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Phytochemical Investigations of the Medicinal Plant Swertia Chirata Ham

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Abstract

The fresh stem of the plant *Swertia chirata* Ham was extracted by rectified spirit. The crude rectified spirit extract was fractionated by using standard chromatographic techniques, on alumina gave several fractions (A, B, C, D, E and F). Fraction D, when subjected to column chromatographic analysis on neutral alumina, yielded a compound, tentatively known as compound AJ-1 m.p. 178°C. In this research work only one compound is isolated. From the spectral evidences, the compound is an alkaloid containing 37 carbon atoms with 52 hydrogen along with secondary or tertiary nitrogen and several OH groups in the molecule. The pure compound AJ-1 has found antidiabetic, analgesic, anti-inflammatory and other different bioactivity tests can be performed for the pure compounds AJ-1.The plant is being used as flok medicine in the treatment of diabetes, jaundice and skin diseases.

Keywords: Gentianaceae; *Swertia chirata* Ham; Spectral characteristics; Pure Compound AJ-1

Introduction

Natural product chemistry is an ancient science. Different medicinal compounds such as antitumor, anticancer, anti AIDS etc were isolated from natural plants. Isolation of any compound from any plants is uncertain. None can assure it before investigation. But generally secondary metabolites are obtained by proper investigation. Plants are unique in their ability to synthesize fats, carbohydrates, proteins that constitute three major food classes for human race [1]. The array of medicines derived from them is impressive and includes hypotensive drugs. analgesics, anaesthetics, anticancer and antiparasitic compounds, anti-inflammatory drugs, steroids, laxatives, diuretics and many others [2]. Theophrastus [3] a student of plato, in his historia plantarum described the use of nearly 500 medicinal plants. A vast compilation of ancient knowledge of medicinal plant, can be seen in the natural history of pliny [4], the famous Roman physician. The Chinese Emperor Shen Nung (3000 BC) [5] compiled a treatise on medicinal plants. The medicinal plants that have been used since ancient time, many have yielded most useful drugs that are very much in use in current medicine. Such as- The most important natural analgesic, morphine [6] was isolated from opium poppy (latex of Papaver somniferum fruit). Quinine [6], an antimalarial alkaloid, was isolated from cinchona bark (Cinchona succirubra) which was used by the South Americans and the Indians. Emetine [7], which is considered as an important medicine for amoebiasis, is the main alkaloid obtained from the root of ipecacuanha (Cephaelis ipecacuanha) was used in Brazil and Far east for dysentery and diarrhoea. Reserpine [8], used as a hypotensive drug and tranquilizer was isolated from the plant Rauwolfia serpentia, considered to be a common remedy for mental illness and snake bite in the Indian sub continent. Bronchodilating effect of ephedrine from Ephedra vulgaris [6].

Curare used as arrow poison by the South American natives has now given as tubocurarine [9], a quaternary alkaloid now being used as an adjunct to anesthesia for surgery. Anti-spasmodic effect of atropine [6], from Atropa belladona. Analeptic effect of strychnine [6] from Nux vomica and pelletierine, from pomegranate. Antileprotic effect of chaulmoogra fruit [6] was well known to the ancient Indians. At present thousands of plant metabolites are also now being successfully used in the treatment of a variety of diseases. A few striking examples of plant metabolites are as follow: Taxol [10], from *Taxus brevifolia*, Vincristine [11] and Vinblastine [11] from *Vinca rosea* Linn. (Periwink Plant), all of which are important anticancer drugs. Arrow poison of foxglove [12] consisting of digitalis glycosides is cardiotonic for man. The root bark of Mexican yam [13] is used in making cortisone and other steroidal drugs. Salicylic acid [14] isolated from willow bark has a variety of pharmacological effects on platelet aggregation, pain and immune system. Artemisinin, a new antimalarial from Chinese herbal medicine [15].

The plant under investigation is *Swertia chirata* Ham which belongs to the family Gentianaceae. The Gentianaceae is a tropical family of small trees, herb and bitter tonic. It consists of 180 species. About 8-10 species exist in India [16]. This plant is indigenous to temperature Himalayas at altitudes above 4000 feet from Kashmir, Nepal and Bhutan [17]. In this family all plants are use as medicine. In the present investigation the plant *Swertia chirata* Ham was selected for phytochemical and biological studies.

The plant *Swertia chirata* (Family: Gentianaceae) Ham was chosen for investigation since it has a folkloric reputation. So far literature surveyed, *Swertia chirata* Ham has tremendous uses in traditional medicines. It has anti-microbial activity against Gram positive and Gram negative bacteria. All the plant are used as astringent, unani-tonic to heart, liver, eyes, cough, scanty-urine, melancholia, dropsy, sciatia, skin diseases, the plant is used as a bitter tonic in gastrointestinal disorders, like dyspepsia/anorexia, it is used as digestive, febrifuge and laxative. It is used to prevent malaria, particularly useful in fever. The plant is also effective against intestinal worms burning of the body, bronchial asthma, regulating the bowels [18] (Figure 1).

