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RESEARCH ARTICLE

Isolation and Characterization of Chemical Constituents from *Alangium Salvifolium* Leaves Extract

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ABSTRACT

Alangium salvifolium is a medicinal plant that has been studied for its medicinal properties. This study aimed to isolate and identify bioactive molecules from A. salvifolium leaves and examine their medicinal properties. Dried leaves of A. salvifolium were extracted with petroleum ether, chloroform, and methanol using successive extraction protocols. The petroleum ether extract was then purified by column chromatography using a solvent gradient, petroleum ether, ethyl acetate, methanol, and silica gel. Isolated compound AS-01 was then analyzed by thin-layer chromatography, high-performance liquid chromatography, high-performance thin-layer chromatography, and different spectroscopic analyses, such as Fourier Transform Infrared, 1H-Nuclear Magnetic Resonance spectroscopy, and mass spectrometry. AS-01 was isolated as a white crystalline compound with a molecular weight of 528 and purity of 90%, as confirmed by HPLC. The compound showed strong structural characteristics in the form of hydroxyl, methoxy, and aromatic groups. The compound was positive for alkaloids, and the retention factor (Rf) value was aligned with the standards in both TLC and HPTLC. This study reports a novel alkaloid AS-01. The isolation and structural elucidation of AS-01 revealed new insights into the pharmacological potential of the plant.

Keywords: Alangium salvifolium; Alkaloids; Isolation; Column Chromatography; Spectroscopic Analysis

INTRODUCTION

Plants contain a wide range of chemicals crucial for various physiological processes. Approximately 60% of the world's health care products rely on plant resources. In India, individuals use medicinal plants as traditional treatments, pharmaceutical forms, and part of indigenous healing systems, such as Siddha, Unani, and Ayurveda, to cure various diseases[1]. The medicinal value of these plants lies in certain chemical substances called phytochemicals that produce definite physiological effects on the human body. The most important plant constituents are alkaloids, tannins, flavonoids, and phenolic compounds[2].

Alangium salvifolium Wang. (A. salvifolium) belongs to the Alangiaceae family and is widely recognised as a sage-leaved Alangium. In India, A. salvifolium is a well-established medicinal plant with a broad range of biological activities, including antidiabetic[3], anti-ulcer[4] analgesic[5], anti-inflammatory[6], antimicrobial[7], antioxidant[8], anti-arthritic[9], diuretic[10], fertility inhibitor[11], anticancer[12], epilepsy treatment, and antifungal properties[13]. Most plant components (roots, stems, leaves, seeds, and fruits) are recognized for their medicinal value and are widely utilized in traditional herbal remedies for various applications. In Sanskrit, it is referred to as "shoedhanam," while in Hindi, it is called "ankoia." This plant is a deciduous shrub or tree that can grow to 3–10 m in height[1].

A. salvifolium contains various alkaloids, including alangines A and B, alangicine, marckine, marckidine, emetin, dimethyl cephaeline, cephaeline, tubulosine, and psychotrine. Its leaves have been found to possess properties that help lower blood sugar levels and manage diabetes, whereas its stem can be utilized to create compounds that alleviate arthritis symptoms and prevent fertility[14,15]. Although many components have already been extracted from various parts of the plant, there is considerable potential for the presence of additional components. Therefore, the current study aimed to isolate and characterize the chemical constituents of the petroleum ether extract of A. salvifolium leaves.

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MATERIALS AND METHODS

Collection and extraction of Plant Material: The authenticated and dried leaves of A. salvifolium were obtained from Green Chem. Private Limited, Bangalore, and voucher specimens were deposited. Powdered leaves of A. salvifolium were successively extracted using petroleum ether, chloroform, and methanol in an extractor. Initially, small-scale extraction was carried out to determine the yield, followed by pilot-scale extraction.

