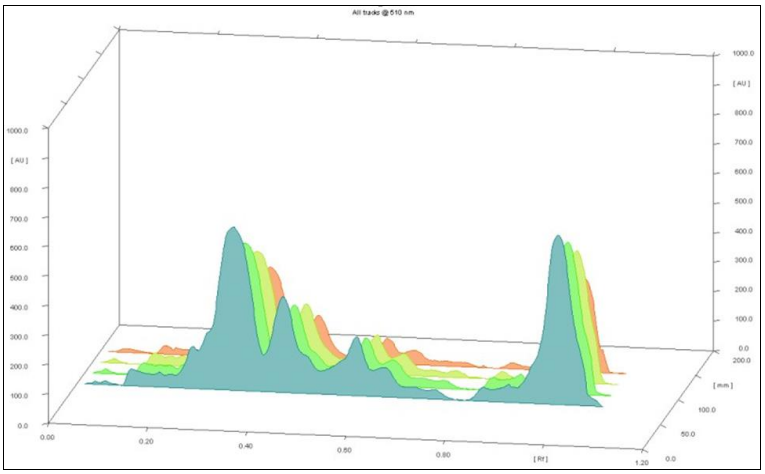
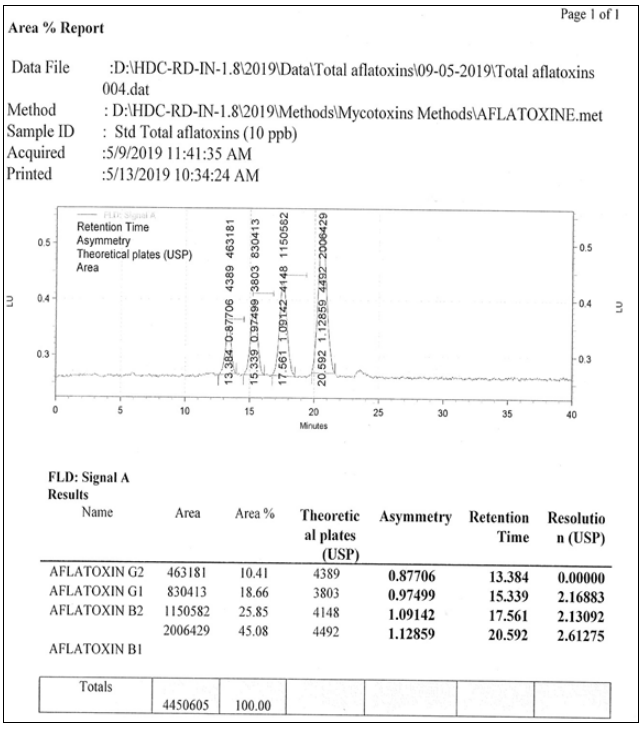


Fig 7: HPTLC 2D Densitometric superimposable chromatogram of methanol extract at White light (Butyl Acetic acid: water 4:1:1).



Page 1 of 1

Area % Report

Data File :D:\HDC-RD-IN-1.8\2019\Data\Total aflatoxins\09-05-2019\Total aflatoxins 004.dat
Method : D:\HDC-RD-IN-1.8\2019\Methods\Mycotoxins Methods\AFLATOXINE.met
Sample ID : Std Total aflatoxins (10 ppb)
Acquired :5/9/2019 11:41:35 AM
Printed :5/13/2019 10:34:24 AM

FLD: Signal A Results

Name	Area	Area %	Theoretical plates (USP)	Asymmetry	Retention Time	Resolution (USP)
AFLATOXIN G2	463181	10.41	4389	**0.87706**	**13.384**	**0.00000**
AFLATOXIN G1	830413	18.66	3803	**0.97499**	**15.339**	**2.16883**
AFLATOXIN B2	1150582	25.85	4148	**1.09142**	**17.561**	**2.13092**
AFLATOXIN B1	2006429	45.08	4492	**1.12859**	**20.592**	**2.61275**
Totals	4450605	100.00				

