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Significance of Analytical Instruments in Industrial Standardization of Herbal Formulations: A Review - *Hemidesmus indicus*

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ABSTRACT: Traditional herbal medicine has been gaining importance in the current times owing to their immense medicinal properties and non-toxic nature. Moreover, they do not cause any harmful side effects. Herbal medicine has become the current need of the hour. *Hemidesmus indicus* is a highly threatened medicinal plant having efficient clinical applications. Standardization of the herbal formulations is essential for the evaluation of its security, potency and quality. It is necessary to establish the quality as well as the safety of the herbal drug for the acceptance by the people, regulatory bodies and for it to be regarded as rational drug. This includes identification and authentication of the bioactive compounds in the herbal extract, evaluating its therapeutic value and assessing its purity which has several criteria like detection of any microbial contaminants or pesticides, adulteration heavy metals and so on. Analytical techniques played a major role in the standardization along with the authentication of the herbal formulations with respect to the regulatory and industrial guidelines. In this review, we will be focusing on how the analytical methods such as TLC, HPTLC, HPLC, LC-MS, GC-MS and ICP-MS have been used the standardization procedures for *Hemidesmus indicus*.

KEYWORDS: Herbal medicine, *Hemidesmus indicus*, standardization, quality, safety, analytical technique

I. INTRODUCTION

Hemidesmus indicus also known as Anantamool (Indian sarsaparilla) is a flora species of *Apocynaceae* family. These are found in several parts of West Bengal in India. It is a semi raised, lean and highly threatened flora. It is well-known for its therapeutic properties namely- anti-cancerous, anti-arthritis, anti-microbial, anti-ulcer, anti-venom, anti-leprotic, immunomodulatory, hepatoprotective or wound healing activity and so on. Its promising therapeutic values can bring *Hemidesmus indicus* because of its significant source of herbal medicine in India. [1]



(a)



(b)

Fig 1: *Hemidesmus indicus* (a) plant (b) dried roots



Despite the modern science has made remarkable advancements, allopathy and technology, we are unable to provide everyone with high-quality healthcare. Traditional medication predominantly herbal medication is still the mode of treatment and healthcare around the world in remote villages and rural areas.[2] Many people, especially in undeveloped and developing nations, rely on traditional/herbal medicine for their main healthcare. Traditional Indian medicinal systems such as Siddha, Ayurveda, and Unani have a long history of efficacy. The value and usefulness of such medication has been recognized by modern study. Traditional medicine or medicinal plants in India are the vital source of newly developed drug which will have no side effects. The public benefits from the mainstreaming of such medicine. In India, numerous initiatives have been taken to promote herbal medicine and integrate it into clinical practice. The evidence-based integration of Indian traditional medicine into clinical practice will help to ensure that everyone receives high-quality treatment. [3]

Medicinal plants have a vast variety of application in the field of cosmetics, pharmaceutical, agricultural and food industries. Medicinal plants are being used as raw drugs or extracts in the commercial market. Phytochemistry is making a rapid progress and the demand for herbal products is increasing. Alkaloids, flavonoids, glycosides, resins, oleoresins, sesquiterpene lactones, saponins, and oils are all found in medicinal herbs. These bioactive components have been identified and their pharmacological action has been determined.[3]. In the health-care delivery system, medicinal plants can be utilized as a single medicine or in combination. *Hemidesmus indicus* is a very rare and endangered plant of Deccan plateau of India and is a vital source of several active constituents of therapeutic value. The root of *Hemidesmus indicus* was used mainly for the preparation of a local drink called as nannari or sugandhipala. The drink has medicinal value; it cools the body, gives good appetite and acts as a blood purifier and roots are used in preparation of pickles and chutney. [4]

Modern analytical methods to study the herbal drugs and formulations are necessary for worldwide to accept the Ayurveda and traditional medicine. Ayurveda products and traditional medicine based on scientific study could be obtained by comprehensive and precise pharmacogenetic assessment. To acquire authentication and standardization on the organoleptic assessment, physicochemical scrutiny and pharmacogenetic analysis are required. The data gathered and reported through microscopic and macroscopic examinations can serve as an extra advantage for identifying adulterants and authenticating genuine herbs, thereby preventing the original herbal ingredients from becoming contaminated. [5]

The upcoming medicinal system purely depends on these alternative types of medication, specifically traditional herbal medicines which are plant-derived. Common medicinal plants which are used mainly, are- *Hemidesmus indicus*, *Zingiber officinale*, *Rauvolfia serpentina*, *Swertia chirayita*, *Cassia angustifolia*, *Acorus calamus*, *Adhatoda vasica*, *Aconitum species*, *Picrorhiza kurroa*, *Colchicum luteum*, *Rheum emodi* etc. The medications are primarily delivered as a water/ethanolic extract in the traditional medicinal system. There are also fresh plant pieces, juice, and crude powder accessible. As a result, medicinal plant components must be genuine and free of contaminants such as pesticides, heavy metals, microbial infection, and radioactivity. The active principle of the bioactive extract should be standardised. The bioactive extract should be standardized on the basis of active principle. Standardization is thus essential and analytical instruments play very significant role in the same. [1]

II. PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical analysis of aqueous extract confirmed the presence of terpenoids, flavonoids, steroids, phenolic compounds, tannins, lignin, carbohydrates, proteins, alkaloids, saponins along with cardiac glycosides were found to be absent. Cardiac glycosides and inulin were also present in aqueous extracts and alcoholic with other components.[6]

Table 1: Qualitative phytochemical assessment of *Hemidesmus indicus* root extracts [6]