Lab-Scale Extraction: Powdered leaves (200 g) were macerated in 200 ml of methanol for 3 h. This procedure was performed twice and then filtered to separate the filtrate. The filtrate was evaporated and the yield was calculated to be 1.2% w/w. Similarly, 200 g of powdered leaves were extracted using chloroform and petroleum ether, resulting in yields of 1.8% w/w and 3.0% w/w, respectively. The chloroform filtrate was then extracted with ethyl acetate to obtain a yield of 2.0% w/w.

Pilot-Scale Extraction: For pilot-scale extraction, 6 kg of powdered A. salvifolium leaves were initially extracted with chloroform. The chloroform extract was then extracted with ethyl acetate for three hours, filtered, and concentrated. Likewise, petroleum ether extract was obtained from 6 kg of powdered leaves, filtered, concentrated, and dried under reduced pressure at 60–80°C.

Optimization of thin layer chromatography (TLC) System: To determine the constituents of the petroleum ether extract, various solvent systems were used for TLC. The solvent system of toluene: ethyl acetate: formic acid (5:5:1) was found to provide maximum separation and was used for further analysis. TLC of petroleum ether extract revealed the same Rf value as the standard compound.

Column Chromatography: The petroleum ether extract was subjected to column chromatography using silica gel as the stationary phase. A glass column packed with 400 g of silica gel was used to load the extract, which was then eluted with a gradient of solvents, beginning with petroleum ether and progressively increasing in polarity with ethyl acetate and methanol. The fractions were collected, concentrated, and analysed by thin-layer chromatography to identify the presence of the desired compounds. Fractions showing promising separation were pooled for further purification.

In the first column, elution began with 100% petroleum ether, gradually increasing the amount of ethyl acetate and methanol. The collected fractions were dried and analysed, and fractions 5–9 were pooled for processing in the second column. In the second column, the pooled fractions (5–9) were eluted using a mixture of petroleum ether and ethyl acetate. Fractions with similar retention factors were pooled for further analysis. Finally, the pooled fractions from the second column were purified using the third column. Precipitates were observed in fractions CC-3/F-7, which were separated and recrystallised in ethanol. The purified compound was analysed by TLC and designated AS-01.

HPTLC Analysis of Isolated Compound: HPTLC analysis was conducted for the isolated compound AS-01 employing a solvent system toluene of toluene: ethyl acetate: formic acid (5:5:1). The compound had an Rf value of 0.76, observed under UV 254 nm, following the application of vanillin sulphuric acid reagent as the spraying agent.

Determination of the Melting Point: The isolated compound was subjected to melting point analysis in a capillary tube. The sample was heated in liquid paraffin, and its melting point was recorded.

HPLC analysis of the isolated compound (AS-01): The isolated compound AS-01 was subjected to HPLC analysis for secondary identification and determination of purity. The chromatographic parameters were C18 column, gradient elution system, and detection at 242 nm. Retention times and peak areas of the samples and standards were used to compute the assay.

Spectroscopic Characterization of the Isolated Compound: The isolated compound was characterized using Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (1H-NMR), and mass spectrometry, with DMSO being employed as the solvent. The above spectroscopic methods were used to verify the structure and identity of AS-01.

HPLC quantitation of AS-01: The quantity of AS-01 isolated from petroleum ether extract was determined using HPLC analysis. Standard solutions were prepared, and the assay was performed by comparing the peak area of the isolated compound with that of the standard. The assay results confirmed the yield and purity of the AS-01.

RESULTS

TLC of the petroleum ether extract was used for the isolation of the constituents from this extract.

Isolation of Phytoconstituents: The petroleum ether extract was subjected to column chromatography using three columns (1-3), with mobile phases of increasing polarity ranging from petroleum ether to ethyl acetate and methanol.

Isolation using Column-1 (CC-1): Eighteen fractions were collected from Column-1 using petroleum ether extract. These fractions were concentrated under vacuum and TLC analysis of these fractions, revealed major spots in fractions CC-1/F-5 to CC-1/F-9. These fractions were selected for purification in Column-2.