Materials and Methods

Dried stem of *Swertia chirata* Ham (Locally known as chirata) were collected from Bhangura Kabiraji Shop of Rajshahi Shaheb Bazar. The stems of the plant were cut into small pieces by a sharp knife. About 650

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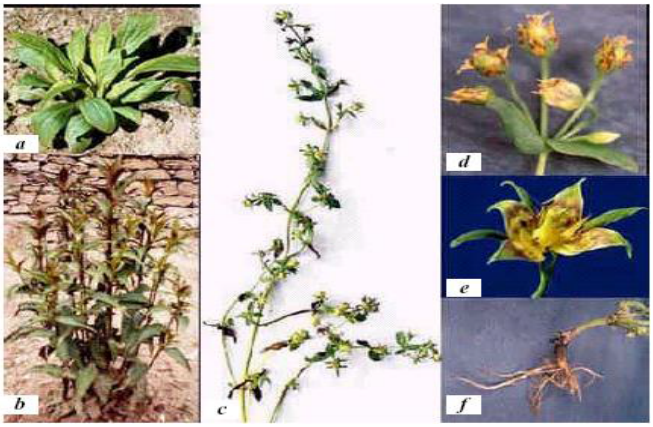

Figure 1: Swertia chirata: a, plant in vegetative phase; b, A 2ft tall plant before flowering; c, Flowering twig; d, Flowering panicle during seed set; e, Single tetramerous flower; f, Root of a mature two year-old plant.

Eluants	Amount of solvent (in ml)	No. of collected beakers
100% n-hexane	300	1-3
25% Petroleum ether in hexane	100	4
50% Petroleum ether in hexane	100	5
100% Petroleum ether	200	6-7
2.5% Ethyl acetate in petroleum ether	100	8
5% Ethyl acetate in petroleum ether	300	9-11
7.5% Ethyl acetate in petroleum ether	100	12
10% Ethyl acetate in petroleum ether	300	13-15
12.5% Ethyl acetate in petroleum ether	100	16
15% Ethyl acetate in petroleum ether	100	17
20% Ethyl acetate in petroleum ether	100	18
25% Ethyl acetate in petroleum ether	100	19
30% Ethyl acetate in petroleum ether	200	20-21
40% Ethyl acetate in petroleum ether	100	22
50% Ethyl acetate in petroleum ether	100	23
60% Ethyl acetate in petroleum ether	100	24
70% Ethyl acetate in petroleum ether	100	25
80% Ethyl acetate in petroleum ether	100	26
90% Ethyl acetate in petroleum ether	100	27
100% Ethyl acetate	100	28
5% Methanol in ethyl acetate	200	29-30
15% Methanol in ethyl acetate	100	31
50% Methanol in ethyl acetate	200	32-33
100% Methanol wash	100	34

The fractions were combined on the basis of their preliminary TLC examination to give combined fractions A, B, C, D, E, F (Table.2). Each combined fraction was evaporated to dryness under reduced pressure.

Table 1: Solvent used for column chromatography.

gm of the plant pieces were weighed by the electric balance and made paste by pestle-moter. The paste materials (stem) were taken in a clean flat bottomed glass container (2.5 litre) and macerated with sufficient amount of rectified spirit and with occasional shaking. After 15 days the solvents was decanted and filtered by Tincture filter press (Karl Kolb, Scientific- Technical Supplies, Frankfurt/M Germany) and then filtered through fresh cotton. The filtrate thus obtained was taken in a 1 litre beaker. The solvent (R.S) of the extract was evaporated under normal pressure and normal temperature to obtain a gummy mass. This mass was preserved in a refrigerator for chemical investigation. Rectified spirit extract of the plant of *Swertia chirata Ham* a crude product. It contained a mixture of compounds. In this chapter, an attempt was made to isolate the individual compounds. TLC examination of rectified spirit extract, petroleum ether: ethyl acetate (1:2) showed 3 spots of R_f values 0.1, 0.54 and 0.73, respectively.

The crude rectified spirit extract was subjected to an alumina column for fractionation. The column containing 10 gm of alumina was prepared as described before. Crude extract (2.5 gm) for the purpose was mixed with a little rectified spirit in a morter to get a free following mass. The sample was then placed carefully on the top of the prepared column and was successively eluted with n-hexane, n-hexane with increasing portions of petroleum ether; ethyl acetate and finally with methanol (Table 1). A number of colour bands were observed during the development of the column. The subsequent eluants were collected in 100 ml beakers (Table 2).

