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Isolation of novel Steroidal Saponin from the stem extract of Andrographis echioides L.Nees

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Abstract— *The field of ethnopharmacology recommends the use of traditional approaches for the development of drugs from natural sources. The herbaceous species Andrographis echioides, commonly found in the dry regions of the Indian subcontinent and Sri Lanka, was the subject of the present study, which aimed to test this hypothesis by isolating and characterizing compounds from the plant. Specimens were collected from Trichy and identified using monographic data. The ethanolic extract was subjected to preliminary analysis, and chromatographic techniques were employed to separate secondary metabolites. The bioactive compounds were then characterized to determine their chemical compositions and molecular structures. This study emphasizes the significance of natural medicines, specifically focusing on diosgenin, a secondary metabolite found in Andrographis echioides. HPTLC analysis of the ethanolic extract revealed promising results, with Rf values similar to those obtained by ultraviolet spectrum analysis. IR analysis indicated the presence of chemical groups with distinct peak formation, while NMR results demonstrated the intricate structure of the molecules and their associated chemical groups. In conclusion, harnessing the power of natural sources in the pharmaceutical industry has the potential to revolutionize drug discovery and development, as long as the challenges associated with standardization, quality control, and safety are addressed.*

Keywords— ***Drug discovery, Ethnopharmacology, Herbaceous, Molecular structures, Standardization.***

I. INTRODUCTION

Drug discovery necessitates balancing safety and health care [1,2]. Institutions have been established to advance drug discovery [3,4], Aspirin's discovery transformed modern medicine. Despite this progress, ongoing challenges remain. Research strategies must be adapted owing to high costs, lengthy processes, low success rates, and high productivity. Natural sources from plants and microbes, utilizing combinational and genomic methods and rapid in vitro screening, have emerged from traditional medicine [5]. An interdisciplinary approach is essential, involving species identification, compound isolation, and extraction using ethnopharmacology [6]. The isolation of morphine from *Pappaver somniferum* (Opium) initiated natural product drug isolation [7]. Numerous scientists have isolated phytochemicals, primarily alkaloids, from medicinal plants [6]. Historical records show the use of over 1000 medicinal plants, such as *Glycyrrhiza glabra* and *Papaver somniferum*, dating back 5000 years [8]. Ethnopharmacological studies suggest that traditional methods can be used to derive drugs from plants, microorganisms, and animals. In India, over 45,000 species have been identified, with 7000-7300 used in clinical applications [9]. Since the discovery of aspirin in Salix trees, the importance of ethnopharmacology has increased [10]. Metformin from *Galega officinalis*, initially used for type II diabetes, shows potential against cancer [11]. Various medicinal

plants enhance community wellness, with some native species serving as edibles, condiments, and spices [12]. Specific plants provide immediate clinical aid to children, elderly individuals, and pregnant women [13]. Indigenous plants contain phytoconstituents in their stems, leaves, roots, bark, flowers, fruits, and seeds, which aid in the treatment of severe diseases [6]. Advancements in secondary metabolite isolation, genetic modifications, animal experiments, and human studies [14] have led to the discovery of specific metabolites for various ailments, such as Taxol from *Taxus officinalis* for cancer [15], Quinone from *Cinchona officinalis* for malaria [16], Emetine from *Carapichea ipecacuanha* for protozoan infections [17], Colchicine from *Colchicum autumnale* [18], and *Gloriosa superba* [19] for gout and rheumatoid arthritis pain, Atropine from *Atropa belladona* as an anti-motility agent [20], and galantamine from *Galanthus woronowii* for early-stage Alzheimer's disease [21]. *Andrographis echioides*, a herbaceous Acanthaceae species, is prevalent in dry regions of the Indian subcontinent and Sri Lanka [22]. The plant, known by various names [23], features an erect habit, with numerous outgrowths, sessile hairy leaves, and sparsely branched raceme inflorescences. The rhombic hairy stem bears white and brownish tubular corollas with hairy lanceolate calyces. Ovoid yellow seeds are found in elliptical, minimally hairy capsules [24,25]. *Andrographis echioides* treats various ailments and is used in traditional Indian medicine [26,27]. Its pharmacological effects are attributed to various phytocompounds, including echioidinin, flavonones, dihydroechioidinin, skullcapflavone derivatives, pinostrobin, androechin, methyl salicylate glucoside, glucopyranoside, and andrographidine [26-29]. The current century underscores the necessity of healthy diets and medications for survival, highlighting dependency on medicines. This indicates a significant interest in naturally occurring medicines, owing to their minimal side effects and accessibility. The present study tested this hypothesis by isolating and characterizing drugs from the natural plant source of *Andrographis echioides.*

II. MATERIALS AND METHODS

2.1 Plant collection and extraction

In this study, stems of *Andrographis echioides* were collected from Trichy. It was identified using keys and plant descriptions from several articles and monographs [30]. The *Andrographis echioides* stem was collected, cleaned, and shaved. The dried stems of the plant were ground using a mechanical grinder and then passed through a 20-mesh sieve. The ethanolic extract of the powdered sample (500 g) was extracted using the hot percolation method. The extract was kept at room temperature (35 °C) for 24 h with mild shaking and then filtered for further analysis [31].