Serial No:	Test	Reagent	Observation	Aqueous extract	Alcoholic extract
1	Alkaloids	Mayer's reagent	Cream precipitate	+	-
2	Steroids	Conc. Sulphuric acid	Reddish brown precipitate	+	+
3	Terpenoids	Chloroform+conc. Sulphuric acid	Reddish brown interface	+	-
4	Flavonoids	Alkaline reagent	Yellow colour to colourless	+	+
5	Saponins	Water+ shake	Foam formation	-	-
6	Phenolic compounds	Alcohol+ Ferric chloride	Bluish green	+	+
7	Tannins	Lead acetate	White ppt	+	+
8	Lignin	Safranine solution	Pink colour	+	+
9	Phlobatannins	1%Aqueous hydrochloric acid	Reddish colour precipitate	-	-
10	Fat and oil	1%Coppersulphate + 10%sodium hydroxide	Clear blue solution	-	-
11	Inulin	Alpha naphthol+ sulphuric acid	Brownish red colour	-	+
12	Cardiac glycosides	Glacial acetic acid+ FeCl ₃ + Conc. Sulphuric acid	Brown ring at the interface	-	+
13	Proteins	Conc. Nitric acid +40% NaOH	Orange colour	+	+
14	Carbohydrates	Barfoed's reagent	Reddish brown ppt	+	+
15	Amino acids	Ninhydrin reagent	Violet colour	+	+
16	Reducing sugars	Benedict's solution	Red precipitate	+	+

III. NEED OF STANDARDIZATION OF HERBAL DRUG

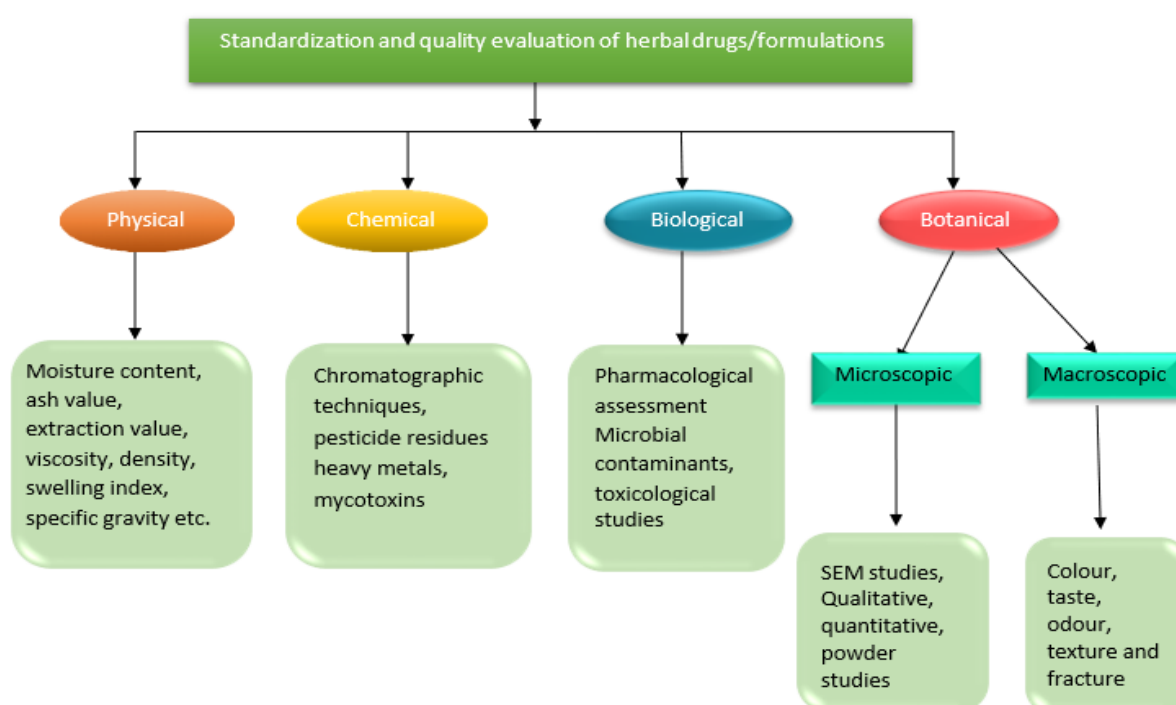
Modern system of medicines is based on thorough analytical and experimental data, human clinical analysis and toxicity analysis. There are no pharmacopeial standards for raw materials or finished products. Neither is the cGMP for herbal sector is well-defined nor are the basic minimum criteria of medicinal plant products maintained or monitored. Hence, lack of quality control has resulted in mild to severe side effects ranging from liver damage to death. Thus, herbal ingredients need tools and techniques helps to get the information of the identity, quality, and purity and tools have to be technically rapid, satisfactory, and economical along with GMP requirements. Specific guidelines have been set by WHO for the study of security, quality, and efficacy of herbal drugs. Standardization of herbal drugs is difficult due to the influence of numerous factors on bio effectiveness. [7,8]

WHO Guidelines for characteristic standardization of herbal Formulations

- Standard control of raw drugs material, shrubs preparations and finished products. [9]
- Stability analysis and shelf life. [10]
- Safety analysis; documentation of safety which are based on experience along with toxicological studies. [11]
- Analysis of efficacy by ethnomedical information with biological activity evaluations. [12]

The bioactive extract is classified on the basis of active principles and major compounds along with the chromatographic fingerprints such as TLC, GC, HPTLC, and HPLC. Safety on the usage of drugs is closely attached with the purity and it also deals with the factors such as heavy metals, ash values, and contaminants (e.g., foreign matter in the form of other herbs). The application of improved and enhanced analytical methods have played a major role in the standardization process. Modern purity examination also includes radioactivity, microbial contamination aflatoxins, and pesticide residues. [13]

