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Research Article

ISOLATION, PURIFICATION AND CHARACTERIZATION OF *HEMIDESMUS INDICUS R.BR.* ROOT EXTRACT

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ABSTRACT
The roots of *Hemidesmus indicus R.Br.* was collected from Maruthamalai hills, Coimbatore and were extracted with 90% ethanol and fractionated by column chromatography using different solvents. A phytochemical Terpenoid was isolated using thin layer chromatography. The infrared spectrum was used for characterization of organic, inorganic and biological compounds. FT-NMR spectroscopy is used to determine the molecular structure based on the chemical environment of the magnetic nuclei like ^{1}H, ^{13}C, ^{31}P, etc., even at low concentrations. The carbon, hydrogen and oxygen content of the compound were analyzed by Erlinmeier flask method. Mass spectrometry has become a vital tool for giving qualitative and quantitative information on molecules based on their structural compositions.
Keywords: *Hemidesmus indicus* R.Br., ethanol, terpenoid, infrared spectrum, FT-NMR spectroscopy, mass spectrometry.

INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. Medicinal herbs are significant source of synthetic and herbal drugs. In the commercial market, medicinal herbs are used as raw drugs or extracts. Isolated active constituents are used for applied research. For the last few decades, phytochemistry has been making rapid progress and herbal products are becoming popular. Medicinal herbs are considered to be chemical factories as they contain multitude of chemical compounds like alkaloids, flavanoids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils. Several bioactive constituents have been isolated and studied for pharmacological activity (Bnouham *et al.,* 2006).

Hemidesmus indicus R.Br. belongs to the family Asclepiadaceae widely used in folk medicine is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody and aromatic. The stem is numerous, slender, terete, thickened at the nodes. The leaves are opposite, short-petioled, very variable, elliptic-oblong to linear-lanceolate. The flowers are greenish outside, purplish inside, crowded in sub-sessile axillary cymes (Acharya *et al.,* 2006).

This review briefly describes the different extraction methodologies used in the preparation of herbal extracts and reviews the utility of chromatography-mass spectrometry for the analysis of their active components.

MATERIALS AND METHODS

Collection of plant material

Hemidesmus indicus roots were collected from Maruthamalai hills, Coimbatore district, Tamil nadu, India. The plant was identified and authenticated by Dr.K.Arumugasamy, Reader, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil nadu, India and voucher specimens were deposited at herbarium.

Extract preparation

The fresh roots collected were washed with distilled water and shade dried. *Hemidesmus indicus* R.Br root was used in the form of crude 90% ethanolic extract and this extract was prepared according to the traditional system of medicine. 750g of the shade dried and powdered root material was extracted with 2000 ml of 90% ethyl alcohol in cold for 72 hr. The extract was filtered and distilled on water bath, the syrupy mass obtained was dried at low temperature under reduced pressure in a rotary evaporator and a crude residue was obtained. The residue was suspended in water and administered orally to the experimental rats.

Qualitative identification of phytochemicals

Identification of active principles in *Hemidesmus indicus* root extract was done by modern method of plant analysis by Peach and Tracey (1955) to identify the presence of alkaloid, flavanoid, saponin, carbohydrate, protein, phenol, steroid, terpenoids, glycoside, resin, tannin and thiols.

Isolation, purification and identification of active constituents in root extract

Column chromatography

12 g of the plant extract was added to 30 g silica gel, (Mesh 70-325). In column packing, 150 g of silica gel (70-325 Mesh), using hexane as solvent was used. The column was eluted with solvent gradually starting from 100 % hexane, followed by increasing order of polarity of solvents like chloroform, then with ethyl alcohol / chloroform (0-50%) was done (Indian Herbal Pharmacopoeia, 1998).

Thin layer chromatography

Thin layer chromatography is an easy technique to adopt for the identification of active constituents. It is highly useful in research laboratories to separate and identify unknown compounds. Thin layer chromatography is the separation of a mixture into individual components using a stationary and mobile phase (Sadasivam and Manikam, 1992).

Ten TLC plates were spotted with 10 microlitre of ethanolic extract and placed in 10 separate TLC chambers with different solvent systems. When the mobile phase had raised to maximum height of the plate, they were removed and sprayed with the spraying reagent.

Infra red Spectroscopy

The infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is also carried out by using Fourier transform technique.

PERKIN ELMER Spectrum one FT-IR Spectrophotometer 450-4000 cm^{-1} was the instrument used and the sample requirement was 50 mg, solid or liquid

Nuclear magnetic resonance

FT-NMR spectroscopy is used to determine the molecular structure based on the chemical environment of the magnetic nuclei like ^{1}H, ^{13}C, ^{31}P, etc., even at low concentrations. This is one of the most powerful nondestructive techniques in elucidating the molecular structure of biological and chemical compounds. JEOL GSX 400 NB, 400 MHz FT NMR Spectrometer was the instrument used and sample requirement was 5 mg for ^{1}H and 15 mg for ^{13}C, Solubility: 10 mg/ml for ^{1}H and 50 mg/ml for ^{13}C and Solvents available: $CDCl_3$, D_2O, C_6D_6, CD_3COCD_3 and $DMSOd_6$.