Examination of the combined fractions

Fraction A: Fraction A gave no spot on TLC examination and was discarded.

Fraction B: The residue (35 mg) from fraction B did not give any discrete spot with different solvent system and tailed badly and was not worked out further.

Fraction C: The fraction showed two spots on the TLC, placed with solvent system n-Hexane: Ethyl acetate (3:1). The spots were pink in color under UV light. The band were contains R_f value=0.67 and 0.583, respectively. The residue from fraction C was subjected to a small chromatographic column fitted with a cotton-plug, using Ethyl acetate: Toluene (1:4). The two eluants were collected and evaporated to get small amount of compounds C_1 and C_2. The compounds C_1 and C_2 were insufficient quantities and were not worked out further.

Fraction D: The fraction showed two spots (R_f=0.53 and 0.73) on TLC plate using the solvent system Petroleum ether: Ethyl acetate (1:2). The spot having R_f=0.73 showed a violet fluorescence under UV light and yellowish spot in iodine vapour. While the other spot having R_f=0.53 was pink under UV light and yellow in iodine vapor. The components on chromatoplate did not react with potassium permanganate reagent and reacted with vanillin sulfuric acid reagent giving bluish violet spots.

The residue (110 mg) from fraction D was subjected on alumina to a small chromatographic column using petroleum ether: ethyl acetate (1:1). The two eluants were colleted and evaporated to get two compounds designated as AJ-1 (45 mg) and AJ-2 (8 mg).

The compound AJ-1 was crystalline but the compound AJ-2 was not crystalline. The compound AJ-2 was insufficient quantities and was not worked out further.

Fraction E and F: These two fractions appeared to be a mixture of components, which had R_f values very close to each other and could not be separated. The fractions were therefore preserved for further studies in future.

Fraction	Beaker number	Yield of residue
Fraction-A	1-6	9mg
Fraction-B	7-11	35mg
Fraction-C	12-17	60mg
Fraction-D	18-21	110mg
Fraction-E	22-25	25mg
Fraction-F	26-30	45mg

Table 2: Name of the fraction obtained from column chromatography.

Isolation of the compound AJ-1 from small chromatographic column

From the TLC analysis of the fraction obtained from small column chromatography, it was observed that the compound AJ-1 contained only one compound. This was recystallized from methanol-ethyl acetate mixture.

Purification of the compound AJ-1: The compound AJ-1 was further recrystallized dissolving in petroleum ether: ethyl acetate (1:1) and the crystals were washed with different solvents of varying polarity when needle-shaped crystals were obtained.

Purity Test: This isolated compound AJ-1 was tested in different solvent systems for its purity. The compound showed a single spot on TLC examination. So this compound is pure. Finally its R_f values were determined using the various solvent systems (Table 3).

Spectral characteristics of the compound AJ-1

Infrared (IR) Spectrum of the compound: Infrared (IR) spectrum (Figure 2) of the compound in KBr (spectroscopic grade) pellet was recorded with a pye-unicam SP^3-300 spectrophotometer. The samples were put in an agate mortar and thoroughly powdered with KBr and then transferred in a disc holder and a disc was made by hydraulic press. The KBr pellet was mounted in the sample cavity of the machine.

The infrared (IR) spectrum of the compound had nine characteristic bands in the functional group region at 3509 (OH-streching); 2993, 2971, 2938 (CH-streching); 1762, 1744; 1705 (>C=O); 1502 (NH) and 1150 (CH-streching) cm^{-1}, respectively.

^{1}N-NMR Spectrum of the compound AJ-1: ^{1}H.NMR spectrum of the compound was recorded on NMR spectrophotometer (500MHz) at the Faculty of Pharmaceutical Sciences, Nagoya City University, Tanbe-dori, Mizuho-Ku, Nagoya 467, Japan. $Pyrd_5$ was used as solvent and the chemical shifts are given in δ-values.

^{1}H-NMR data of the compound

^{1}HMR ($Pyrd_5$, 500 MHz)

δ : 7.73 (1H,S, ArH), 7.65~7.67. (3H, m, ArH)

δ : 6.70 (2H, dd, J=1.3 and 12.2 Hz, terminal=CH_2 olefinic)

Solvent system and ratio	R_f value
Petroleum ether: Ethyl acetate (1:2)	0.73
Chloroform: Ethyl acetate (49:1)	0.92
Ethyl acetate: Chloroform (19:1)	0.79
Toluene: Ethyl acetate (3:1)	0.88
Ethyl acetate: Pyridine: Water (5:1:4)	0.98
Ethyl acetate: Chloroform: Methanol (2:2:1)	0.95
Benzene : Ethyl acetate (19:1)	0.86
Chloroform: Ethyl acetate (4:1)	0.91
Ethyl acetate: Acetone (9:1)	0.94

Table 3: TLC analysis of the compound on silica gel G.