IV. METHODS OF HERBAL STANDARDIZATION



In this review, we will be focusing on the analytical techniques used in the standardization and characterization process. Modern analytical methods used in the standardization procedure include high-performance thin layer chromatography i.e., HPTLC, gas chromatography i.e., GC, high performance liquid chromatography i.e., HPLC, and hyphenated techniques such as liquid chromatography-mass spectroscopy i.e., LC-MS, liquid chromatography-nuclear magnetic resonance spectroscopy that is LC-NMR and gas chromatography-mass spectroscopy (GC-MS). Basically, they're used to determine the quality of herbal items. HPTLC and HPLC fingerprints are useful tools for identifying marker chemicals in phytoconstituents and for quality control in the creation of herbal medicines. Application and developments of analytical techniques aid in rapid analysis of herbal formulations in herbal sectors as well as help to maintain the therapeutic safety along with efficacy of Ayurvedic medications. [14]

Thin layer chromatography (TLC) is a simple fingerprinting method for identifying the phytoconstituents present in pharmaceuticals, which aids in the identification of distinct plant species. HPTLC is a popular modern analytical technique for analyzing low or moderately polar substances. This approach is commonly used in the pharmaceutical industry to identify and detect adulterants and substitutes in Ayurvedic medicines. Isolation, purification, and quantification of phytoconstituents in herbal formulations are accomplished using preparative and analytical HPLC procedures. Better resolution, sensitivity, and quick analysis are all important factors in HPLC analysis. The most powerful tool for quality control of Chinese herbal medicine is the combination of HPLC and MS. Because of its high separation efficiency and sensitive detection, gas chromatography is used to characterize volatile substances. GC- MS analysis is used to identify and quantify the compounds found in essential oils. Another analytical technique for determining medicine quality is liquid chromatography-mass spectroscopy (LC-MS).[15]

V. THIN LAYER CHROMATOGRAPHY (TLC)

TLC was the most popular and practical approach for herbal analysis before the development of instrumental chromatography methods such as gas chromatography and HPLC. TLC is still used for herbal medicine analysis nowadays. The solute is distributed between two phases in this technique: a stationary phase that operates through adsorption and a mobile phase that takes the shape of a liquid. Partition or a mix of partition and adsorptions can be used to produce separation. More than 30 sample places can be analyzed per plate.

In a study done by Saryam et al where TLC was used for characterization of *Hemidesmus indicus*, TLC analysis was performed on ethanol and aqueous extracts. The extracts were created up to 10ml in various test tubes with ethanol and distilled water, then spotted in preparative TLC plates coated with silica gel G in a capillary tube. The plates were grown in a saturated chamber with several solvent solutions. Various spots produced in each solvent solution were recognized using UV light at a maximum wavelength of 254 nm, and the R_f value was calculated. The retention factors (R_f) of ethanol and aqueous extracts in various solvent systems are shown in the table below. [16]

Table 2: TLC profile of ethanol and aqueous extract of *Hemidesmus indicus* root [16]

Extract	Solvent	No: of spots	R _f value
Ethanol extract	Ethyl acetate: Methanol (4:6)	3	0.78; 0.71; 0.38
Aqueous extract	Hexane: dichloromethane: methanol (2:3:5)	2	0.75; 0.51

TLC investigations revealed the existence of the active ingredient hemidesmin in the extract in another work by Shanthy A et al (2010), with identical r_f values (nm). The R_f values were 0.04, 0.14, 0.16, 0.26, 0.35, 0.52, 0.7, 0.78, 0.88, indicating that the root portion includes nine active ingredients. [17]

Fig 2: TLC finger print of *hemidesmus indicus* root extract [17]

VI. HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC fingerprinting profile is a major quality control aspect in herbal medicine and Siddha to establish the pharmacopeial standards. The formulations' phytochemical components may be identified, and efficacy, safety, and quality can all be guaranteed. In the studies conducted by Manasi et al (2017), a sample and convenient HPTLC method was developed for standardization of *Hemidesmus indicus* (Root). Silica gel 60F254 Aluminium plates (Merck) was used as stationary phase. Soxhlet extraction was done for the dried plant roots by using different solvents with gradually increasing the polarity of the solvents starting from pet ether, ethyl acetate, methanol, water for 6 hours and extracts were filtered and concentrated and then taken for following HPTLC profiles. [18]

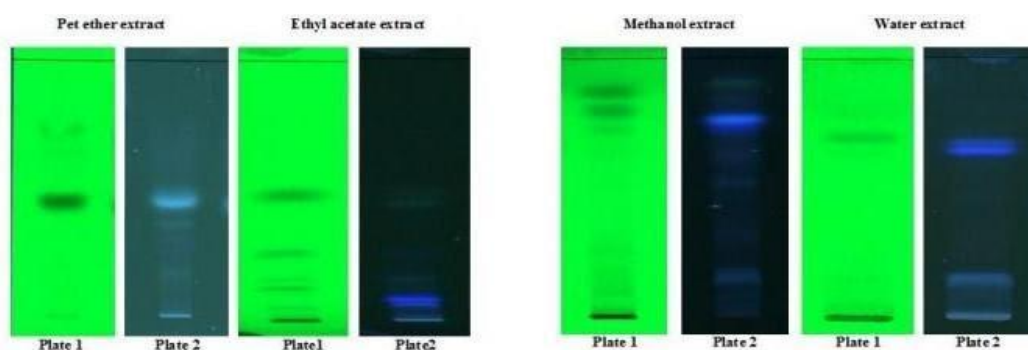


Fig 3: Photography of HPTLC plates at different wave length [18] Plate 1 (Observed at 254 nm) and Plate 2 (Observed at 366 nm)

In the study mentioned above, four different solvent extracts were used starting from less polar to more polar that is, polarity of Pet ether extract < Ethyl acetate extract < Methanol extract < Aqueous extract. By phytochemical analysis and HPTLC screening, it was found that a large number of secondary metabolites are present in methanol extract rather than other three extracts. In HPTLC study it was found that number of R_f values are maximum in methanol extract. We can conclude that the biological potential of methanol extract is more than other extracts because of the presence of large number of secondary metabolites in it. As the HPTLC profiling of methanolic extract of *Hemidesmus indicus* (roots) was found to show maximum no. of R_f values indicating that maximum number of phytoconstituents are present in this extract, in near future, researcher can isolate a large number of bioactive compounds from the methanolic extract through column chromatography. [18]