Identification of the elements by elemental analysis

The carbon, hydrogen and oxygen content of the compound were analyzed by Erlinmeier flask method.

Gas chromatography – Mass Spectroscopy

Mass spectrometry has become a vital tool in the hands of organic chemists and biochemists because of its potential to supply definitive, qualitative and quantitative information on molecules based on their structural compositions. Finnigan MAT 8230 JEOL GC Mate GC-MS Spectrometer was the instrument used and the sample requirement was about 1mg. The sample required can be in the solid or liquid state. Sample should be pure and free from solvents and metal ions.

RESULTS AND DISCUSSION

Hemidesmus indicus R.Br. is a perennial prostrate or twining shrub, root-stock woody, with aromatic smell, stems woody, leaves opposite, smooth, shining, dark green, flowers are small, green outside, purple within, fruits are long, slender, tapering, spreading and seeds with silvery white (Acharya *et al.,* 2006) So, the selected plant possesses similar characters described by Indian herbal pharmacopoeia.

The yield of the product from the plant material after the complete extraction of 750 gm of the root powder in 2000 ml of 90% ethanol was found to be 15 g.

The phytochemical analysis obtained qualitatively by 90% ethanolic crude extract of *Hemidesmus indicus* are presented in the following table 1**.**

The phytochemical study of the alcoholic extract of *Hemidesmus indicus* showed the presence of alkaloids, flavanoids, saponins, phenols, glycosides, steroids, terpenoids and tannins.

Kumar *et al.* (2007) have reported that the medicinal values of herbs are due to the presence of phenols, flavanoids, tannins, terpenoids and glycosides.

85mg of the pure compound was obtained in 51-54th fraction at 15% $EtOH/CHCl_3$ elution. The pure compound obtained was found to be yellow and semi solid.

Thin layer chromatography is one of the most widely used technique for rapid identification of drugs. It is equally applicable to drugs in their pure state, to those extracted from pharmaceutical formulations and to biological samples. The time required to demonstrate the presence of constituents in any extract by thin layer chromatography is very short. It allows the possibility of separating wide classes of drugs and is surprisingly versatile in its various fields of application (Sethi, 1992).

The R_f value of the isolated compound was found to be 0.56. The blue colour developed confirms the presence of terpenoid in the root extract.

Infra red spectrum of the isolated compound showed the absorption bands at 2956 cm^{-1}**,** 2925 cm^{-1} for C-H stretching vibration and sharp peak at 1731 cm^{-1} for ring based carbonyl group. Another broad band at 1378 cm^{-1} for gem dimethyl stretching vibration and the broad band at 1122cm^{-1} for C-O-C stretching vibration are seen. It also, registered C=C stretching vibration at 760 cm^{-1}.

The infra red spectrum indicates, that the isolated compound contain, one ring based carbonyl group (ester) one gem dimethyl group C-O-C and C=C bond nature. It is represented in Figure 1.

H^1 NMR spectra can be quite complex, for example, due to the interaction between protons attached to adjacent carbon atoms, the spectral signals may appear as ‘ singlet’ or ‘ multiplet’ instead of as single peaks.

The ^{1}H–NMR spectrum of the isolated compound registered two multiplets at δ 7.25 for C_{20}-H and δ 4.24 for C_{22}-H aromatic protons. The two singlets were obtained in C_4 for gem-dimethyl protons and also three singlets observed at δ 1.57 for C_{23} methyl protons, and δ 1.40 for C_{17} and C_{18} methyl protons.

Also, registered the two multiplets at δ 4.23 and δ 4.25 for =CH proton (C_{10} and C_{14}) and other data are depicted in Table 2, Figure 2 and Figure 3.

The ^{13}C–NMR spectrum of the compound showed intensive peak at δ 14.06 and 14.14 for methyl carbon (C_{17} and C_{18}). Also the peaks at δ 27.55 and δ 27.66 for gem dimethyl carbon (C_{24} and C_{25}) and one more peak at δ 22.34 for C_{23} methyl carbon. The peak showed at δ 167.66 for the carbonyl peak for C_{21} and δ 68.07 for C-O at C_{22} carbon. All the other peaks are listed in the following table 3 and Figure 4.

From the results of ^{13}C–NMR spectrum, it is confirmed that the isolated compound contain one gem dimethyl group, three methyl groups and four=CH nature. The compound also contains O-C=O linkage.

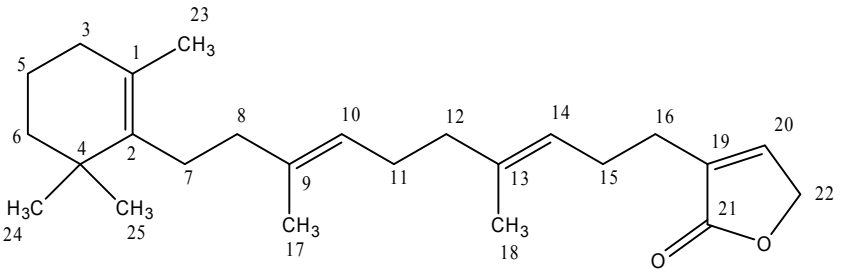
The mass spectrum of the isolated compound exhibited a peak at *m/e* 370 and the other fragmentation peaks at *m/e* values at 355, 327, 311, 287, 247 and 164. The mass spectral fragmentation pattern of the isolated compound is as in figure 5 and 6.

