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RESEARCH ARTICLE

Isolation And Characterization of Major Phytoconstituents from Aerial Parts of Achyranthes Aspera Extract

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ABSTRACT

India has a long history of using medicinal plants for therapeutic purposes. Despite advancements in contemporary medicine, plant-derived substances remain crucial for the discovery of new drugs. Achyranthes aspera, a widespread plant in tropical regions, exhibits several medicinal activities, including anti-inflammatory, anti-cancer, and liver-protective activities. Nevertheless, few studies have investigated the isolation and characterisation of bioactive compounds from the aerial parts of Achyranthes aspera. Phytoconstituents of the aerial parts of Achyranthes aspera were isolated using two methods: Column Chromatography (isolation-I) and extraction and recrystallisation (isolation-II). Petroleum ether, methanol, water, and ethyl acetate were used for plant extraction. Column chromatography fractions were examined by Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), and spectroscopic methods (IR, NMR, and MS) for structural elucidation. The phytosterol compound (AA1) was purified and identified. The compound exhibited a melting point ranging from 198 to 202°C, and its molecular weight was calculated to be 412.37 g/mol. Its sterol structure was verified by spectroscopic analysis using IR, NMR, and MS, offering detailed information on its molecular configuration. HPLC analysis revealed that AA1 was of high purity. This study isolated and characterised a new phytosterol compound from Achyranthes aspera, highlighting the medicinal potential of the plant.

Keywords: Achyranthes aspera; Phytosterols; Column Chromatography; HPLC; Spectroscopic Analysis

INTRODUCTION

India is home to affluent plant and animal diversity, and plants have been a vital medicinal resource for millennia. Herbal medicine has played a major role in the treatment of diseases throughout history, and is currently internationally acclaimed for the development of pharmaceuticals, nutraceuticals, phytochemicals, and cosmetics. Drug discovery through natural sources is still important, even in the era of modern medicine. More than 80% of the world's population depends on traditional herbal medicine for primary health care[1].

The transition towards herbal remedies has increased owing to the long-term side effects of conventional drugs. Increasing population, urbanisation, and overexploitation have caused a decrease in medicinal plant reserves and traditional knowledge[2]. Although herbal medicines are of long-standing use, the levels of active compounds are influenced by geography, climate, season, and plant parts, so their efficacy can be impacted. The isolation of natural products addresses these problems by allowing standardised dosing, potency determination, and structural elucidation. Isolates can also be used as models for synthetic drugs to enhance consistency and effectiveness[3,4].

Achyranthes aspera (A. aspera), which is a member of the family Amaranthaceae, exists extensively in tropical parts of Asia, Africa, Australia, and the Americas as a common wasteland weed or herb[5]. This plant plays an important role in traditional Indian medicine owing to its medicinal properties. It is generally a one-meter-tall plant with simple, opposite, obovate, and elliptical leaves. Its greenish flowers occur in spikes, and its oblong fruits (utricles) have one inverted seed. It is commonly seen growing on roadsides and other disturbed grounds[6–8].

A. aspera is reported to have many biological activities, ranging from hepatoprotective effects and possible

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anticancer activity,[9] anti-inflammatory activity,[10] thyroid-stimulating action,[11] to antioxidant, reproductive, and abortifacient activities[12]. The plant has also shown promise in the treatment of leprosy,[13] bacterial infections, and autoimmune diseases. Saponins affect heart function. Traditionally, the root has been used to treat diseases such as malaria, asthma, hypertension,[14] and diabetes[15]. The decoctions from plants have also been utilised to treat pneumonia and serve as diuretics[14].

Considering the medicinal potential of A. aspera, different parts of the plant have been evaluated for different biological activities by using their extracts. To date, only a limited number of chemical compounds have been isolated. As the aerial parts of A. aspera have not been well explored, the present study focused on isolating and identifying the bioactive constituents of these parts.

MATERIALS AND METHODS

The phytoconstituents of A. aspera were isolated in two different ways: isolation I by column chromatography and isolation II by extraction and recrystallisation. The aerial portion of the plant was obtained from Green Chem Pvt. Ltd., Bangalore, and processed using extraction and isolation procedures, as detailed below.

Isolation-I: Column Chromatography Method: The dried aerial parts of A. aspera (300 g) were initially powdered and defatted with petroleum ether (60–80°C) in a Soxhlet apparatus. The obtained dark greenish-brown oily mass (5.6 g) was designated as petroleum ether extract (PEE) and was chilled. Defatted marc was air-dried and successively extracted using methanol, water, and ethyl acetate. Methanolic extraction was performed by refluxing marc thrice for 1h with fresh methanol and recovered to a yield of 3.9 g. Similarly, aqueous extraction recovered 1.8 g and ethyl acetate extraction recovered 4.2 g. Among these, the petroleum ether extract exhibited prominent spots on TLC and was therefore chosen for further fractionation.

TLC Optimization: TLC analysis was performed with different solvent systems like chloroform: ethanol (9.8:0.2), petroleum ether: ethyl acetate (8:2), and toluene: ethyl acetate: formic acid (4.5:4.5:1). The plates were developed, sprayed with an anisaldehyde reagent, and visualised at 254 nm. The chloroform: ethanol (9.8:0.2) and petroleum ether: ethyl acetate (8:2) systems showed the best separation and were chosen for analysis.

Pilot Scale Extraction and Saponification: Approximately 400 g of the raw material was extracted with petroleum ether, yielding 46 g of the extract. This was saponified using 1M alcoholic KOH to eliminate the fatty components. The unsaponifiable material (41.3 g) was separated and selected for column chromatography because of its reduced composition.