In another study done by Parita et al, The Department of Pharmaceutical Sciences at Saurashtra University used HPTLC to fingerprint a methanolic extract of *Hemidesmus indicus* root. Because it yielded the most acceptable TLC fingerprint, the mobile phase employed was Chloroform: Methanol: Ethyl acetate (13:1:2). Stationary phase used was silica gel 60 F 254 (E. Merck KGaA) For HPTLC fingerprinting, four tracks of the same extract were run at varying concentrations and scanned under visible light, UV 254nm, and UV 366nm. 9 peaks were observed at 254nm (Table 3, Fig. 4,5) and 4 peaks were detected at 366nm (Table 4, Fig. 6, 7) [19]

Table 3: Rf values at 254 nm

Peak	Rf	Area under the curve	Area%
1	0.03	22380.8	58.02
2	0.09	3053.2	7.92
3	0.13	1290.1	946
4	0.21	1290.1	3.34
5	0.9	575.6	1.49
6	0.38	979.8	2.54
7	0.68	1188.5	3.08
8	0.75	4766.1	12.36
9	0.86	689.2	1.79

Table 4: Rf values at 366 nm

Peak	Rf	Area under the curve	Area%
1	0.03	14221.9	87.85
2	0.11	701.3	4.33
3	0.13	470.2	2.90
4	0.79	796.0	4.92

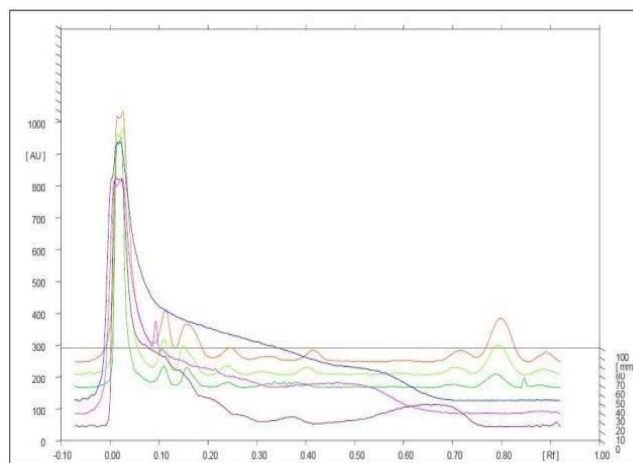


Fig 4: HPTLC 2D densitometric superimposable chromatogram of methanol extract at 254 nm (Chloroform: Methanol: Ethyl acetate (13:1:2))

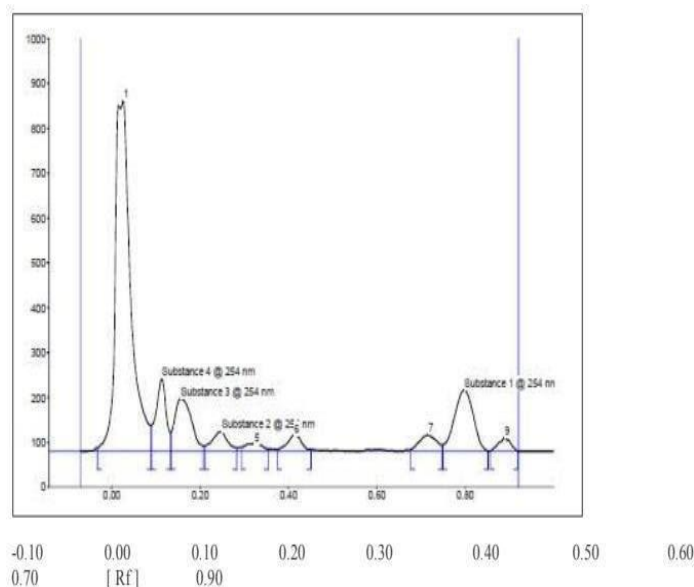


Fig 5: HPTLC chromatogram of methanol extract at 254 nm (Chloroform: Methanol: Ethyl acetate (13:1:2))

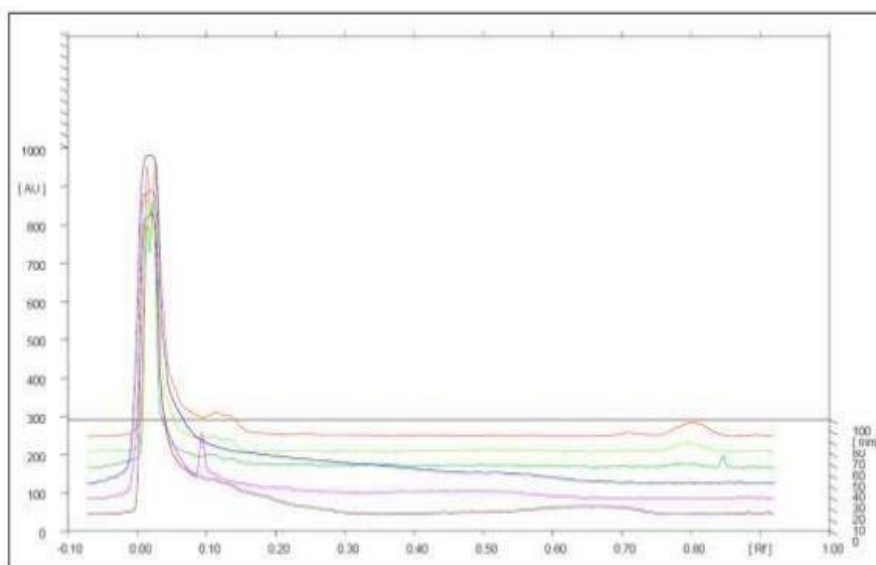


Fig 6: HPTLC 2Ddensitometric superimposable chromatogram of methanol extract at 366 nm (Chloroform: Methanol: Ethyl acetate (13:1:2))