Fig 8: Standard Aflatoxins Chromatogram

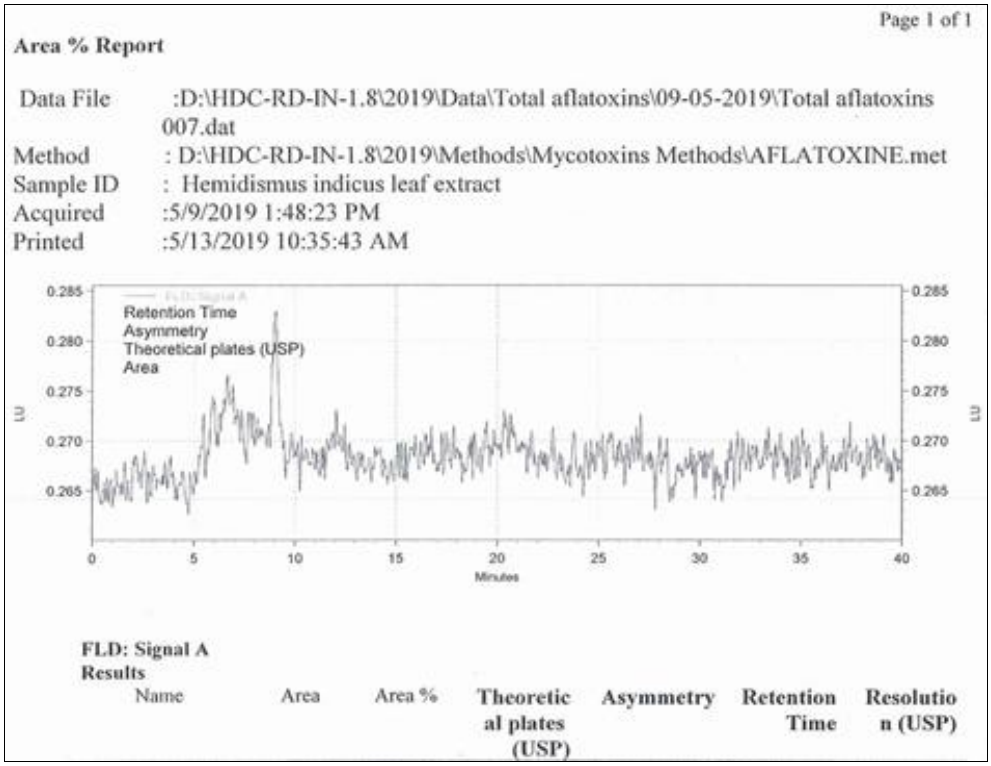


Fig 9: Sample Aflatoxins Chromatogram (Leaf)

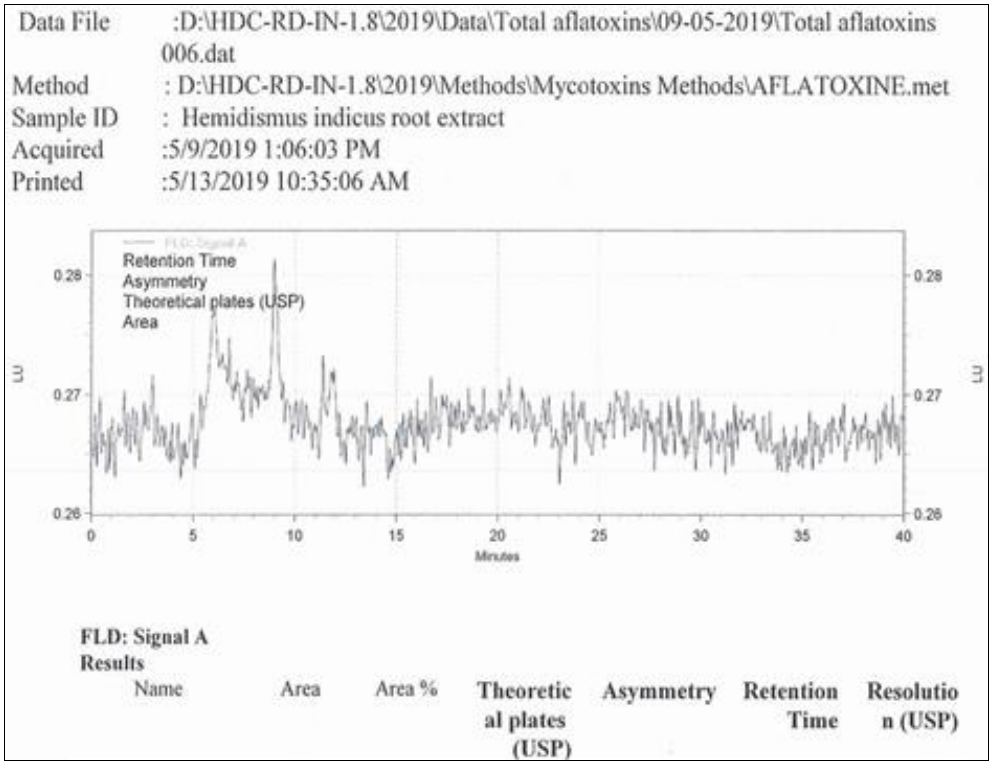
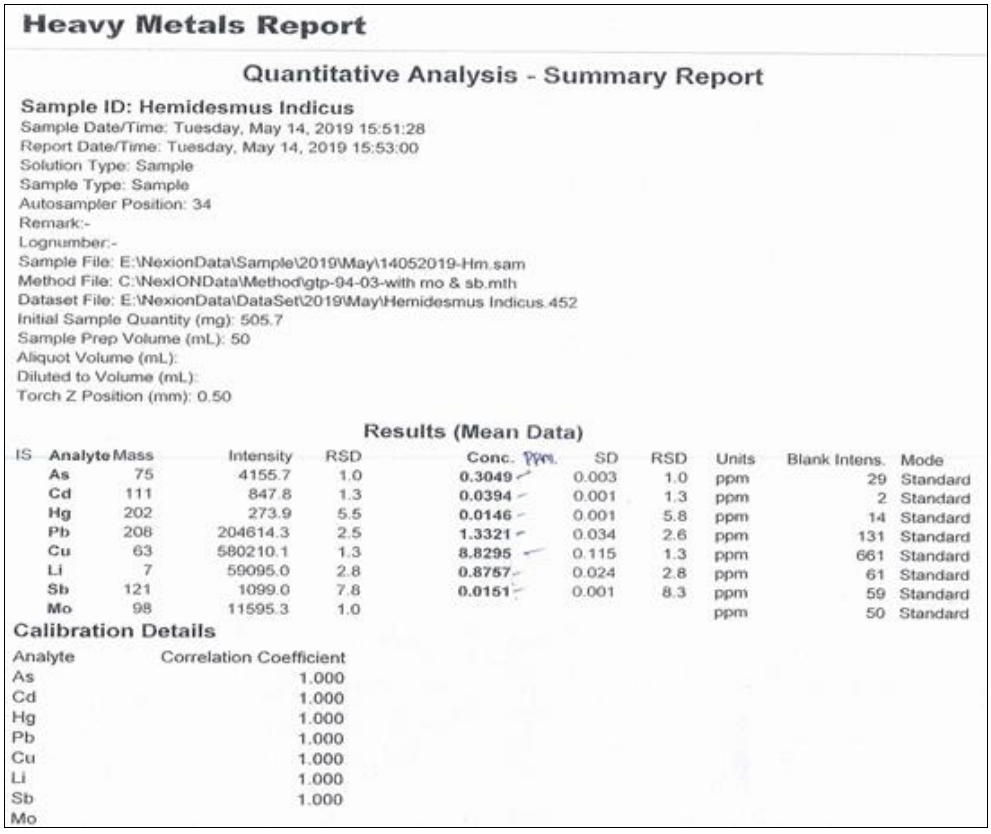