2.2 Phytochemical analysis:

2.2.1 Qualitative analysis:

To evaluate the presence of bioactive compounds, including flavonoids, saponins, terpenoids, tannins, alkaloids, steroids, glycosides, phytosterols, protein, coumarin, emodin, anthraquinone, anthocyanin, carbohydrates, leucoanthocyanin, cardiac glycosides, xanthoproteins, and phenols in the *Andrographis echioides* stem extract, qualitative analysis is performed using specific tests tailored to each chemical compound, along with appropriate solvents [32,33].

2.2.2 *Quantitative analysis:*

Quantitative analysis was performed to determine the concentration of bioactive compounds in the stems of *Andrographis echioides*, enumerating the phytochemicals present, such as flavonoids, saponins, phenols, terpenoids, alkaloids, and tannins, by conducting specific tests for each compound [32,33].

2.3 Preparations of ethanolic extracts

The ethanol extracts were prepared by immersing 500 g of dried plant material in 1 liter of ethanol for a continuous 10-hour period utilizing a Soxhlet extractor. Subsequently, the samples were strained through Whatman filter paper no. 42 which is 125 mm, concentrated, and dried using a rotary evaporator at a reduced pressure. The resulting dried samples, weighing 50 g, were then securely stored in sterile bottles at -20 °C [22].

2.4 Isolation techniques

2.4.1 Column chromotography

The ethanol extract of the samples was used for column chromatography analysis to separate the compounds using thin-layer chromatography with silica gel (Figure 1). The column was eluted with n-hexane, which resulted in an increased amount of ethyl alcohol, and fractions were obtained along with methanol. The solvents used to generate disogenin (56 mg/ 403 g) and ethyl alcohol: methanol (60:40 v/v) were 13 fractions. The secondary metabolites were identified TLC plates by spraying with the Libermann-Burchard reagent and heating at 100 °C for 10 min [34].

Fig: 1 Column chromatography

2.5 Purification techniques of the Isolated compounds:

2.5.1 High perfomance Thin-layer chromotography (HPTLC)

Individual compounds were subsequently dissolved in an appropriate solvent. Of the isolated compounds, 5 µL of disogenin was submerged in silica plates (measuring 20 × 20 centimeters and 0.25 millimeters in thickness) procured from Merck (Germany). A solvent system comprising n-hexane and ethyl acetate at a ratio of 7:3 (v/v) was used to develop the plates for disogenin. Application of the newly prepared Liebermann reagent resulted in distinct zones. The reagent was heated at 100 °C for 10 min. The chromatograms were inspected under normal daylight conditions within the stipulated timeframe [35].

2.5.2 High-performance liquid chromatography (HPLC)

The HPLC System was found with a diode-array detector, and 200 x 4.6 mm C18 column. Methanol (HPLC grade, 0.2 mm filtered) was used as the mobile phase. Disogenin was isolated using a mobile phase consisting of acetonitrile and water (80:20 v/v) at a flow rate of 1.0 ml/min and temperature of 30o C. The injection volume was 40 µL and detection was performed at 346 nm [36].

2.6 Characterization techniques of the separated compound:

Numerous assessments have been performed to identify the isolated substances. Spectroscopic methods employed included ultraviolet analysis, Fourier transform infrared analysis, Hydrogen-1 Nuclear Magnetic Resonance, and Carbon - 13 Nuclear Magnetic Resonance, and gas chromatography-mass spectrometry. The UV–visible spectrum of the isolated compounds in methanol was recorded using a 160A UV–visible spectrophotometer [37]. FTIR spectra were obtained using the Kbr technique, with a nominal resolution of 4 cm^{-1} and a range of 400–4000 cm^{-1} [38]. 1H and 13C NMR spectra were acquired on Bruker WP 200 SY and AM 200 SY instruments (1H, 200.13 MHz; 13C, 50.32 MHz) with TMS serving as the internal standard and CDCL3 as the solvent [39].