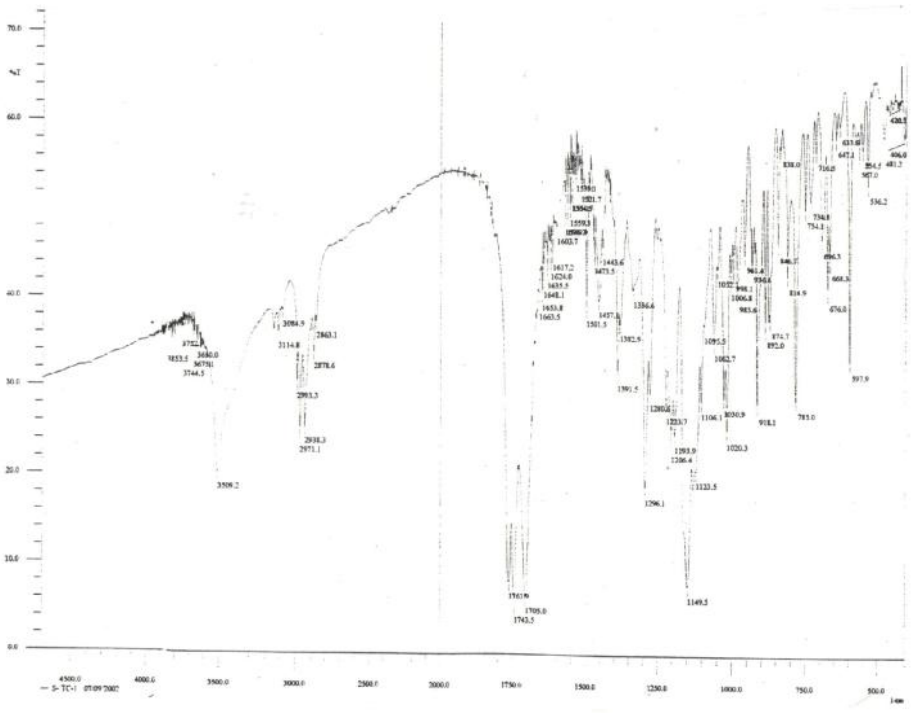

Figure 2: IR Spectrum of Compound AJ-1.

δ : 6.51 (1H, dd, J=2.2 and 9.6 Hz, =CH)

δ : 6.43 (1H, dd, J=6.4 and 9.6 Hz =CH)

δ : 5.99 (1H, dd, J=5.1 and 15.3 Hz, =CH) : Two olefinic terminal

δ : 5.92 (1H, dd, J=5.1 and 15.3 Hz, =CH)=CH_2

δ : 5.45 (1H, bd, J=5.1 Hz, =CH): Five olefinic=CH

δ : 5.26 (2H,S, terminal olefinic CH_2)

δ : 2.94~2.87 (3H, m, CH_2)

δ : 2.60 (2H, bd, J=Hz,=CH_2)

δ : 2.51 (2H, tdt, J=Hz,=CH_2)

δ : 2.34 (2H, S, CH_2)

δ : 1.98~2.14 (10H, m, CH_2)

δ : 1.75~1.85 (4H, m, CH_2)

δ : 1.52 (4H, S, either NH or OH, or both protons)

δ : 1.33 (6H, S, 2XCH_3)

δ : 1.32 (3H, S, CH_3)

δ : 1.31 (3H, S, CH_3)

^{13}C-NMR (Pyr d_5, 500 MHz)

^{13}C-NMR spectrum of the compound was also recorded (Figure 3) on NMR- spectrophotometer (500MHz) with 100 MHz frequency and pyrd$_5$ was sued as solvent and the data are given below.

<C=O at 176.02, 174.41, 174.36 and 173.08 ppm.

ArCH at 144.63, 140.86, 138.08 and 130.55 ppm.

ArC at 126.59 and 126.49 ppm

=CH at 109.75 and 109.72 (Down field signals may be due to the presence of neighbouring electronegative group, such as OH)

=CH at 74.47, 71.56 and 71.47 (olefinic CH)

–CH at 52.65, 50.53, 47.53, 47.59, 46.88, 45.09 and 44.60 (aliphatic)

=CH_2 at 81.97 and 81.90 ppm (olefinic=CH_2)

–CH_2 at 42.26, 42.15, 41.79, 38.33, 36.93, 35.52, 27.19, 27.04, 18.27 and 17.60, CH_2 aliphatic)

–CH_3 at 28.93, 28.41, 24.98 and 23.87 ppm

Result and Discussion

Preliminary extraction

The paste materials were taken in a clean flat bottomed glass container (2.5 litre) and macerated with sufficient amount of rectified spirit and with occasional shaking. After 15 days the solvents was decanted and filtered by tincture press. Then the extract was evaporated under normal pressure and normal temperature to obtain a gummy mass.