Isolation using Column-2 (CC-2): Twelve fractions were collected from Column-2 using fractions from Column-1. These fractions were concentrated under vacuum and TLC analysis indicated that major spots were found in fractions CC-2/F-6 to CC-2/F-9, which were selected for purification in Column-3.

Isolation using Column-3 (CC-3): Eight fractions were collected from Column-3, using fractions from Column-2. These fractions were concentrated under vacuum and TLC

analysis of these fractions led to the isolation of a compound, coded as AS-01, from fraction CC-3/F-7.

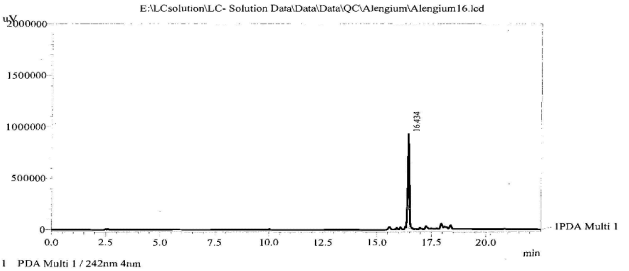
Physical Properties of the Isolated Compound (AS-01): AS-01 is a crystalline solid, white in colour, soluble in acetic acid, and sparingly soluble in water. Its melting point was 280°C and its molecular weight was determined to be 528. The retention factor (Rf) of AS-01 was 0.76.

HPTLC and HPLC analysis of the isolated compound (AS-01): The isolated compound was subjected to HPTLC and HPLC analyses. An HPLC gradient system (solvent A: 0.1% acetic acid, solvent B: acetonitrile) was used, and the chromatographic profile indicated that AS-01 eluted at a retention time of 16.43 minutes. The HPLC analysis confirmed the purity and identity of AS-01 at a concentration of 90%.

Identification tests of the isolated compound: The isolated compound was tested for alkaloids, and positive results were obtained with Dragendorff's, Mayer's, Wagner's, and Hager's reagents, indicating the presence of alkaloids.

Quantification of AS-01 Using HPLC: The isolated compound AS-01 was quantified by HPLC, and peaks corresponding to the standards confirmed its presence. The yield of AS-01 from petroleum ether extract was 1.6% w/w.

HPLC Chromatograms: The HPLC chromatograms of the isolated compound and petroleum ether extract are presented in Figure 1 and Figure 2 , respectively.



PeakTable

PDA Ch1 242nm 4nm

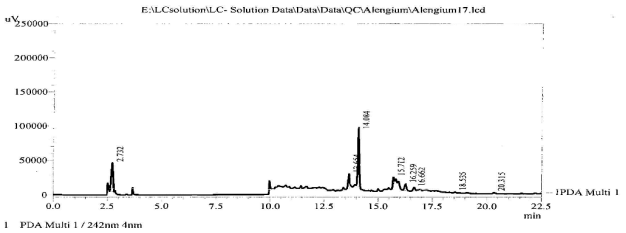
Peak#	Ret. Time	Area	Height	Area %	Height %
1	16.434	4340281	915659	100.000	100.000
Total		4340281	915659	100.000	100.000

Fig. 1: HPLC chromatogram of isolated compound

Spectral data of isolated compound (AS-01)

The FTIR spectral data of the isolated compound (AS-01) revealed several characteristic absorption peaks: 3412.25 cm^{-1} (Ar.OH stretch), 1651.55 cm^{-1} (C=C stretch), 2923.41 cm^{-1} (C-H stretch), 1273 cm^{-1} (OH stretch), 1903 cm^{-1} (C=O stretch), 2151.01 cm^{-1} (C=C=O stretch), 1318 cm^{-1} (C-N stretch), 1436 cm^{-1} (C-H bend), and 710 cm^{-1} (Ar. C-H stretch) (Figure 3).