III. RESULT AND DISCUSSION

3.1 Phytochemical analysis

3.1.1 Qualitative analysis:

The ethanolic extract of the stem contains various phytoconstituents, including tannins, saponins, flavonoids, terpenoids, alkaloids, steroids, proteins, coumarins, phenols, xanthoproteins, glycosides, emodin, anthocyanins, cardiac glycosides, and leucoanthocyanins shown in TABLE 1. These active phytoconstituents are responsible for the therapeutic properties of the plants. A study conducted in Shahada yielded anticipated outcomes. This investigation utilized *Elytraria acaulis* from the family Acanthaceae. A preliminary investigation was conducted on three types of extraction: chloroform, petroleum ether, and methanol. The two initial extractions resulted in a predominance of steroids and alkaloids, consistent with the aforementioned outcomes [40]. Comparable results were found in another study on *Astercantha longifolia*, which demonstrated the abundance of sterols in petroleum ether extraction, whereas ethanolic extraction revealed alkaloids, tannins, and sterols [41]. Another study on *Andrographis lineata* also reported the presence of steroids and flavonoids in all five types of extracts [42]. Additionally, *Dyschoriste pedicellata* showed increased levels of steroids, terpenoids, and glycosides [43].

Table 1: Qualitative analysis of Andrographis echoides stem

S.No	Phytochemical constituents	Andrographis echoides
1	Terpenoids	+++
2	Flavanoids	+++
3	Saponin	+++
4	Tannin	+++
5	Alkaloids	+++
6	Steroids	+++
7	Glycosides	+++
8	Phlobotannins	-

9	Protein	+++
10	Coumarin	+++
11	Emodin	++
12	Anthroquinone	+++
13	Anthocyanin	+++
14	Carbohydrate	++
15	Leucoanthocyanin	-
16	Cardiac glycosides	+++
17	Xanthoproteins	+++
18	Phenol	++

[+ = slightly present, ++ = moderately present, +++ = strongly present, - =absence]

3.1.2 Quantitative analysis

A quantitative investigation of some significant phytochemicals in the ethanolic extract of *Andrographis echoides* revealed the presence of various quantities of these phytochemicals. As shown in the TABLE 2, phytochemical steroids were found to be the most abundant, followed by flavonoids, tannins, saponins, alkaloids, and terpenoids. Several investigations have been conducted on *Strobilanthes kunthias*, including phytochemical analyses. Quantitative analysis revealed that steroids exhibited the highest values, which was consistent with the aforementioned findings [44]. In another study, *Andrographis echoides* leaves demonstrated the highest yield of flavonoids, tannins, and steroids [45]. The steroids and glycosides present in *Adathoda vasica* and *Adathoda beddomei* were found at significantly higher levels [46]. In addition, *Tubiflora acaulis* from Acanthaceae yielded the highest number of steroid compounds [40].

Table 2: Quantitative analysis of Andrographis echoides stem

S.No	Phytocompounds	Empty Value (mg/g)	Yield Value (mg/g)	Final Value (mg/g)
1.	Phenol	18.830	18.846	0.016
2.	Flavonoid	20.656	20.669	0.013
3.	Tannin	20.286	20.326	0.04
4.	Saponin	21.046	21.088	0.42
5.	Alkaloids	19.325	19.595	0.27
6.	Terpenoids	18.771	18.785	0.014

3.2 Isolation techniques

3.2.1 Column chromatography:

In the present study, 29 fractions were isolated. Notably, the final 29th fraction contained the desired secondary metabolite, disogenin, which had a strong brown color. The initial fraction was transparent, while the 2nd and 3rd fractions were dark green. The color gradually faded throughout the subsequent fractionations, with transparency returning to the 13th fraction before reappearing as a dark brown color in the 24th fraction. The next two fractions were transparent once more, before a brown color began to emerge in the 27th fraction. Finally, the 29th fraction yielded crude disogenin with a thick, strongly brown color shown in Figure 2. A study was conducted to isolate bioactive compounds from *Tagetes erecta* of the Asteraceae family using column chromatography. These findings are consistent with previously mentioned outcomes. The final fractions were brownish and exhibited promising yields [47]. Similarly, a study on *Careya arborea* from the Lecythidaceae family confirmed the aforementioned results by providing expected yield values with a light yellow appearance [48]. Column chromatography isolation of *Curcuma longa* also produced a brownish color, suggesting promising results [49]. *Odontonema strictum* exhibited white-colored formations in the final fractionation, partially supporting previously mentioned findings [50]. Biological investigation of Andrographis panniculata revealed a dark green appearance in the final fractionation, which was consistent with the results described above [51].

