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Research Article

PHYTOCHEMICAL SCREENING AND FINGERPRINTING OF ROOT EXTRACTS OF *Hemidesmus Indicus* (L.) R.BR.

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A B S T R A C T

Background: *Hemidesmus indicus* is a well known traditional medicinal plant found distributed throughout India. It belongs to Asclepiadaceae family. There are numerous scientific reports on *Hemidesmus indicus*. The roots of this medicinal plant find extensive used in Ayurvedic medicine. **Aim:** The present study deals with the fingerprinting of root extract of *Hemidesmus indicus* **Materials and Methods:** In this work extractive value, preliminary phytochemical screening, spectral and chromatographic fingerprinting of extract is documented. **Results:** The polar extracts contain steroids, terpenoids and flavonoids prominently. IR and NMR fingerprints are indicative of the small molecules 2-hydroxy 4-methoxy benzoic acid,2-hydroxy 4-methoxy bezaldehyde, lupeol and amyrin. **Conclusion:** In view of the need for standardized extracts of *Hemidesmus indicus* in Ayurvedic formulations, the present study was carried out to fingerprint the root extracts of *Hemidesmus indicus.* The study will aid herbal practitioners to obtain authentic roots of valuable plant.

INTRODUCTION

Hemidesmus indicus is derived from the Latin word hemidesmos which means half bond, indicus stands for India. Roots and root bark are mostly used in traditional medicine. This plant has been scientifically analysed by many. Literature reports on phytochemical studies[1], quantification of phytochemical contents[2][3],quantification of compounds-lupeol octacosanoate[4], ferulic acid[5],2-hydroxy-4-methoxybenzaldehyde and 2- hydroxy-4-methoxybenzoic acid[6] and biological activity studies[1][7]are available. There are nine reviews on this plant published till date[1][7][8][9][10][11][12][13][14]. A detailed review on compounds isolated from *Hemidesmus indicus* is reported in the review by Avjit Banerji *et al*., 2017[8]. In view of the need for standardized extracts of roots of *Hemidesmus indicus* in various medicinal formulations, it is necessary to document the various parameters related to standardization. The pharmacological potential, dosage and safety aspects of extracts, morphological studies, ethnobotanical studies and pharmacognostical aspects have been reported. However, fingerprinting of the extracts is not yet reported. The results of this study will be useful in selecting authentic sample of roots of *Hemidesmus indicus* when medicinal preparations are made.

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MATERIALS AND METHODS

Authentification of Plant Material

Authenticated sample of powdered roots of *Hemidesmus indicus* was obtained from the Arya Vaidya Pharmacy Pvt. Ltd. Coimbatore.

Extraction of Plant

Extraction of roots of *Hemidesmus indicus* was done by cold extraction, soxhlet extraction and direct refluxing.

Cold extraction

Cold extraction of the pulverized roots of *H.indicus* (5g) was done with hexane (H), chloroform (CH), acetone (A), ethyl acetate (EA), ethanol (E), hydro ethanol (HE) and water (W). In each case the root powder was soaked for one hour in the solvent with frequent maceration. The extracts were filtered and concentrated. The weight of the extract was taken. The extract concentrates were designated as: HEIN-H, HEIN-CH, HEIN-A, HEIN-EA, HEIN-E, HEIN-HE and HEIN-W

Hot extraction

The root powder (100g) was extracted by refluxing with hexane for 6 hours. The hexane extract was filtered and concentrated. The residual plant material was extracted with ethanol-water mixture (90:10) (2x6h). The extract was filtered and concentrated. The extract concentrates were designated as

HEIN-H and HEIN-HE_d. Sequential soxhlet extraction was carried out with 250g of root powder with solvents hexane, ethyl acetate and ethanol-water (90:10) mixture. The extract concentrates were designated as HEIN-H, HEIN-EA_S, and HEIN-HE_S

Phytochemical Screening

Qualitative phytochemical screening of the extract concentrates of roots of *Hemidesmus indicus* was done as per standard procedure [15]. The following colour tests were done with each of the extract concentrates.