Fig 10: Sample Aflatoxins Chromatogram (Root)



Heavy Metals Report

Quantitative Analysis - Summary Report

Sample ID: Hemidesmus Indicus

Sample Date/Time: Tuesday, May 14, 2019 15:51:28
Report Date/Time: Tuesday, May 14, 2019 15:53:00
Solution Type: Sample
Sample Type: Sample
Autosampler Position: 34
Remark:-
Lognumber:-
Sample File: E:\NexionData\Sample\2019\May\14052019-Hm.sam
Method File: C:\NexIONData\Method\gtp-94-03-with mo & sb.mth
Dataset File: E:\NexionData\DataSet\2019\May\Hemidesmus Indicus.452
Initial Sample Quantity (mg): 505.7
Sample Prep Volume (mL): 50
Aliquot Volume (mL):
Diluted to Volume (mL):
Torch Z Position (mm): 0.50

Results (Mean Data)

IS	Analyte	Mass	Intensity	RSD	Conc. ppm	SD	RSD	Units	Blank Intens.	Mode
	As	75	4155.7	1.0	0.3049	0.003	1.0	ppm	29	Standard
	Cd	111	847.8	1.3	0.0394	0.001	1.3	ppm	2	Standard
	Hg	202	273.9	5.5	0.0146	0.001	5.8	ppm	14	Standard
	Pb	208	204614.3	2.5	1.3321	0.034	2.6	ppm	131	Standard
	Cu	63	580210.1	1.3	8.8295	0.115	1.3	ppm	661	Standard
	Li	7	59095.0	2.8	0.8757	0.024	2.8	ppm	61	Standard
	Sb	121	1099.0	7.8	0.0151	0.001	8.3	ppm	59	Standard
	Mo	98	11595.3	1.0				ppm	50	Standard

Calibration Details

Analyte	Correlation Coefficient
As	1.000
Cd	1.000
Hg	1.000
Pb	1.000
Cu	1.000
Li	1.000
Sb	1.000
Mo	

Fig 10: Heavy metal Report

4. Discussion

The *Hemidismus Indicus* roots are the part of several herbal drug formulations and food supplements as they are indicated as broad spectrum of diseases (Pandy Devaraj *et al*. 2018). The present work can be useful to herbal industry as an important standardization parameter of *Hemidismus Indicus* roots. This work can specifically useful for the authentication of the raw materials and the parameter can be considered as the quality control features. The precise and robust methods used in the study proven the safety and efficacy of the *Hemidismus Indicus* roots. The results are complies with the regulatory guidelines which are helpful in the commercialization of the drug and food products. The method developed can be used in regulatory studies like stability and stress studies to derive the expiration date of the herbal products.

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