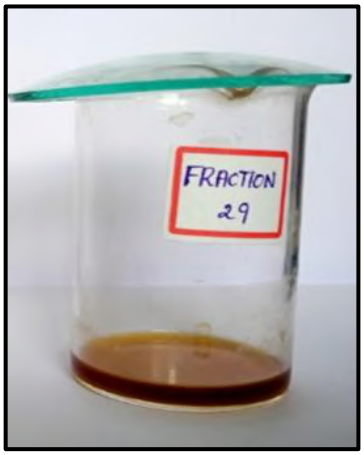

Fig: 2 Isolated end product from Column chromatography

3.3 Purification techniques of the Isolated compounds:

3.3.1 High perfomance Thin liquid chromatography (HPTLC)

The ethanolic extract of diosgenin was subjected to High-Performance Thin-Layer Chromatography (HPTLC) analysis using a 1-D method with silica gel and

various solvent systems. Specifically, n-hexane, ethyl acetate, and methanol were used as solvents. The ratios of these solvents were optimized to 3:7 (ethyl acetate/methanol) and 2:8 (ethyl acetate/methanol). Movement of the active compound was expressed using a retention factor (Rf) value of 0.69. A study on *Periploca aphylla* was initiated to investigate the presence of bioactive compounds. The investigation yielded promising Rf values, with each compound displaying different values that were partially aligned with previously mentioned results [52]. It is plausible that an evaluation of steroidal compounds in *Ipomoea sagittifolia* would yield similar results. The Rf values varied across compounds, displaying several similarities with previously reported outcomes [53]. Another investigation of the same plant provided in-depth results, including the standard Rf values [54]. Researchers used *Holoptelea integrifolia* to investigate the presence of phytosterols, which were subjected to HPTLC analysis at 227 nm to yield the expected results [55]. A study on *Achyranthes aspera* focused on steroidal compounds, and the results indicated that the sixth peak exhibited an Rf value similar to that of the present study [56].

3.3.2 High Perfomance Liquid Chromatography (HPLC):

HPLC analysis yielded a noteworthy peak, as indicated by the retention time of 2.463 min. This measurement was performed at a wavelength of 270 nm. The peak height was 75,000 V, indicating a significant concentration of the analyte. The sharp and well-defined peak coupled with a robust baseline demonstrates strong chromatographic and detector performance (Figure 3). The compound was identified using a combination of retention time and other structural interpretation methods, which were employed to confirm its identity. A study employing HPLC on *Trigonella foenum* seeds yielded expected results. A total of 23 distinct peaks were identified, representing various bioactive compounds with different retention times aligned with the aforementioned outcomes [57]. Similarly, *Tribulus terrestris* and steroidal compounds were examined using HPLC, resulting in 7-8 peaks with different retention times [58]. Investigations into bioactive compounds in several fruit species have demonstrated promising retention times, which supports the findings of the current study [59]. *Mangifera indica* was used to quantify bioactive compounds, revealing two peaks with different retention times [60]. Additionally, *Andrographis paniculata* was subjected to HPLC with various solvents, each of which yielded 20-23 peaks with different retention times [61].

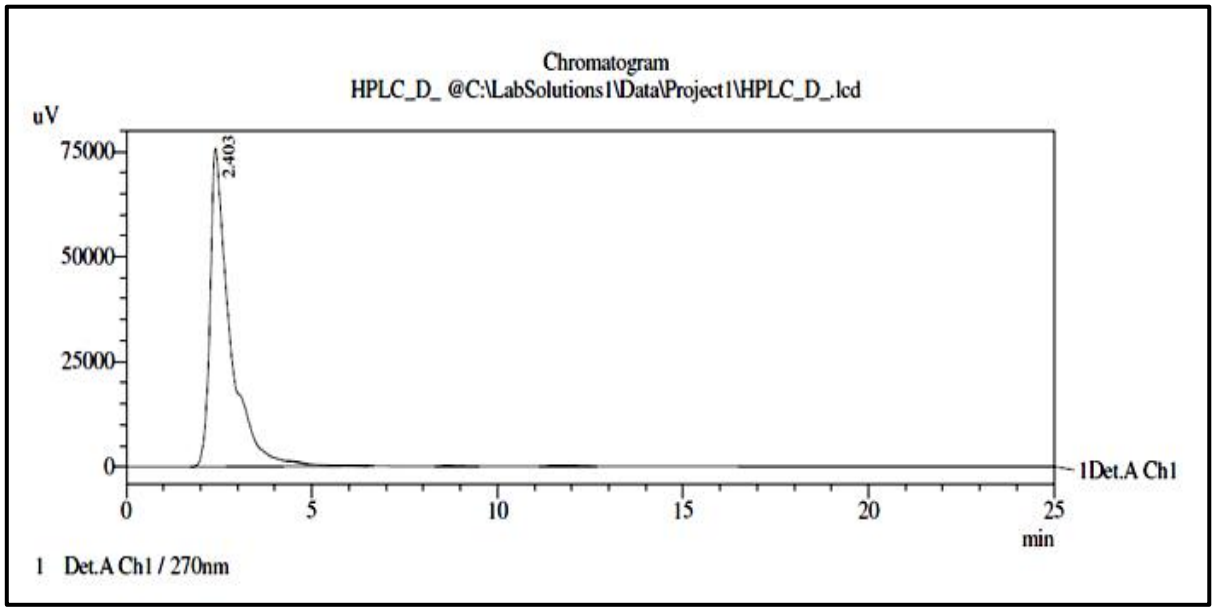

Fig: 3 Chromotogram of High perfomance Liquid chromatography (HPLC)