1. Test for alkaloids (Hager's Test)
2. Test for steroids (Salkowski test)
3. Test for flavonoids (Shinoda test)
4. Test for terpenoids (Liebermann-Burchard test, Salkowski test)
5. Test for carbohydrates (Molisch's test)
6. Test for phenolic compounds (Ferric chloride test)
7. Test for saponins (Foam test)

Chromatographic Fingerprinting of Extracts

Thin layer chromatographic analysis (TLC)

TLC analysis was performed for all extracts [15].The non-polar extract (HEIN-H) and polar extracts (HEIN-HE_d), (HEIN-EA_S), (HEIN-HE_S) were dissolved in ethanol one by one separately and were spotted on the TLC plates manually with a capillary tube. Developing solvent systems optimized for the samples are mentioned in [Table-1]

Table 1 Developing solvent system for TLC analysis

Extract	Solvent system for TLC
HEIN-H	Hexane: Ethyl acetate (8:2)
HEIN-HEd	Hexane: Ethyl acetate (7:3)
HEIN-EAs	Hexane: Ethyl acetate (7:3)
HEIN-HEs	Hexane: Ethyl acetate (6:4)

GC-MS-Analysis

GC-MS analysis of extracts was done in Thermo Fisher make GC-MS spectrometer. Interpretation of mass spectrum was done with reference to National Institute of Standard and Technology (NIST) database. The GC-MS protocol followed is given below in [Table-2]

Table 2 GC-MS protocol

Column used	Silica column packed with Elite5MS 30.0m x 250µm
Carrier gas	Helium
Flow rate	1 ml/min
Sample injection	Split 10:1
Solvent delay	2.80 mins
Oven (hold 6 min)	Initial temp 60°,ramp 10°C/min to 290°C
Transfer temp	240°C
Source temp	240°C
Concentration of sample	1 µL
Run time	35 mins

Spectral Fingerprinting of Extracts

UV-Visible spectral fingerprinting: The extracts were dissolved in distilled alcohol and their respective UV-Visible spectra were recorded in a double beam UV-Visible double beam spectrophotometer-Systronics(AU-2701). Few drops of sodium ethoxide was added as shift reagent to each test sample and the UV-spectrum was recorded again.

FT-IR spectral fingerprinting: Extracts were analysed in Shimadzu FT-IR spectrometer. Spectrum was recorded in the range of 4000 to 750 cm^{-1}.

^{1}H NMR spectral fingerprinting: ^{1}H NMR spectrum of the extracts was recorded in Bruker 400 MHz proton nuclear magnetic resonance spectrometer at room temperature.

RESULTS

Extractive Values of Extracts

[Table -3] gives the percentage extractive values obtained for the various extracts. Hot extraction for six hours provided a higher yield of the hexane extract and the polar hydroethanol extract.

Table 3 Extractive value of extracts

Extracts	Extract code	Extractive value (%)
Cold extraction of roots of *Hemidesmus indicus* (HEIN)		
Hexane	HEIN (H)	0.1
Chloroform	HEIN (CH)	2.4
Acetone	HEIN (A)	2.9
Ethyl Acetate	HEIN (EA)	1.6
Ethanol	HEIN (E)	6.3
Hydroethanol	HEIN (HE)	7.2
Water	HEIN (W)	9.3
Hot extraction of roots of *Hemidesmus indicus* (HEIN)		
Hexane	HEIN-H	4.5
Hydroethanol	HEIN-HEd	6.2
Ethyl acetate	HEIN-EAs	1.4
Hydroethanol	HEIN-HEs	3.6