The mass spectrum of the compound exhibited a molecular ion peak at m/z 528, corresponding to the molecular formula $C_{27}H_{29}O_{10}N$, indicating the molecular structure of AS-01. An M+H peak was observed with a base peak in the spectrum at 348.6, confirming the identity of the



PeakTable

PDA Ch1 242nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.732	232850	41331	27.343	23.561
2	13.654	112857	20561	13.252	11.721
3	14.084	357068	86108	41.929	49.086
4	15.712	44270	10771	5.198	6.140
5	16.259	59303	9264	6.964	5.281
6	16.662	26705	4560	3.136	2.600
7	18.535	5792	1172	0.680	0.668
8	20.315	12754	1656	1.498	0.944
Total		851599	175424	100.000	100.000

Fig. 2: HPLC Chromatogram of petroleum ether extract

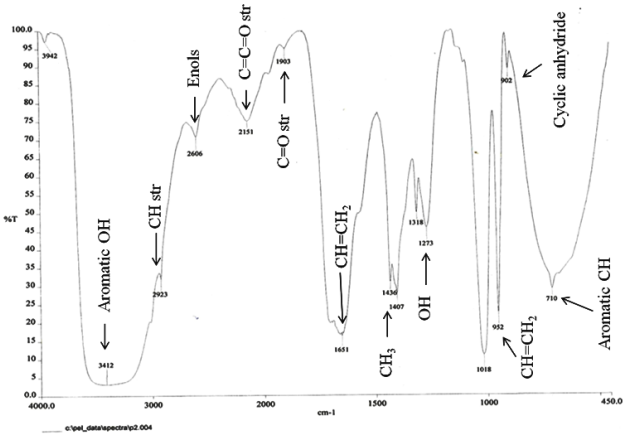


Fig. 3: FTIR spectrum of isolated compound

compound. Based on these spectral data, the structure of the isolated compound was determined to be AS-01 (Figure 4).

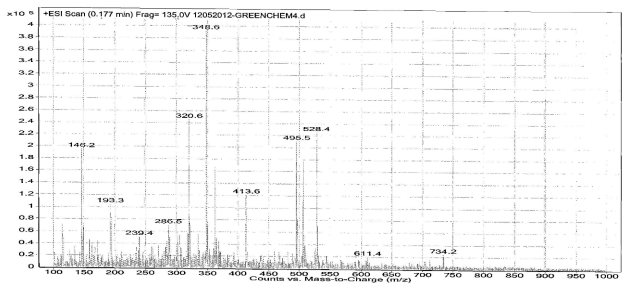


Fig. 4: Mass spectrum of isolated compound

The 1H NMR spectrum showed several significant peaks: δ 5.27 (1H, t, vinyl a), δ 2.89 (3H, s, OCH3 b), δ 0.93 (3H, d, CH3 c), δ 0.89 (3H, d, CH3 d), δ 5.10 (1H, t, CH e), δ 4.83 (2H, m, CH2 f), δ 3.47 (1H, d, CH g), δ 3.59 (1H, d, CH h), δ 3.36 (1H, d, OH i), δ 4.96 (1H, d, OH j,k), δ 4.83 (1H, t, CH2OH l), δ 6.71 (1H, d, CH m), δ 7.55 (1H, d, CH n), δ 9.38 (1H, s, Ar.OH o), δ 6.84 (2H, d, CH2 p), δ 5.42 (1H, d,

CH q), and δ 7.31 (1H, s, CH r) (Figure 5).