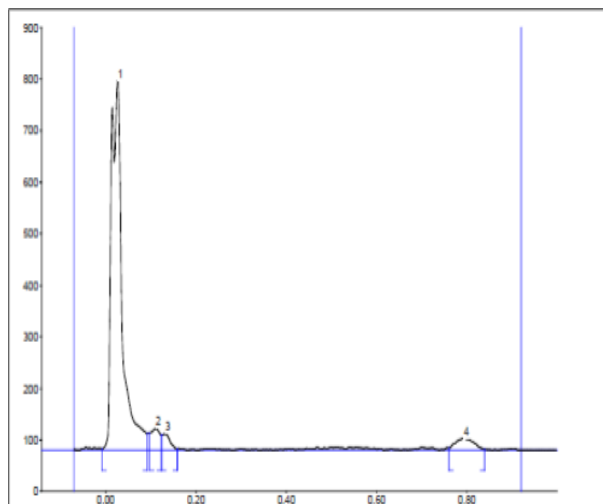


Fig 7: HPTLC chromatogram of methanol extract at 366 nm (Chloroform: Methanol: Ethyl acetate (13:1:2))

The retention factor (R_f) values obtained from different extracts can be used to identify each compound which is due to their uniqueness. This study serves as a ready reference for authentication of *Hemidesmus indicus* (roots). This is through macroscopic study, microscopic study and also it gives indication about the secondary metabolite's presences such as flavonoids, alkaloids, tannins, carbohydrates, terpenoids, steroids, polyphenols and saponins in different solvent extracts. The presence of many secondary metabolites proves that the roots of *Hemidesmus indicus* can be used to treat and prevent several diseases.

This research will aid in the identification of raw root material and the detection of adulteration, ultimately benefiting those who ingest *Hemidesmus indicus* root formulations. [18,19]

VII. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The chemical components of herbs and traditional medicinal preparations can be assessed using the HPLC methodology. It is possible to carry out quality assurance. As quantifications of numerous constituents from herbal medical formulations have challenges related with safety and efficacy of the active elements, the standardization methodology with the marker profile is of higher advantage for the identification of phytoconstituents in herbal goods.

The presence of active chemical and biological indicators in complicated traditional herbal extracts and formulations can be determined using HPLC. It is beneficial for standardization with both qualitative and quantitative assessment. HPLC methodology for the examination of the thermo-labile compounds contained in traditional herbal formulations with advances of isocratic and gradient elution is one of the most popular methods of analysis. RP-HPLC (reverse phase-high-performance liquid chromatography) may also be used to analyze multi-component medicinal herbal items, which is more efficient. In the identification of numerous ingredients in herbal medicines, HPLC and RP-HPLC provide great repeatability and ease of automation.

In the study done by Ashalatha et al (2019), The peak was detected at 3.936 minutes in the chromatogram of the 2H4MB standard compound. The peaks of the methanolic root extract show a retention time of 3.891 and the root oil shows a retention time of 3.861, respectively. [20]

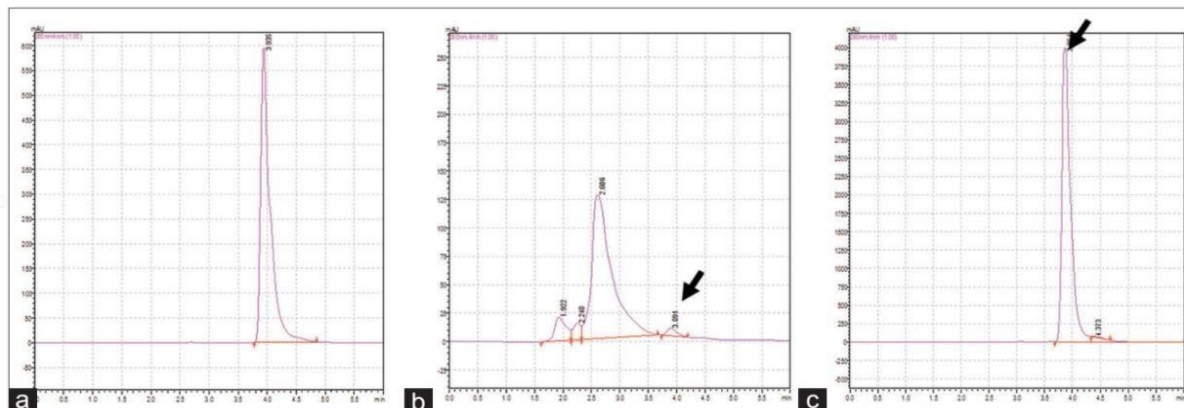


Fig 8: High-performance liquid chromatography assessment result of (a) standard 2-hydroxy 4-methoxy benzaldehyde and (b) methanolic root extract (c) root oil of *Hemidesmus indicus* [20]

VIII. LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY (LC-MS)

Structure characterization, molecular mass determination, fragmentation information, retention time, and a broad range of detection and excellent separation of analytical chemicals are all capabilities of LC-MS.

The complete recording of the data required for online qualitative analysis of the herbal extract can be achieved by executing the LC fingerprinting method. The hyphenated LC-MS methodology is used when individual structural elucidation of chemical elements of the herbal extract is not possible with the HPLC analytical approach. The LC-MS technology can be used to identify chromatographic peaks and conduct an online comparative research.