Phytochemical Screening of Roots of H.Indicus

The colour tests carried out for the extracts revealed the presence of terpenoids, steroids and flavonoids predominantly. Of particular mention in the appearance of a bright rose red coloured solution in Shinoda test for the polar extracts [Fig.1][Fig.2][Fig.3]. The intensity of the colour was high for the hydroethanol extract. The results of the colour tests are presented in the [Table-4]

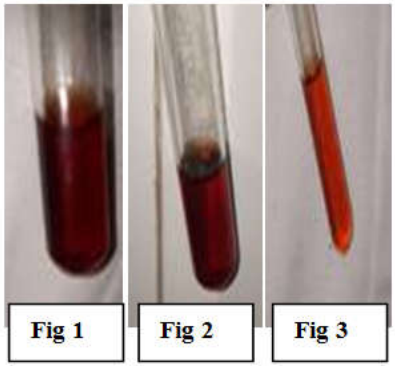


Fig 1 Shinoda test for HEIN-HE_d
Fig 2 Shinoda test for HEIN-EAs
Fig 3 Shinoda test for HEIN-HEs

Table 4 Results of qualitative colour test

Tests	HEIN-HE_d	HEIN-H	HEIN-EA_S	HEIN-HE_S
Hager's test	+	-	-	-
Liebermann Burchard test	+	+	++	+++
Salkowski test	++	+	++	+++
Shinoda test	+++	-	+++	+++
Molisch's test	-	-	+	-
$FeCl_3$ test	-	-	-	-
Foam test	-	-	-	+

- negative, + slight positive,++ moderately positive , +++ intense colour

Chromatographic Fingerprinting of Extracts

TLC analysis of the extracts was done with the optimized developing solvent systems.

TLC chromatograms of the various extracts are represented in [Fig.4][Fig.5][Fig.6][Fig.7]. The relative factor (R_f) of the major spots are given in the [Table 5]

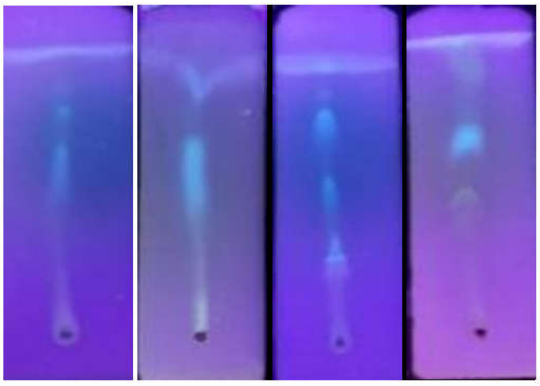


Fig.4 **Fig.5** **Fig.6** **Fig.7**

Fig 4 Thin layer chromatogram of HEIN-H,
Fig 5 Thin layer chromatogram of HEIN-HE_d
Fig 6 Thin layer chromatogram of HEIN-EAs
Fig 7 Thin layer chromatogram of HEIN-HE_S

Table 5 R_f values of major spots in the TLC of the extract

S.No.	Sample	Rf value of major spots
1	HEIN-H	0.2, 0.5
2	HEIN-HEd	0.1,0.14, 0.80
3	HEIN-EAS	0.05, 0.26, 0.4
4	HEIN-HES	0.2, 0.4

GC-MS fingerprints

The GC-MS fingerprints of the hexane and hydroethanol extracts are represented in [Fig.8]and [Fig.9]. From the chromatograms obtained under the conditions of the adopted GC protocol, the peaks with intensity greater than 10% were considered. For the hexane extract, peaks due to oleanyl acetate and lupeol acetate could be identified by the mass spectral comparison with NIST database. Similarly for the polar HEIN-HE extract major peaks due to oleic acid and lupeol could be identified. The compounds identified are listed in [Tables-6] and [Table-7]