Examination of the rectified spirit extract

The rectified spirit extract showed at least three components (R_f=0.4, 0.54 and 0.73) on thin layer chromatographic plates with Petroleum ether: Ethyl acetate (1:2). The rectified spirit extract was then subjected to column chromatography on alumina. The column was eluted successively with n-hexane, n-hexane-petroleum ether mixtures and finally with methanol (Table 1). The fractions were combined on the basis of their preliminary TLC examination to give combined fractions A, B, C, D, E and F (Table 2). The combined fractions were evaporated to dryness under reduced pressure. Fraction A did not give any residue.

Fraction D: Fraction D showed two spots (R_f=0.53 and 0.73) on

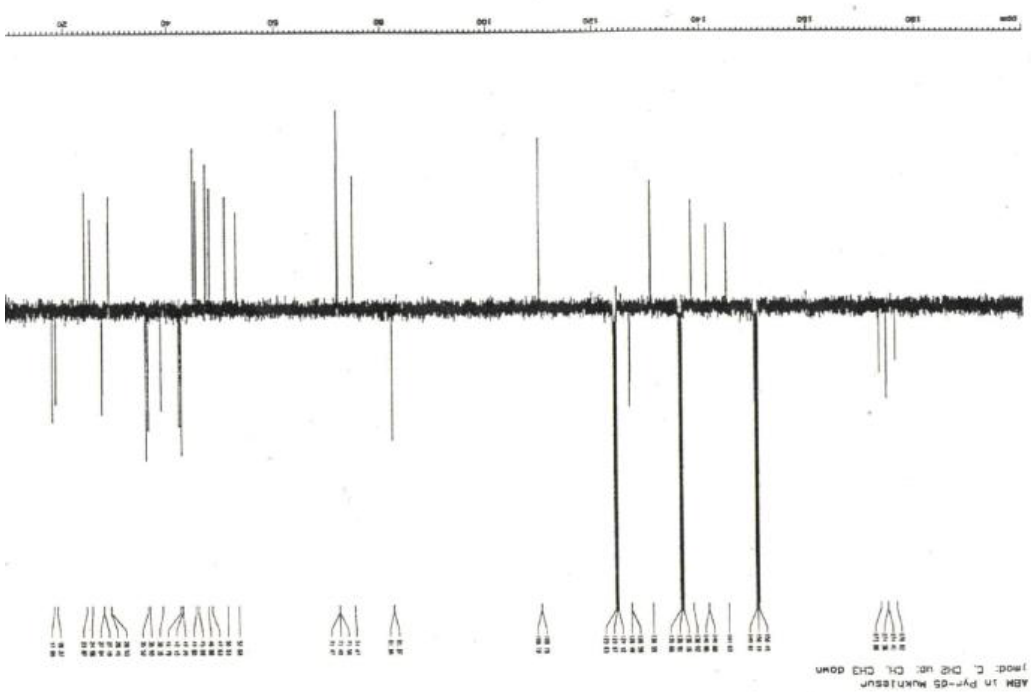

Figure 3: ^{13}C-NMR Spectrum (500 MHz, pyr-d_5) of Compound AJ-1.

TLC plates using solvent system Petroleum ether: Ethyl acetate (1:2). The fraction D was further subjected on mini column chromatography using Petroleum ether: Ethyl acetate (1:1). The two eluants were collected and evaporated to get two components designated as AJ-1 (40 gm) and AJ-2 (5 mg). The component AJ-1 was crystalline but the component AJ-2 was not crystalline and was insufficient quantities.

Isolation and purification of pure compound

The compound AJ-1 showed single spot on TLC analysis with some impurities. The compound AJ-2 was recrystallized dissolving in Petroleum ether: Ethyl acetate (1:1) and the crystals were washed with different solvents of varying polarity. The isolated compound AJ-1 was tested in different solvent systems (Table 1) for its purity. The compound showed a single spot on TLC examination. So, this compound was pure. Finally its R_f values (Table 3) were determined using the various solvent systems.

Characteristics of the compound AJ-1

(A) Physical characteristics

Physical form: Fine crystals (needles shaped) having single R_f value was obtained.

Color: The compound was all most colorless.

Solubility: The solubility data of the compound have been summarized below.

The compound is highly soluble in ethyl acetate and chloroform.

The compound is insoluble in n hexane, petroleum ether.

The compound is sparingly soluble in ethanol, diethyl ether.

Melting point: Melting point of the compound was observed in REICHERT AUSTRIA, Nr. 341910 melting point apparatus; melting point of the compound is 178°C.