Furthermore, thanks to the helpful LC-MS technology used for standardization, detecting adulterants in extracts and herbal products, as well as phytochemical evaluation of herbs, has become easier. It is possible to identify the separation and identification procedure of structurally similar molecules. Among the several tools for the analysis of various herbs and adulterants, LC-MS is one of the most excellent qualitative tools. With the advancements in the LC-MS methodology, the detection and identification of novel analogue adulterants (both unknown and known) can also be detected and recognized by using it in the quality control of extract samples. In the LC-MS analytical technique, UHPLC is one of the more advanced varieties. Complex herbal extracts can be analyzed with great resolution and sensitivity using UHPLC-Q-TOF/MS (ultra-high-performance liquid chromatography coupled with electrospray ionization tandem quadrupole-time of flight/mass spectroscopy). [21,22,23,24]

In a study by Joshi et al (2018), The methanolic extract of *Hemidesmus indicus* roots was subjected to LC-MS analysis. 5 microliters of methanolic extract were used for the chromatographic separation. The sample was injected with needle wash onto the UHPLC system. [25]

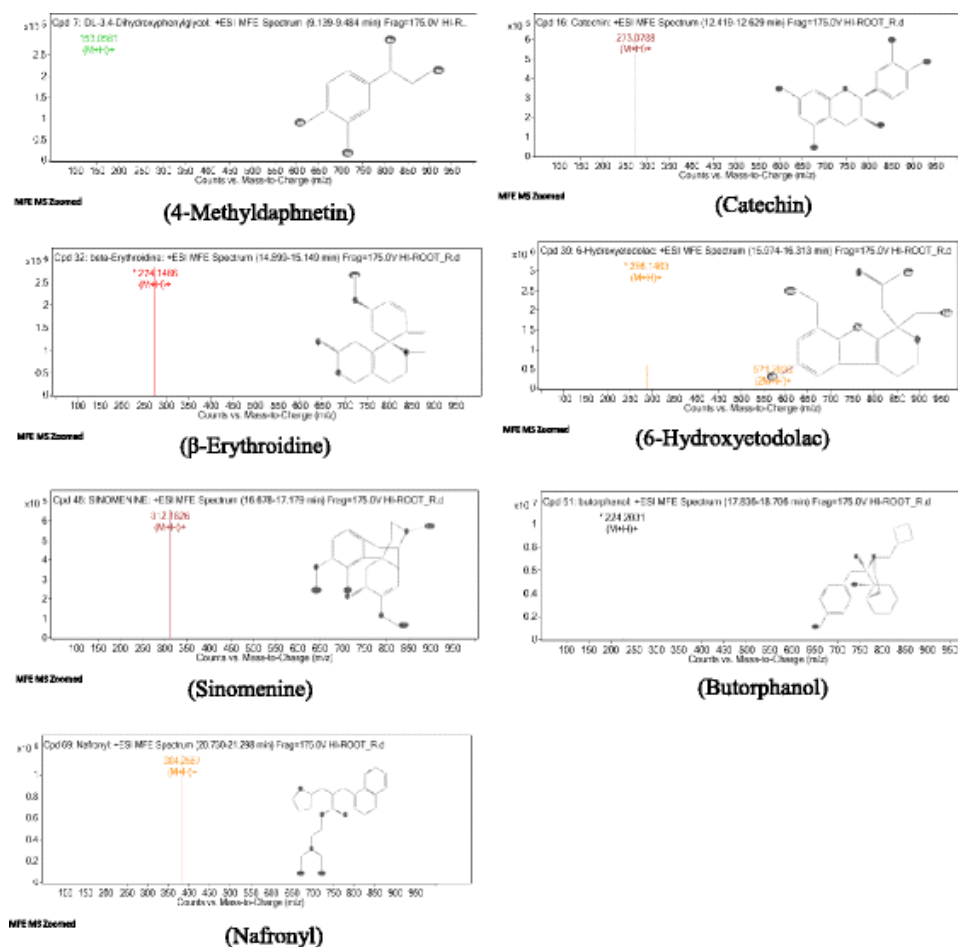


Fig 9: MFE MS spectrum of some compounds of HIE detected by LC-MS

LC-MS examination of the phytochemical composition of *H.indicus* roots revealed the presence of phenols, flavonoids, terpenoids, and around 40 distinct phytoconstituents. The flavonoid and triterpenoid content of a methanolic extract of *H.indicus* roots was found to be higher than the phenolic content. This contributes to the antioxidant capacity of the extract. The presence of phenolic compounds and flavonoids was discovered using LC-MS and putative compound identification such as catechin, DL-3,4-dihydroxyphenyl glycol, amiloxate (cinnamic acid derivative); 4-methyldaphnetin (coumarin), terpenoid such as punctaporin B and podocarpatriene derivative. Several alkaloids were found and identified, including ecgonine, homotropine, butorphanol, beta-erythroidine, and securinine, as well as phytosterols, lactones, prostaglandins, lipids, amino acids, and fatty acids. [25]

IX. GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

GC-MS is a combination of gas chromatography (GC) and MS (Mass spectroscopy). GC separates the various compounds in a mixture while the MS analyses the constituents that are separated by GC. To identify and analyze the chief compound in an herbal extract, GC-MS can be used. It is also used in the cosmetic industry, pharmaceutical industry and forensic science for compound analysis.

The root extract methanolic of broad-leafed *H. indicus* had shown the presence of a variety of compounds as compared to that of published in earlier literature of *H. indicus*. The relative concentrations of distinct chemicals being eluted as a function of retention time were characterized by the gas chromatogram obtained following GC-MS analysis. The peak

heights revealed the relative amounts of various compounds present in the methanolic extract, while the mass spectrometer analyzed the chemicals and determined their type and structure depending on the time of elution

A total of 40 substances were discovered in the GC-MS analysis (figure 10) and the compounds in the extract were identified based on the peak area, which denoted the proportion of that particular molecule deduced from the chromatogram and molecular weight of the compound.