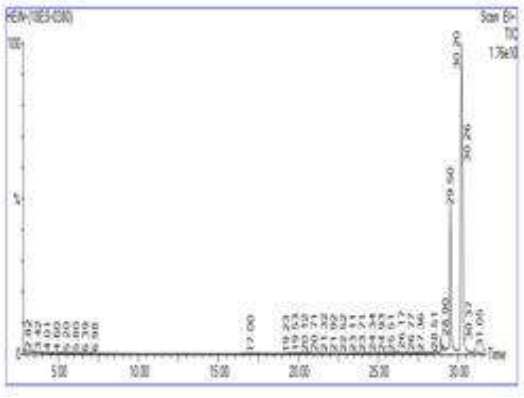


Fig 8 GC-MS fingerprints of HEIN-H

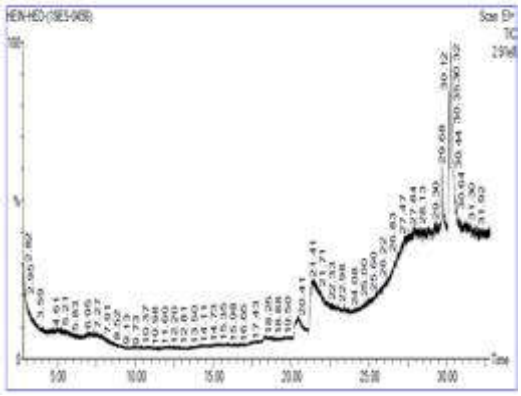


Fig 9 GC-MS fingerprints of HEIN-HE_d

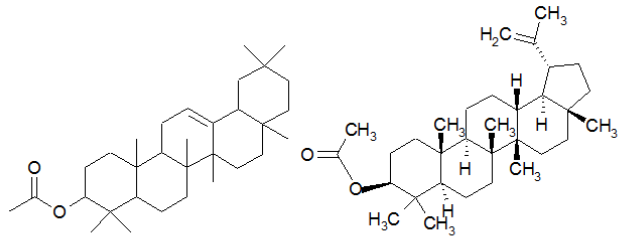
Table 6 GC-MS result of HEIN-H

Retention time	Predicted compounds
29.50	Oleanyl acetate
30.20	Lupeol acetate

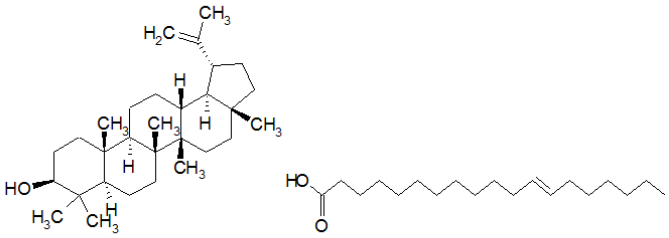
Table 7 GC-MS result of HEIN-HE_d

Retention Time	Predicted Compounds
21.59	Oleic acid
29.68	Urs-12-ene-28-ol
30.12	Lupeol

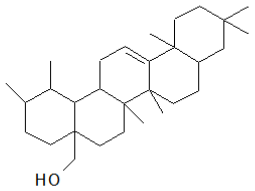
The structure of compounds predicted by comparison with NIST database are given in [chart 1].



Oleanyl acetate **Lupeol acetate**



Lupeol **oleic acid**



Urs-12-en-28-ol

Chart 1

Spectral Fingerprinting of Extracts

UV-Visible spectral fingerprint

[Fig.10][Fig.11][Fig.12][Fig.13] respectively represent the UV-Visible fingerprints of the extracts HEIN-H, HEIN-HE_d, HEIN-EAs, HEIN-HEs

[Fig.14][Fig.15][Fig.16] represents the overlay spectra of the UV spectra of the above extracts with that obtained with sodium ethoxide as shift reagent.