Table: 3 HPLC Chromotogram interpretation

Peak #	**Ret. Time**	**Area**	**Height**	**Area%**	**Height%**
1	2.403	2863901	76505	100.000	100.000
Total		2863901	76505	100.000	100.000

(Detector A Ch 1 270 nm)

3.4 Characterization techniques of the separated compound

3.4.1 Ultra-violet spectral analysis:

The identified compound was a light-crystalline powder that was dissolved in an appropriate solvent. Disogenin is associated with the 203oC melting point and the molecular formula is C27H42O3. The λmax value of diosgenin was 212 nm (Figure 4). The reported outcomes have been examined in other studies on various plant species. *Elaeocarpus tectorius* is a plant that can be used to treat tract infections. Ultraviolet spectral analysis was conducted between 250 nm and 800 nm. The two extraction methods resulted in different peak absorptions. Each peak corresponded to distinct compounds present in the plant samples [62]. *Bauhinia purpurea* was subjected to isoflavonoid isolation, which led to structural elucidation. The results of the ultraviolet spectral analysis showed four distinct peaks, which were attributed to two different ranges: 300–380 nm and 240–340 nm [63]. The leaves of *Peronema canescens* were used to isolate the bioactive compounds. Ultraviolet characterization revealed two significant bands at 329 and 270 nm. These two bands represented each group of bioactive compounds [64]. *Cordia dichotoma* from Boraginaceae was subjected to isolation studies. Ultraviolet peaks that resulted in the identification of two active compounds were observed. Upon the addition of sodium hydroxide, band shifting was observed [65]. The isolation of secondary metabolites from *Mamordica balsamina* from Cucurbitaceae was subjected to ultraviolet analysis, which resulted in the identification of ten distinct peak ranges between 200 and 750 nm [66].