(B) Chemical characteristics:

The compound gave positive test for ketone group, tertiary amine group, alcohol, ester, unsaturation, alkaloid and negative test for carbohydrate, phenol and hydrocarbon.

^{1}H and ^{13}C-NMR (Neuclear Magnetic Resonance) Spectra: ^{1}H and ^{13}C-NMR spectra were recorded on a JEOL-AX-500 (500 MHz) and JEOL-JNM AX 400 (400MHz), FT NMR spectrometers, Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan. CDC13 was used solvent with Tetramethylsilane (TMS) as an internal standard and the chemical shifts are given in δ-values. All organic extracts were dried over anhydrous Na_2SO_4 and solvent was evaporated off under reduced pressure in a rotary evaporator.

Study of spectral data: The Infrared (IR) spectrum (Figure 2) of the compound in KBr disc showed the absorption bands at 3509; 2993, 2971, 2938; 1762; 1744; 1705; 1502 and 1150 cm^{-1}, respectively. The absorption bands observed could be assigned to the following:

The band at 3509 cm^{-1} showed O–H stretching vibration in hydrogen bonded alcohols and phenols.

Bands at 2993, 2971 and 2938 cm^{-1} showed alkane type C–H stretching (C–H stretching vibration in CH_3).

The bands at 1705 cm^{-1}, 1502 cm^{-1} and 1150 cm^{-1} indicated the presence of >C=O, NH and CH–stretching, respectively.

From the complete decoupling ^{13}C-NMR spectrum of the compound, a total of 37 carbon signals were detected (Figure 3).

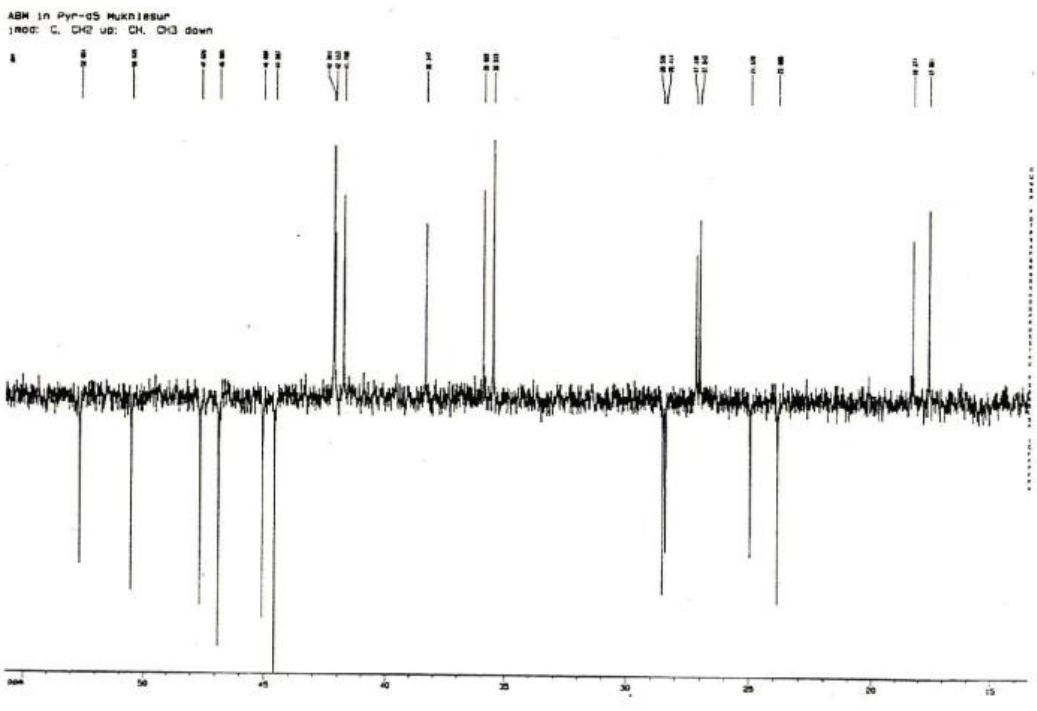

Figure 4: Expanded ^{13}C-NMR Spectrum (500 MHz, pyr-d_5) of Compound AJ-1.

In J resolved DEPT 45° spectrum four down field signals appeared at 173.08, 174.36, 174.41 and 176.02 ppm and are assumed to be carbonyl carbon. Chemical examination showed the positive test for carbonyl group and absence of aldehyde which is also supported by the appearance of three peaks in its IR spectrum at 1762, 1743 and 1705 cm^{-1} for carboxyl functions. Signal for aldehyde group proton did not appear in ^{1}H-NMR spectrum. From the above findings, four ketone functions are present in the molecule. Six aromatic carbons appeared at 126.49, 126.5, 130.55, 138.03, 140.85 and 144.63 among which two are quaternary and the other four are methane carbon atoms, identified by DEPT 135° spectrum, which indicates the presence of a benzene ring in the molecule.