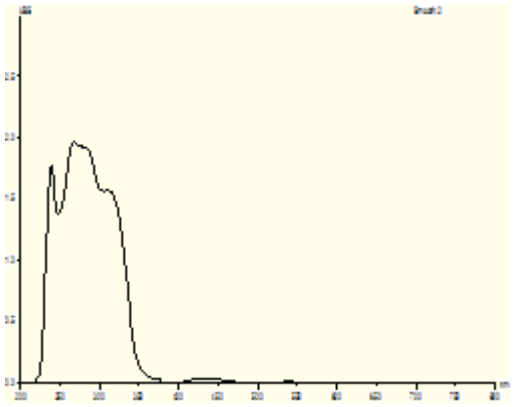


Fig 10 UV-Visible fingerprints HEIN-H

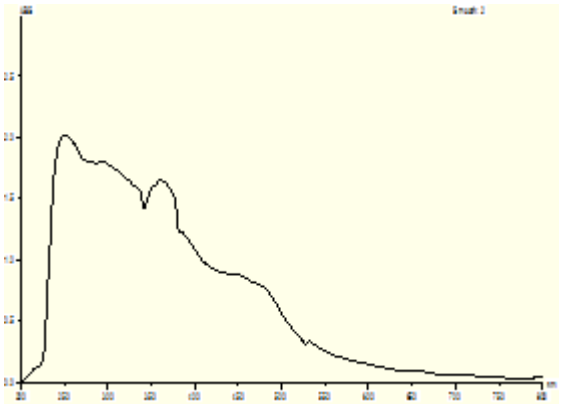


Fig.11 UV-Visible fingerprints HEIN-HE$_d$

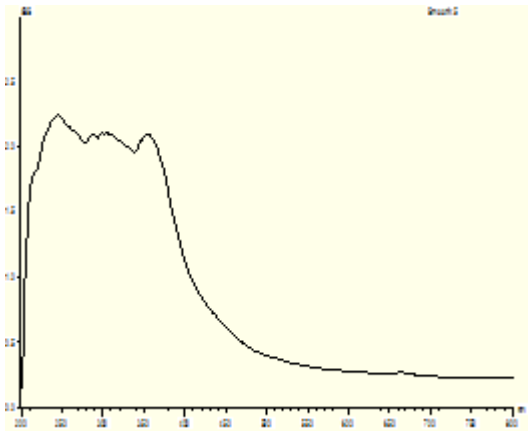


Fig.12 UV-Visible fingerprints HEIN-EAs

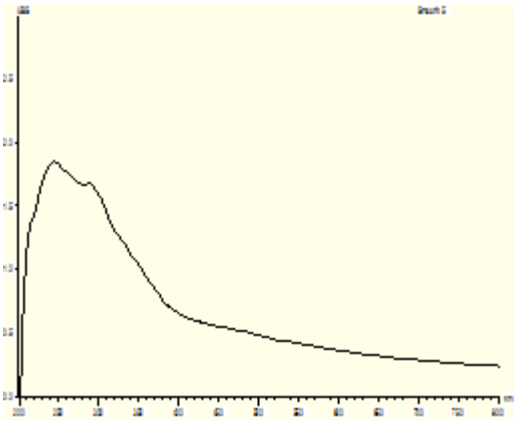


Fig.13 UV-Visible fingerprints HEIN-HEs

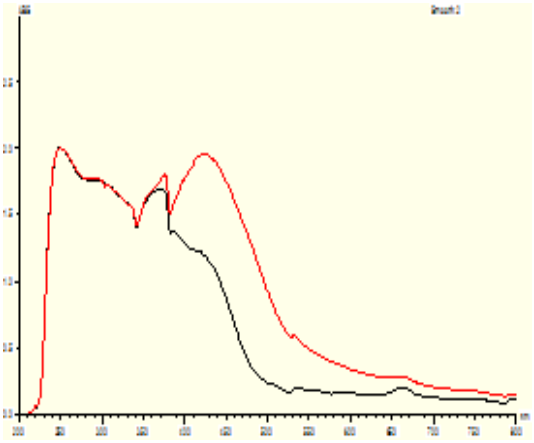


Fig.14 Black-HEIN-HEd, Red-HEIN-HEd+Sodium Ethoxide

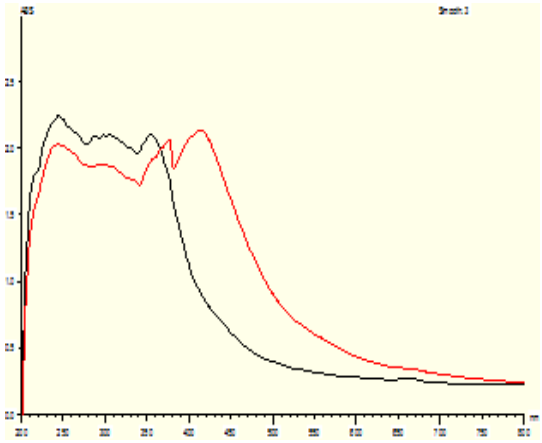