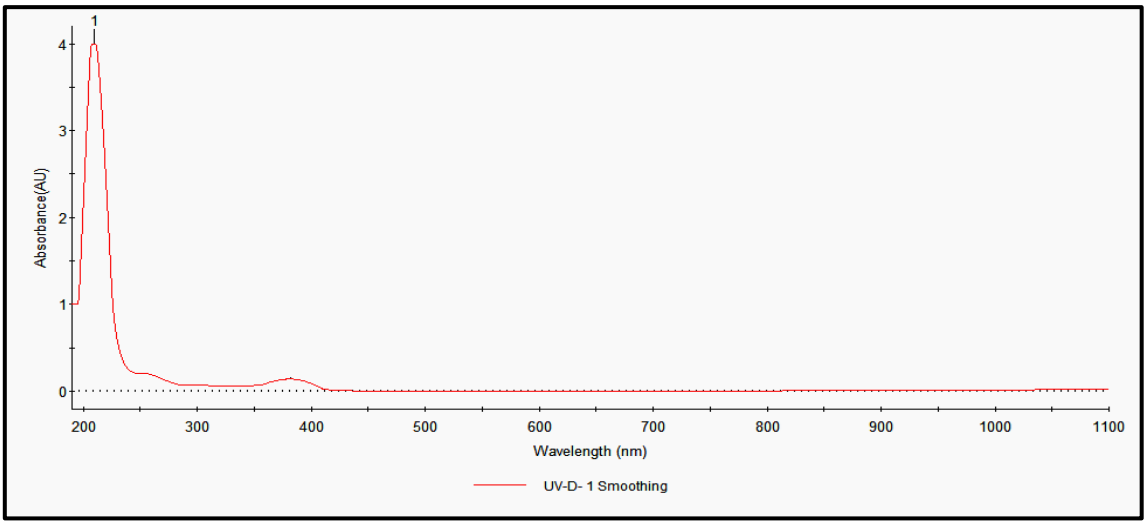

Fig: 4 Ultra violet spectroscpy analysis on the plant extract

3.4.2 Fourier transform infrared analysis

The FT-IR spectrum (KBr, vmax, cm^{-1}): diosgenin 3449.78 cm^{-1} shows the strong alcohol group, 2075.38 cm^{-1} express the C=C=C stretching with medium band spectrum, 1639.62 cm^{-1} denotes the C=C stretching with medium to weak band peak, 1457.48 cm^{-1} shows the methylene vibration with medium band peak, 1116.87 cm^{-1} denotes the C-O stretching with strong band, 1055.02 cm^{-1} strong peak express the C-O stretching, 1014.25 cm^{-1} strong denotes sulfoxide groups and the last strong 625.18 cm^{-1} denotes the presence of halo compound groups (Figure 5) TABLE 4. The IR analysis shows promising results with four strong bands, three medium bands, and one medium to weak band. Phytochemicals from the Lamiaceae family, including *Colebrookea oppositifolia*, were isolated by Fourier Transform Infrared Spectroscopy (FTIR). Analysis of the stem resulted in two strong bands, five medium-strong bands, and four weak bands within the range–750-3500 cm^{-1} [67]. Additionally, FTIR analysis in the range of 400-4000 cm^{-1} identified 10 different peaks in medicinally rich formulations, with three being strong, five medium, and the remaining two weak [68]. *Albizia coriaria*, a medicinal plant, was subjected to FTIR characterization in the range of 500-4000 cm^{-1}, resulting in two strong bands, four medium bands, and one weak band [69]. *Fritillaria roylei*, a plant belonging to the Liliaceae family, was used for the isolation of stigmasterol and subjected to FTIR analysis, which resulted in one strong band, three medium-to-strong bands, and six medium bands [70]. Finally, *D. diversifolia* was used for the isolation of secondary metabolites, and Fourier Transform Infrared Spectroscopy was used to investigate the chemical compounds in the range of 450-4000 cm^{-1}, resulting in two

strong and medium-to-strong intensity bands and four medium bands [71].

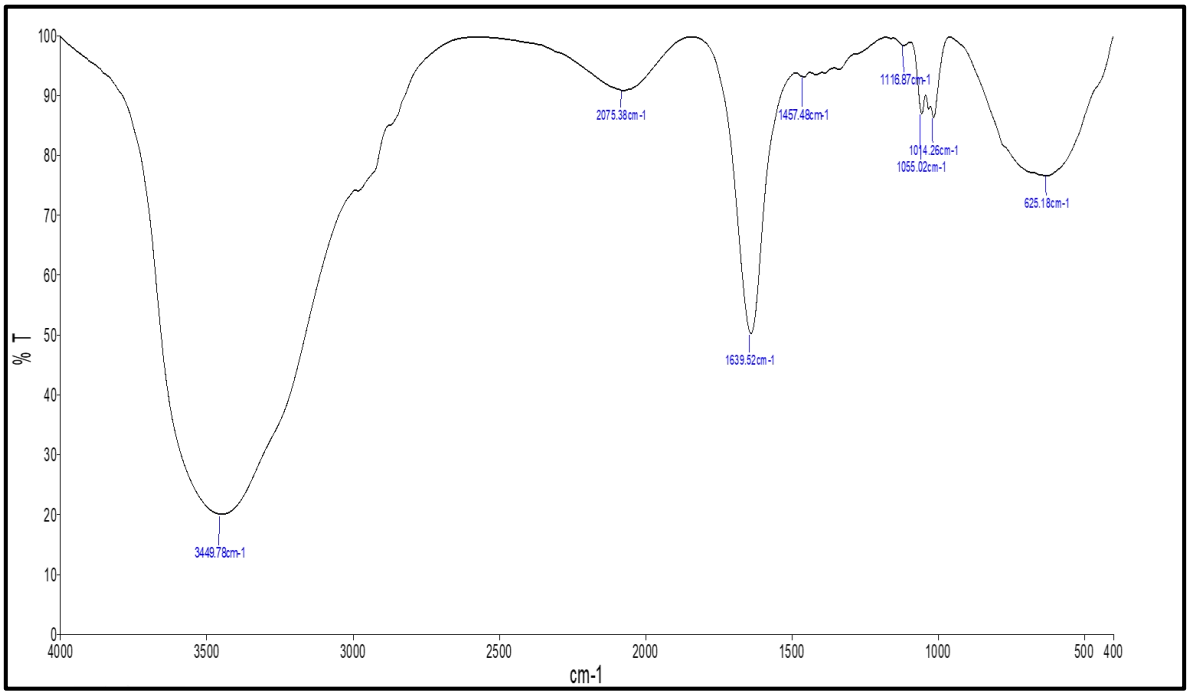

Fig: 5 FTIR analysis on the plant extract

Table: 4 FTIR analysis on the plant extract

Peak no	Wavelength	Group	Compound Class	Band type
1.	3449.78 cm^{-1}	OH	Alcohol	Strong
2.	2075.38 cm^{-1}	C=C=C	Alkene	Medium
3.	1639.62 cm^{-1}	C=C	Alkene	Medium to weak
4.	1457.48 cm^{-1}	CH2	Methylene class	Medium
5.	1116.87 cm^{-1}	C-O	Alcohol	Strong
6.	1055.02 cm^{-1}	C-O	Alcohol	Strong
7.	1014.25 cm^{-1}	Spiroketal ring	Steroidal sapogenin	Strong
8.	625.18 cm^{-1}	Halo group	Halogenated compound class	Strong