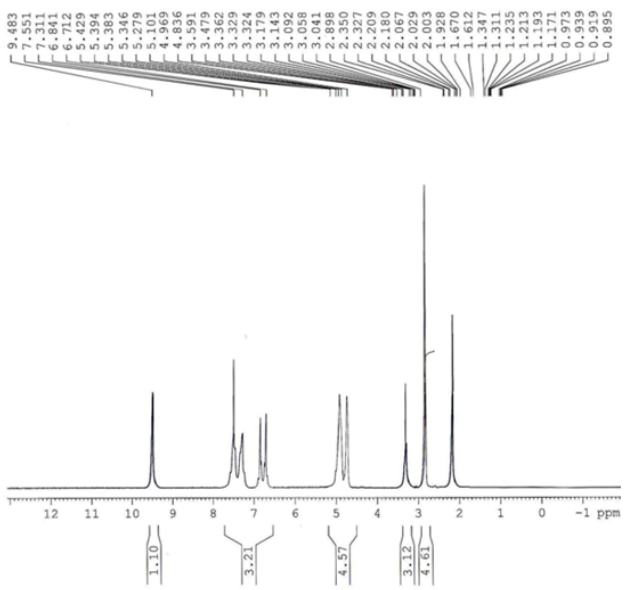


Fig. 5: NMR spectrum of isolated compound

DISCUSSION

The current study focused on isolating and identifying the chemical compounds from A. salvifolium leaf extract using column chromatography methods and subsequent structural and spectral characterisation of the isolated compound, AS-01. Isolation was a stepwise process involving three columns (CC-1, CC-2, and CC-3) with increasing mobile phase polarity from petroleum ether to ethyl acetate and methanol. AS-01 was isolated after rigorous purification.

The preliminary isolation of A. salvifolium leaves was carried out using petroleum ether extract, and 18 fractions were obtained from Column-1 (CC-1), among which fractions CC-1/F-5 to CC-1/F-9 had prominent spots on TLC. These fractions were further purified in Column-2 (CC-2), and fractions CC-2/F-6 to CC-2/F-9 were selected for purification in Column-3. Fraction CC-3/F-7 from Column-3 was found to be the compound of interest and was isolated and characterised as AS-01. The isolation of AS-01 signifies the effectiveness of the stepwise chromatographic method for purifying bioactive compounds from plant extracts.

AS-01 was found to be a white crystalline solid with a melting point of 280°C, soluble in acetic acid and sparingly in water, and with a molecular weight of 528, as established by mass spectrometry. The molecular formula $C_{27}H_{29}O_{10}N$ was established from the mass spectrum with peaks at m/z 528 and 348.6. Purity and identity were established by TLC, HPTLC, and HPLC, and AS-01 exhibited 90% purity with a retention time of 16.43 minutes. HPLC quantification of AS-01 showed a yield of 1.6% w/w from the petroleum ether extract, which is the normal yield for alkaloidal compounds, as reported by Sharma et al[16]. The FTIR spectrum of AS-01 revealed a number of characteristic peaks like the O-H stretch at 3412.25 cm^{-1}, C=C stretch at 1651.55 cm^{-1}, and C-H stretch at 2923.41 cm^{-1}, and so on, which are all typical of functional groups present in alkaloids as well as other plant-derived bioactive molecules. The existence of these functional groups was confirmed from the mass spectra and NMR data.

The 1H NMR spectrum of AS-01 shows a number of distinctive peaks, such as methyl (CH_3) and methoxy (OCH_3) group signals and aromatic protons, which are characteristic of alkaloidal structures. The findings are consistent with Venkateshwarlu et al., findings on the alkaloids obtained from A. salvifolium[13]. NMR data provided information about the proton surroundings in the molecule, which helped determine the molecular structure.

The alkaloid positive results, as authenticated by Dragendorff's, Mayer's, Wagner's, and Hager's reagents, indicate that AS-01 is an alkaloidal compound. Alkaloids possess a wide range of pharmacological activities, including anti-inflammatory, analgesic, and antidiabetic activities[16,17]. These properties are consistent with the traditional uses of A. salvifolium in ethnomedicine, where various parts of the plant are used to treat a range of ailments, including diabetes, gastrointestinal disorders, and pain[18].

CONCLUSION

The isolation and characterisation of AS-01 from A. salvifolium leaves provided a better understanding of the chemical composition of the plant. The findings of HPLC, FTIR, NMR, and mass spectrometry analyses support the fact that AS-01 is a new alkaloidal compound with great promise for pharmacological studies. This study not only shows the phytochemical potency of A. salvifolium, but also opens up avenues for future studies to investigate the therapeutic potential of AS-01 and other plant phytoconstituents.

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