Fig.15 Black-HEIN-EAs, Red-HEIN-EAs+Sodium Ethoxide

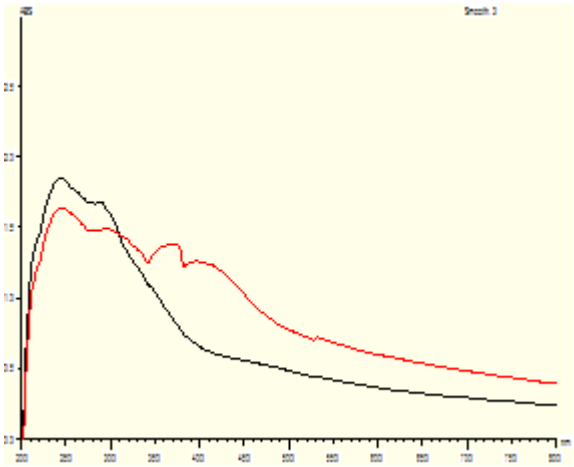


Figure 16 Black-HEIN-HEs Red-HEIN-HEs+Sodium Ethoxide

A shift in the longer wavelength band was observed with addition of sodium ethoxide this indicates the presence of flavonoidal moieties in polar extracts specially the presence of hydroxyl substituent in the flavone moiety in the extract.

FT-IR spectral fingerprints

IR spectral fingerprints of extracts are represented in the [Fig.17][Fig.18][Fig.19][Fig.20]