3.4.3 NMR analysis:

(a) 1H NMR Analysis

The molecule structures from 1HNMR (δ, CDCl3 as solvent, 300 MHz analysis shows 0.692 (s, C-18 methyl), 0.698 (d, J = 6.2 Hz; C-27 methyl), 0.881 (J = 7.1 Hz;C-21 methyl), 1.361 (s, C-19 methyl), 1.894 (t,J = 10.6 Hz; C-26a-H), 5.245 (d, dd, J = 10.5 Hz and J approx. 4Hz; C-26,B-H), 5.253 (broad, C-3a-H), 3.277 (q, J=7.lHz; C-16H), 5.245 (broad d,J = 5.3Hz; C-6H). The Proton H1-NMR analysis clearly shows the expected output in Figure 6 & TABLE 5. Two plant species from the Euphorbiaceae and Asteraceae families were investigated for their ability to isolate bioactive compounds. This paper proposes a characterization technique involving 1H (500 MHz) and 13C (125 MHz) proton NMR analyses. The results indicate the presence of methyl, methine, and methylene proton groups, whereas 13C proton analysis revealed the presence of acetyl and tiglyol groups [72]. Analysis of saffron plants from the Iridaceae family was conducted to separate the secondary metabolite crocetin. Heteronuclear single-quantum coherence (HSQC) revealed the presence of methylene, methyl, and anomeric protons [73].

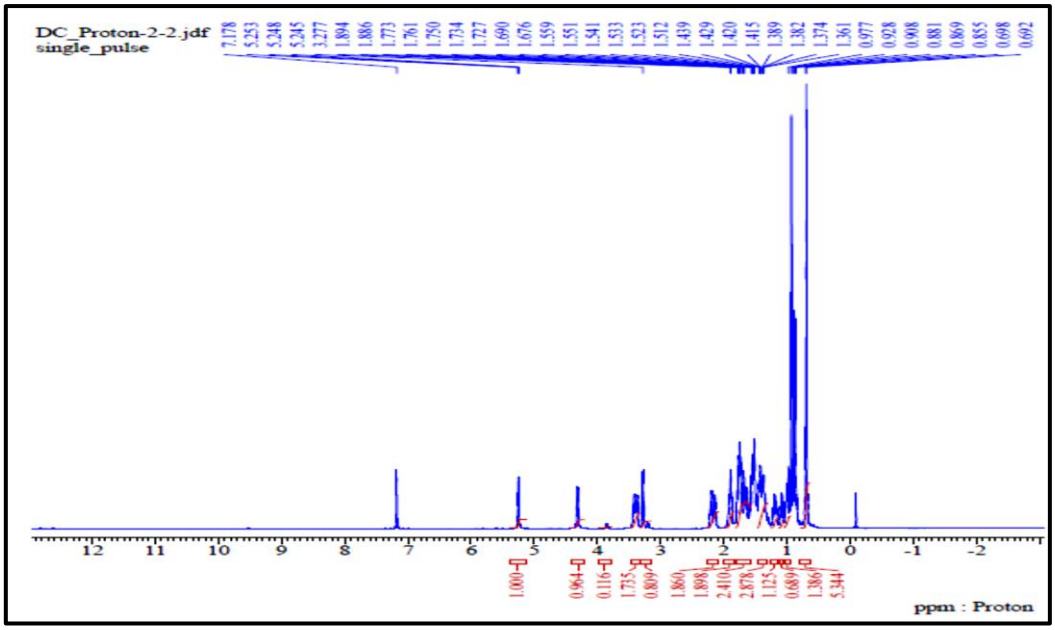

Fig.6 1H NMR analysis on the plant extract

Table: 5 1H NMR analysis result interpretation

Sample No	Chemical shift	Multiplet	J-Coupling Hz
1	0.692	s	-
	0.698	d	6.2
	0.881	-	7.1
	1.361	s	-
	1.894	t	10.6
	5.245	ddd	10.5 -4
	5.253	broad	-
	3.277	q	7
	5.245	broad,d	5.3

(s-singlet, d-doublet,t-triplet,q-quartet, ddd-doublet of doublets of doublets, broad d-broad doublet)