Four benzene ring protons are present in the ^{1}H-NMR spectrum and their splitting pattern is 1:3 i.e. a singlet equivalent to one proton and a multiplet equivalent to three protons. From the above ^{13}C-NMR and ^{1}H-NMR spectra, the benzene ring, substituted in two portions which are presumed to be ortho and para and are evident form the assymmetrical splitting pattern of the benzene ring protons (1:3) (Figure 4).

Eleven methane carbon signals appeared in the ^{13}C-NMR and are identical from the DEPT 135° spectrum–out of which 5 carbons are double banded carbon and the rest are saturated CH carbons. Of the five doubly bonded methane carbons, two carbons showed down field shift probably due to the presence of electro negative groups or atoms present adjacent to these two carbons which appeared at 109.72 and 109.75 ppm and the rest three carbons appeared at 71.47, 71.56 and 74.47 ppm. Six saturated methane carbons appeared in higher field at 44.60, 45.09, 46.88, 47.53, 50.53 and 52.65 ppm.

Two low field methylene carbon signals appeared at 81.97 and 81.90 ppm indicated the presence of two terminal CH_2 groups with double bond in the side chain of the molecule. The other 10 saturated CH_2 carbon signals appeared between 17.60 and 42.26 ppm.

From the methyl carbon signals at 23.87, 24.98, 28.41 and 28.93 ppm, it has been confirmed that four methyl groups are present in the molecule. This is also supported by its ^{1}H-NMR (Figure 5) spectrum. Four methyl group protons appeared at δ: 1.33 (6H, $2XCH_3$), 1.32 (3H, $1XCH_3$) and 1.31 (as 3H, $1XCH_3$) singlets.

From the ^{1}H-NMR spectrum of the compound four benzene ring protons appeared in an unsymmetrical pattern, one as singlet at δ: 7.73 and other three as multiplet between δ: 7.65~7.67. Five doubly bouded methane protons appeared at δ: 6.51 (1H, dd, J=2.2 and 9.6 Hz), 6.43 (1H, dd, J=6.4 and 9.6 Hz), 5.99 (1H, dd, J=5.1 and 15.3 Hz), 5.92 (1H, dd, J=5.1 and 15.3 Hz) and 5.45 (1H, bd, J=5.1 Hz) (Figure 6). One doublet, equivalent to two protons, appeared at δ: 6.70 with J values of 1.3 and 12.2 Hz and other singlet of two protons appeared at δ: 5.26. These two sets of protons seems to be a doubly bonded terminal methylene group present in the molecule. The above discussion field proton signals satisfy all the carbon signals appeared in the down field region in ^{13}C-NMR spectrum.

Rest of the saturated CH_2 protons appeared between δ: 1.75 and 2.94 ppm (plesse, vide experimental). A peak of four protons appeared as singlet at δ: 1.52 which seems to be NH or OH protons or mixture of these two. Positive test with alkaloidal reagents indicated the presence of nitrogen and the presence of OH group can also be predicted from the IR spectrum since a broad peak for OH stretching centered at 3509 cm^{-1} appeared in the IR spectrum. Four methyl protons signals also appeared which have been discussed above.