The elemental analysis of the isolated compound is given in table 4.

The percentage of elements showed the presence of carbon, hydrogen and oxygen in the isolated compound.

From the elemental analysis and mass spectrum, the molecular formula and molecular weight are $C_{25}H_{38}O_2$ 370 respectively.

From all the above spectral studies (IR, NMR, Mass spectrum and elemental analysis) the isolated compound was characterized and the assumed structure of the compound is as follows:



Structure of the isolated compound

3-((3*E*, 7*E*)-4, 8-dimethyl-10-(2, 6, 6-trimethylcyclohex-1-enyl) deca-3, 7-dienyl) furan-2(5*H*)-one

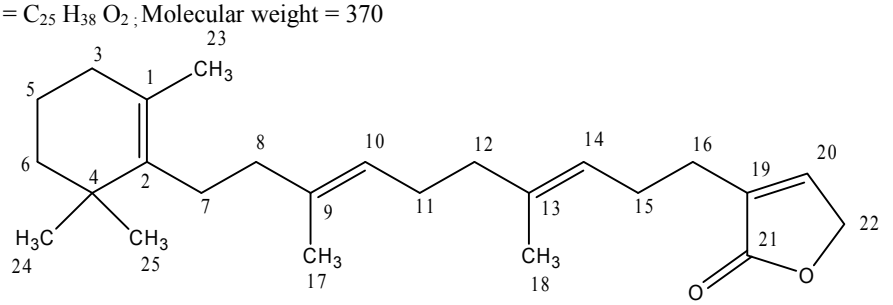
Molecular formula = C_{25} H_{38} O_2 ; Molecular weight = 370.

CONCLUSION

The compound was fractionated using column chromatography,which was yellow in color and semisolid in nature, the Rf value of the isolated compound was found to be 0.56 and the presence of terpenoid was detected using thin layer chromatography.

From the infra red, nuclear magnetic resonance, gas chromatography-mass spectra and elemental analysis the molecular formula, molecular weight and the structure of the isolated compound are analyzed as follows

Molecular formula = C_{25} H_{38} O_2 ; Molecular weight = 370



Structure of isolated compound: 3-((3E, 7E)-4, 8-dimethyl-10-(2, 6, 6-trimethylcyclohex-1-enyl) deca-3, 7-dienyl) furan-2(5H)-one

Table 1: Preliminary phytochemical screening of root extract of *Hemidesmus indicus*

Tests	Observation
ALKALOIDS	
Dragendroff's test	+
Wagnar's test	+
Meyer's test	+
FLAVANOIDS	++
SAPONINS	+++
PHENOLS	
Ferric chloride test	+
Lead acetate test	+
Libermann's test	+
GLYCOSIDES	++
RESINS	+
STEROIDS	
Libermann Burchad's test	++
TERPENOIDS	
Salkowski test	+++
TANNINS	
Ferric chloride test	++
Lead acetate test	++
CARBOHYDRATES	
Fehling's test	-
Benedict's test	-
Molisch's test	-
PROTEINS	
Million's test	+
Biuret's test	+
THIOLS	+

+ = trace, ++, +++ = excess , - = Nil

Table 2: ^{1}H NMR Spectrum of the isolated compound from *Hemidesmus indicus* root extract

Protons	δ, ppm
-	-
-	-
H-3	2.37 (2H, m)
-	-
H-5	1.55 (2H, m)
H-6	1.34 (2H, m)
H-7	2.66 (2H, m)
H-8	2.64 (2H, m)
-	-
H-10	4.23 (1H, m)
H-11	1.40 (2H, m)
H-12	1.54 (2H, m)
-	-
H-14	4.25 (1H, m)
H-15	2.37 (2H, m)
H-16	2.45 (2H, m)
H-17	1.40 (3H, s)
H-18	1.40 (3H, s)
-	-
H-20	7.25 (1H, m)
-	-
H-22	4.24 (2H, m)
H-23	1.57 (3H, s)
H-24	0.93 (3H, s)
H-25	1.26 (3H, s)

s-singlet, m – multiplet

Table 3: ^{13}C NMR Spectrum of the isolated compound from *Hemidesmus indicus* root extract

Carbons	δ, ppm
C-1	127.69
C-2	145.98
C-3	32.02
C-4	36.62
C-5	20.73
C-6	39.31
C-7	23.78
C-8	31.82
C-9	132.52
C-10	125.71
C-11	27.33
C-12	39.97
C-13	130.77
C-14	125.66
C-15	28.95
C-16	27.63
C-17	14.06
C-18	14.14
C-19	146.27
C-20	147.88
C-21	167.66
C-22	68.07
C-23	22.34
C-24	27.55
C-25	27.66

Table 4: Elemental analysis of the isolated compound

Elements	Percentage
Carbon	81.08 %
Hydrogen	10.27 %
Oxygen	8.65 %

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