(b) 13C NMR Analysis

Then the another proton 13C NMR (CDCl3, 500 MHz); 37.183 (C1), 31.798 (C2), 71.648 (C3), 42.220 (C4), 140.773 (C5), 121.360 (C6), 31.338 (C7), 31.391 (C8), 50.009 (C9), 36.599 (C10), 20.830 (C11), 39.745 (C12), 41.559 (C13), 56.476 (C14), 31.558 (C15), 80.782 (C16), 62.039 (C17), 16.248 (C18), 19.379 (C19), 40.214 (C20), 14.486 (C21), 109.253 (C22), 31.558 (C23), 28.753 (C24), 30.251 (C25), 66.798 (C26), 16.248 (C27) provide the promising results Figure 7. *Capparis ovates* were subjected to steroid and triterpenoid isolation and 1H and 13C proton NMR analyses were performed at 600 MHz and 150 MHz, respectively. The outcome was an oleanane triterpene connected to long-chain methyl singlets, with ends showing methyl triplets with long chains [74]. *Euphorbia cotinilia* from Euphorbiaceae was used to separate and characterize bioactive compounds, and hydrogen and carbon proton NMR analyses were performed at 300 MHz. The results indicated the presence of aliphatic chains, methyl and methoxy groups, amine and alcohol groups, aliphatic and olefinic carbons, and aromatic protons [75]. *Cola lateritia* from Sterculiaceae were involved in the separation of bioactive compounds, and heteronuclear single-quantum coherence (HQSC) was performed at 500 MHz (1H) and 600 MHz (13C). Eight different compounds were characterized, and each showed promising results, partially supporting the findings of the current study [76].

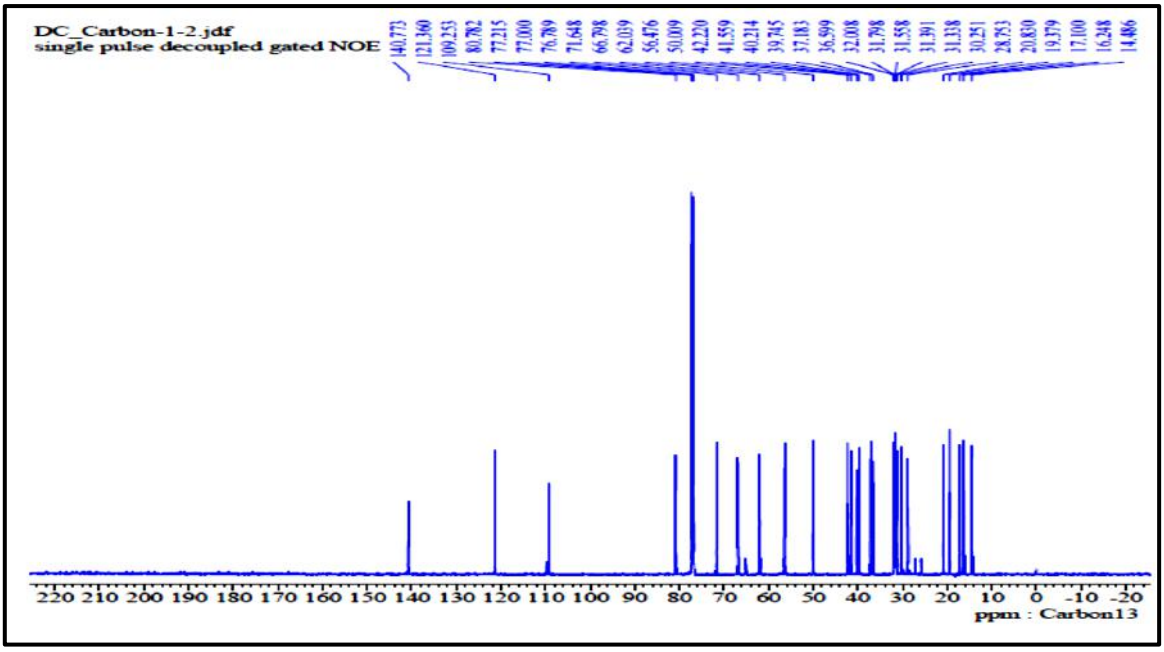

Fig: 7 13C NMR analysis on the plant extract

IV. CONCLUSION

This study focused on diosgenin, a secondary metabolite isolated and characterized from Andrographis echioides, to emphasize the value of naturally occurring medicines. The ethanolic extract of the plant was analyzed using HPTLC, revealing an Rf value of 0.69, whereas HPLC analysis showed a significant peak at 2.463 min, 270 nm, and 75,000 V peak height. The melting point of diosgenin (C27H42O3) was 203°C and λmax was measured at 212 nm. IR analysis revealed strong, medium, and weak bands, whereas the NMR results indicated the presence of methyl, methine, methylene groups, aliphatic regions, oxygenated carbons, and aromatic and olefinic carbons, which were further subjected to pharmacological activities to analyze their viability. Currently, there is a need to develop herbal and naturally synthesized drugs. An effective method to advance the pharmaceutical industry is to utilize the secondary metabolites found in plants and microorganisms, which can provide clinical assistance in treating unwanted infections and other harmful diseases. However, despite the potential benefits of using natural resources, challenges still need to be addressed in order to fully exploit these sources. By overcoming these obstacles, a new pharmaceutical world can be created that leverages the advantages of natural sources without drawbacks.

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