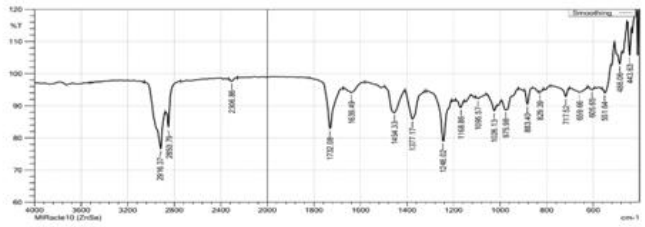


Fig 17 IR spectral fingerprint of HEIN-H

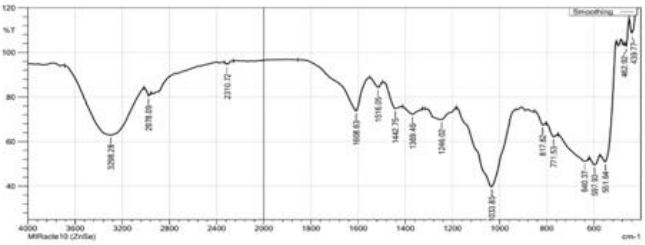


Fig 18 IR spectral fingerprint of HEIN-HE_d

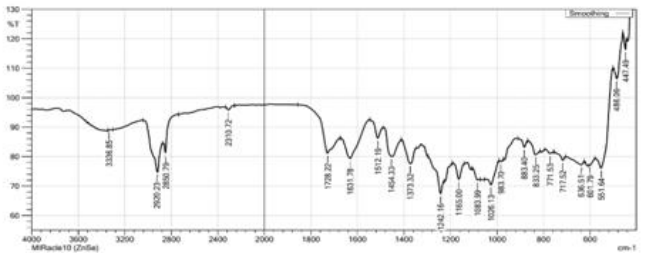


Fig 19 IR spectral fingerprint of HEIN-EAs

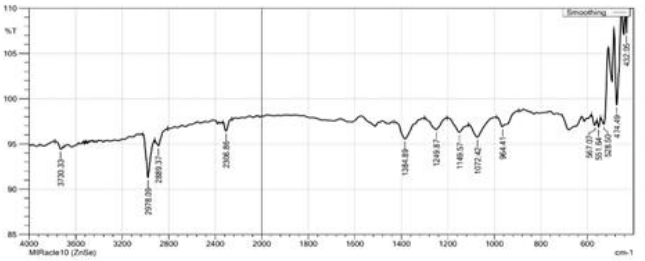


Fig 20 IR spectral fingerprint of HEIN-HEs

The non polar hexane extract showed the predominant presence of aliphatic moieties with peaks at 2916 and 2850 cm^{-1} which were conspicuously absent in the polar extract HEIN-HE_d. This assumes the complete separation of non polar constituents from the polar constituents.

^{1}H NMR spectral fingerprints

[Fig.21] and [Fig.22] represent the ^{1}H NMR spectral fingerprints of HEIN-H and HEIN-HE_d

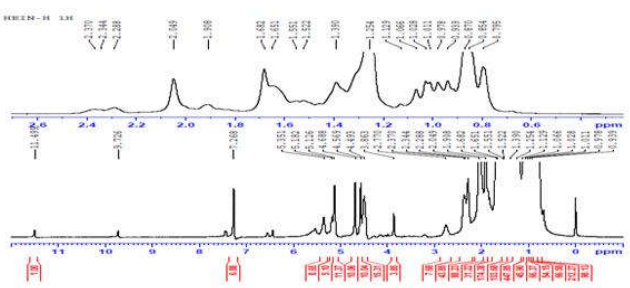


Fig 21 ^{1}H NMR spectral fingerprint of HEIN-H in $CHCl_3$

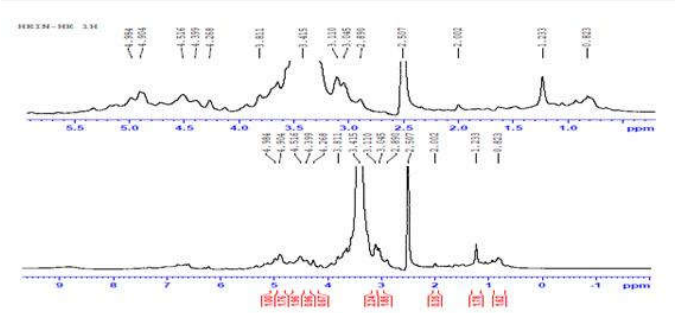


Fig 22 ^{1}H NMR spectral fingerprint of HEIN-HE in DMSO

The ^{1}H NMR spectral fingerprint of the non-polar extract HEIN-H showed peaks at δ 9.73 and 11.49 probably indicative of the presence of the aromatic molecules 2-hydroxy 4-methoxy benzaldehyde and 2-hydroxy 4-methoxy benzoic acid which have been reported to be isolated earlier from the roots[6]. It is worthy of mention that the above small molecule 2-hydroxy 4-methoxy benzoic acid is reported to possess anti venom activity [16].

CONCLUSION

In view of the need for standardized extracts of *Hemidesmus indicus* in Ayurvedic formulations, the present study was carried out to fingerprint the root extracts of *Hemidesmus indicus.* The results of this study will be useful in selecting authentic sample of roots of *Hemidesmus indicus* when medicinal preparations are made.

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