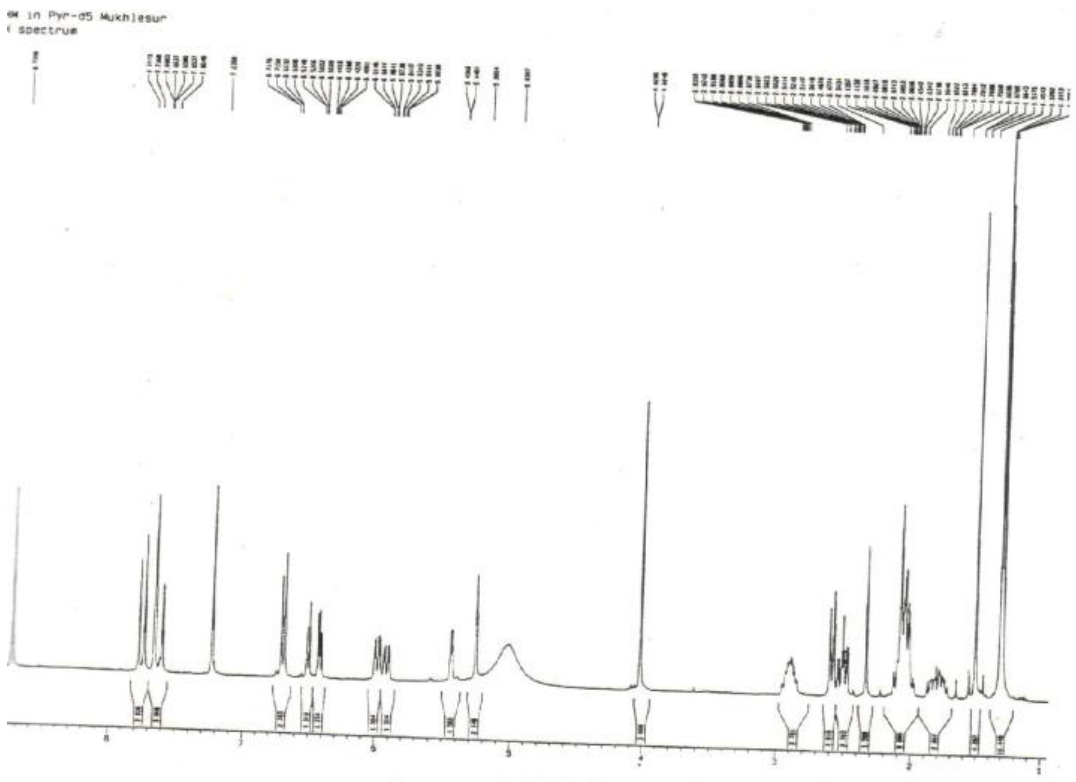

Figure 5: ^{1}H-NMR Spectrum (500 MHz, pyr-d_5) of Compound AJ-1.

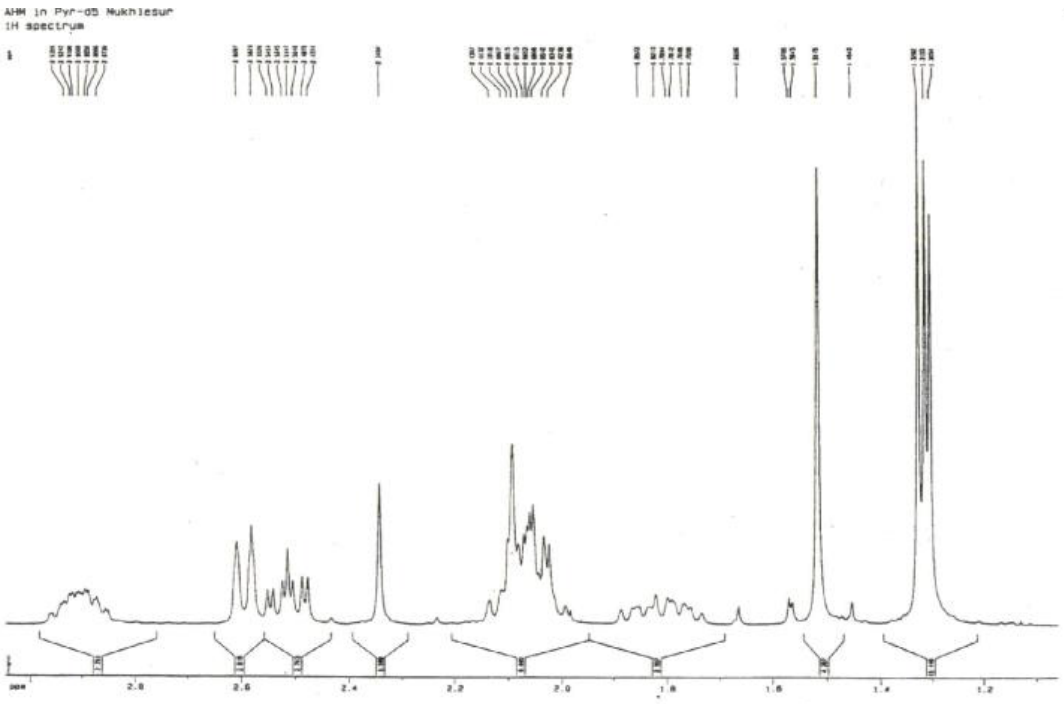

Figure 6: Expanded ^{1}H-NMR Spectrum (500 MHz, pyr-d_5) of Compound AJ-1.

Form the above spectral evidences; the compound is an alkaloid containing 37 carbon atoms with 52 hydrogen along with secondary or tertiary nitrogen and several OH groups in the molecule.

Conclusion

From the research work only one compound is isolated and the above spectral evidence, the compound is an alkaloid containing 37 carbon atoms with 52 hydrogen along with secondary or tertiary nitrogen and several OH groups in the molecule. For complete structure elucidation, Mass spectrum, HSCOSY, HSQC and HMBC spectra will be required. From the above discussion, it is clear that this plant may contain other medicinal compounds. Further research may be extended to isolate active compounds specially from fractions B, C, E and F.

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