In the GC-MS analysis, a total of 40 compounds were detected (figure 10) and the compounds in the extract were recognized based on the peak area, which denoted the percentage of that particular chemical deciphered from the chromatogram and the molecular weight of the chemical. The primary volatile component, 2-hydroxy-4-methoxy benzaldehyde (peak area 11.071 percent), was discovered to be present in abundance among all the components, creating the primary entity. MBALD could be used in a variety of industries, including medicines, food, antimicrobial formulations, and so on, due to its high concentration.

The most distinguished analysis carried out by GC-MS is the assessment of thermo-stable volatile compounds and the volatile derivatives. Even though it is more sensitive than GC-MS, LC-MS can only analyze thermally unstable volatile molecules and not thermostable volatile chemicals. The GC-MS analytical technique can be used to evaluate volatile oil in both qualitative and quantitative ways. Furthermore, it is capable of identifying multiple parts of the drug metabolites. Simultaneous analysis of different compounds can also be carried out [26]

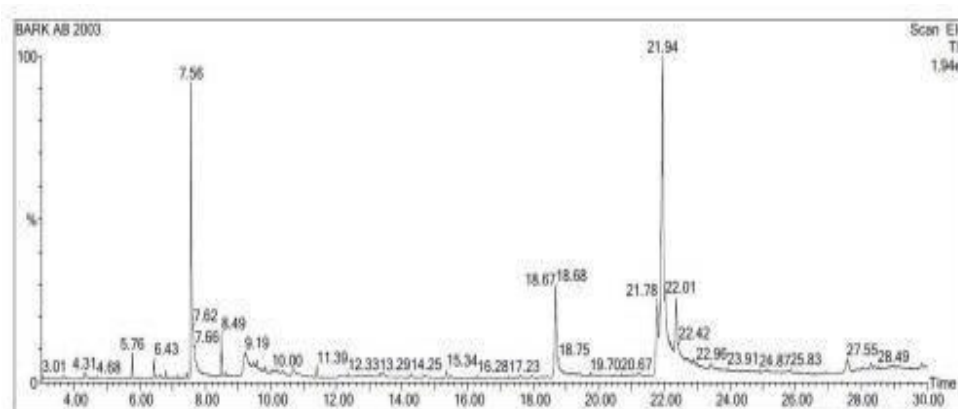
Table 5: Chemical constituents present in the methanolic root extract of broad-leafed *Hemidesmus indicus* [26]

Peak	Retention time	Area	Area%	Compound
1	3.213	6,992,248.5	0.864	Decane, 4-methyl
2	3.669	3,183,574.0	0.393	Undecane
3	3.809	3,117,720.5	0.385	Melezitose
4	4.139	3,276,007.5	0.405	Undecane
5	5.764	4,431,423.5	0.547	Dodecane
6	6.435	3,287,432.5	0.406	Benzene, 1, 3-bis(1,1-dimethylethyl)-
7	7.555	89,619,072.0	11.071	Benzaldehyde, 2-hydroxy-4-methoxy-
8	7.885	2,587,966.5	0.320	Arsine, oxophenyl-
9	8.486	6,022,166.0	0.744	Tetradecane
10	9.201	25,824,714.0	3.190	Guanosine
11	9.456	9,164,683.0	1.132	4-Methyl(trimethylene)silyoxyoctane
12	9.566	6,292,480.5	0.777	Tridecane, 2-methyl-
13	9.671	2,534,709.0	0.313	Dodecane, 5,8-diethyl-



14	9.746	2,271,470.5	0.281	Undecane, 6-ethyl-
15	9.821	5,062,362.5	0.625	2,4-Di-tert-butylphenol
16	10.026	3,298,825.8	0.408	Methyl-4-O-acetyl-2,3,6-tri-O-ethyl-à-d- galactopyranoside
17	10.191	2,665,982.8	0.319	Dodecane, 2,6,10-trimethyl-
18	10.366	2,400,660.5	0.297	Benzaldehyde, 3,4-dimethoxy-, methylmonoacetal
19	10.676	4,810,796.5	0.594	2H-1-Benzopyran-3,4-diol,2-(3,4- dimethoxyphenyl)3,4-dihydro-6-methyl-, (2à,3à,4à)-
20	11.397	2,413,610.8	0.298	Hexadecane
21	18.680	41,335,456.0	5.106	n-Hexadecanoic acid
22	18.875	3,974,250.0	0.491	Octadecanoic acid, 4-hydroxybutyl ester
23	21.776	35,683,764.0	4.408	9,12-Octadecadienoic acid (Z,Z)-
24	21.941	222,793,568.0	27.522	9-Octadecenoic acid, (E)-
25	22.351	83,508,704.0	10.316	Octadecanoic acid
26	22.891	27,773,410.0	3.431	Trans-13-Octadecenoic acid
27	23.401	19,335,120.0	2.388	9-12-Octadecadienoic acid (Z,Z)-
28	23.806	7,194,610.5	0.889	Chromone, 5-hydroxy-6,7,8-trimethoxy- 2,3dimethyl-
29	24.047	2,900,577.2	0.358	17-Pentatriacontene
30	24.162	4,038,792.5	0.499	Cyclopropaneoctanoic acid, 2-(2- pentylcyclopropyl, methyl)-, methyl-ester
31	24.292	2,521,489.8	0.311	Dasycarpidan-1-methanol, acetate (ester)
32	24.532	2,842,063.0	0.351	2,3-Dihydroxypropyl elaidate
33	24.692	2,906,400.5	0.359	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
34	24.832	3,326,499.8	0.411	10-Acetonxy-2-hydroxy-1,2,6a,6b,9,9,12a-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4acarboxylicacid. Methyl ester

35	25.137	6,330,487.0	0.782	Oxiraneoctanoic acid, 3-octyl-,cis-
36	25.277	2,491,672.5	0.308	4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-3(2-hydroxyethyl)-1H-indol-2-yl)- α -methyl-,methyl ester
37	25.782	3,111,850.0	0.384	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
38	27.583	8,563,851.0	1.058	Squalene
39	28.288	3,233,305.0	0.399	Diisooctyl phthalate
40	28.373	2,643,616.8	0.327	Oleyl oleate

Fig 9: Chromatogram (GC/MS) of the methanolic extract of *Hemidesmus indicus* [26]

In the analysis, the retention period for the standard 2-hydroxy-4-methoxy benzaldehyde at 10 ppm as 54.400 min. at 280 nm.

X. INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

Medicinal herbs are utilized all over the world to treat a variety of diseases. The beneficial ingredients in these plants, such as alkaloids, tannins, flavonoids, and phenolic compounds, make them more appealing to be ingested and have garnered people's contentment as cures.

They are exposed to heavy metal contamination through the land, water, and air. Heavy metals are likely to be digested by plants since they take up water from the soil and accumulate in plant tissue. When ingested, it causes a number of chronic disorders. As a result, the analytical method is required to confirm that the herbs are free of heavy metal or contain a safe or allowed limit of heavy metal for human consumption. The primary approach for analyzing multi-elements in a liquid or aqueous solution has been chosen as ICP-MS. [27]

Herbs are linked to the transfer of trace elements from the soil to humans. Heavy metal contamination levels in herbs and therapeutic plants differ. They differ in terms of concentration and location. This is due to the fact that their contents are determined by the soil's geographical context. World Health Organization (WHO) have recommended a limit for different medicinal flora with not more than 0.3 mg/kg for cadmium and 10 mg/kg for lead. To protect customers, the safety and quality of herbs to be taken must be evaluated.

As a result, heavy metal analysis is a key criterion for determining whether or not the herbs are safe to consume. In order to assure the safety of consumers, an analytical approach to analyze the presence of heavy metal and quantify its level is required. Because of its great sensitivity and specificity, the ICPMS has become the most widely used approach. It is the most dependable and effective way for identifying several elements.

ICPMS is a relatively recent method for analyzing and identifying trace multi-elemental and isotopic concentrations in liquid, solid, and gaseous samples. Parts per trillion (ppt) is the detection limit, thus the sample volume required is exceedingly small. The detecting time is also short. In addition, quadrupole ICPMS and high-resolution ICPMS are two different forms of ICPMS. [28,29,30]

In a study conducted by Patnaik et al (2018), the inductively coupled plasma mass spectrometry (ICP-MS) technology was used to quantify *Hemidesmus indicus*. The analytical study allows totally 20 elements (Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cs, Ba and Ti) from this plant extract.

The findings of this study can be used to develop new guidelines for prescribing herbal medicine dosages. Root: stem: Leaf was utilized in a 1:1:1 ratio. 1g of the sample was digested in a 6:1 solution of nitric acid and perchloric acid using the wet digestion method, which involved slowly heating the sample on a hotplate until white residue was produced. The residue was dissolved in 0.1N nitric acid and diluted to a volume of 10 ml. The digested sample was analyzed in ICP-MS [31]. The list of micro-elements and macro-elements identified in *Hemidesmus indicus* by ICP-MS technique along with their average elemental concentration is given in the table below [31]

Table 8: Micro-elements and macro-elements identified in *Hemidesmus indicus* by ICP-MS technique

Serial No:	Element	Average concentration in (ppm)
1.	Li	0.2856
2.	Be	0.0573
3.	Al	910.1811
4.	V	1.3104
5.	Cr	3.7809
6.	Mn	46.0071
7.	Fe	803.8353
8.	Co	0.3669
9.	Ni	1.0818
10.	Cu	3.6711
11.	Zn	31.8423
12.	Ga	10.6188
13.	As	0.7194
14.	Se	0.1428
15.	Rb	10.1466
16.	Sr	116.9598
17.	Ag	0.2178
18.	Cs	0.0675
19.	Ba	56.7672
20.	Ti	0.0255



The findings revealed that heavy and dangerous metal concentrations were below the World Health Organization's allowed range, indicating that they may not be harmful to human health. The trace of medicinal elements has been observed in different concentrations. These help to support pharmacological properties of these plants. From the evaluation of these results, it could be understood that the elemental concentration was in proper amount which are useful to prove medicinal value and ability to cure from these plants against several illness. [31]

XI. CONCLUSION AND FUTURE SCOPE

Herbal medicine is gaining importance world-wide. They are utilized as a raw ingredient in the pharmaceutical industry, as well as in home remedies and over-the-counter medicine items, in both developed and developing countries. Plant extracts are a considerable part of the global medicinal market. The plants are exposed to different types of pollutants, like microbial contaminants, pesticides, chemical toxins, adulterated and heavy metals with conventional drugs during the period of growth and processing. So, there is a concern regarding its safety and efficacy. The quality of herbal medicine is determined by the sum of all aspects that contribute directly or indirectly to the product's safety, efficacy, and acceptance. Thus, standardization of the herbal drugs is vital and the analytical techniques like HP-TLC, HPLC, LC-MS, GC-MS play an important role. There's still a lot to learn about the subject of standardization and in optimization of each of these analytical techniques. All the parameters like heavy metal concentration, pesticide residue, ash-value and so on must be analyzed and the results will prove whether or not the herbal formulation has therapeutic and curative ability against various ailments. These results will help to plan novel herbal medicines which will be more efficient to prevent and cure the existing and emerging diseases of the future. The herbal sector is increasing rapidly, so the advancement of analytical techniques of standardization is essential. This will serve as a rapid tool in the field of herbal research. As a result, regulatory authorities will approve the manufacturing and marketing of herbal pharmaceuticals for therapeutic efficacy, safety, and shelf life. The developments should be made by looking at the current trends and difficulties faced in any technique should be troubleshooted by them. Monitoring the quality of an herbal drug from collection to processing and finally to the final packaged product is required to ensure its safety and efficacy. All agencies should follow a universal approach in establishing the quality of herbal drugs by adopting the WHO guidelines thereby ensuring the safety of the consumers.

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