Column Chromatography: A glass column (120 cm height, 10 cm diameter) was filled using wet packing with silica gel (60–120 mesh). Petroleum ether extract (15 g) was mixed with silica gel and loaded onto a column. Gradient elution was performed using solvents in the following order of increasing polarity: hexane, chloroform, ethyl acetate, and methanol. Elution was performed at 20 drops per minute and 158 fractions (25 ml each) were collected. TLC was performed for each fraction and identical fractions were combined. Fractions 54–57 exhibited two spots at Rf 0.43 and 0.95, with the latter being predominant. The white residue of these fractions has a melting point of 298–302°C. The compound was sterol positive and yielded 4.6 g.

Preliminary Phytochemical Screening: Qualitative examination of the petroleum ether extract showed no carbohydrates, alkaloids, glycosides, flavonoids, proteins, amino acids, or saponins. Positive results were obtained for steroids, sterols, and triterpenoids. This proved the selective extraction of the lipid-solvent compounds.

Isolation II: Extraction and Recrystallization Method: Approximately 4–5 g of powdered aerial parts was extracted using 2 L of 90% ethanol for 3–4 h. The filtered extract was concentrated under reduced pressure to obtain 18 g of the crude residue. The residue was hydrolysed with 50% H_2SO_4 at 80°C for 4 h, filtered, washed with water, and refluxed with 100 ml of 90% alcohol and 0.2 g of activated charcoal. The solution was concentrated, and the crude compound thus obtained was recrystallised from 90% alcohol to yield a pure compound (1.5 g) with a melting point of 198–202°C. The compound was soluble in hexane, toluene, dibromomethane, methanol, and acetic acid.

HPTLC Analysis: HPTLC was conducted on an isolated compound (AA1), a crude extract, and a standard (β-sitosterol). The stationary phase was silica gel 60 F254 and the mobile phase was chloroform: ethanol (9.8:0.2). The samples were applied using a Linomat 5 applicator and post-tank saturation was developed.

Melting Point Determination: The melting points of the isolated compounds were analysed using the capillary tube technique.

HPLC Analysis: The compound AA1 was subsequently screened by HPLC (Agilent-1100) to determine its purity and identity, which demonstrated conformity with expected sterol patterns.

Spectral analysis: The isolated pure compound (AA1) was characterised using extensive spectroscopic analyses, such as infrared (IR) spectroscopy, Proton Nuclear Magnetic Resonance (1H NMR) spectroscopy, and Mass Spectrometry (MS), to determine its structural aspects. Dimethyl sulfoxide (DMSO) was used as the solvent. All spectroscopic analyses were performed at a Sophisticated Instrumentation Facility.

RESULTS

Preliminary phytochemical screening of petroleum ether extract of A. aspera indicated the presence of certain constituents, such as phytosterols, triterpenoids, tannins,

phenols, and fats and oils, but carbohydrates, amino acids, vitamin C, saponins, alkaloids, and flavonoids were not present. Standard qualitative tests were performed to determine the presence of sterols. The Liebermann-Burchard test indicated the formation of a reddish-brown ring, while the Salkowski test gave a red colour in the lower chloroform layer, both of which established the presence of phytosterols.

Column chromatography fractionation of the extract provided multiple fractions, of which fractions 54–57 had equal retention factors on TLC and were hence combined. The pooled fraction yielded a white solid residue which was subjected to chemical tests, thin-layer chromatography (TLC) analysis, and melting point determination. The compound was found to be a white solid with a melting point of 198–202°C, Rf value of 0.95, and molecular weight of 412. It is soluble in solvents such as hexane, toluene, dibromomethane, methanol, and acetic acid (Figure 1).

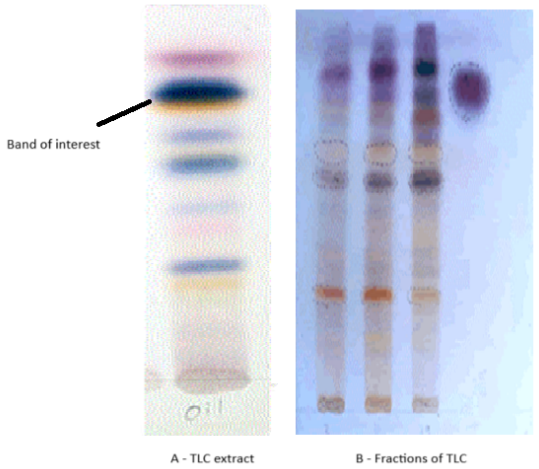

Fig. 1: TLC profiling of column chromatography fractions showing separation patterns under UV light (254 nm) and post-spraying with anisaldehyde-sulfuric acid reagent

The pure compound was subjected to High-Performance Thin-Layer Chromatography (HPTLC). Using a mobile phase of chloroform: ethanol (9.8:0.2), AA1 was detected at 254 nm under UV light after spraying with vanillin sulfuric acid reagent, yielding an Rf value of 0.95 (Figure 2).

The isolated compound in Isolation-I displayed a melting point between 298 and 302°C, whereas that in Isolation-II had a melting point between 198°C and 202°C.

HPLC analysis using a gradient system of mobile phases consisting of water and acetonitrile revealed a single sharp peak at 14.040 min of retention, reflecting high purity (Figure 3).

Elemental analysis of AA1 compound by combustion also verified the empirical formula as $C_{29}H_4O$ with a molecular weight of 412.37 g/mol. The percentage composition was carbon 84.39%, hydrogen 11.73%, and oxygen 3.88%.

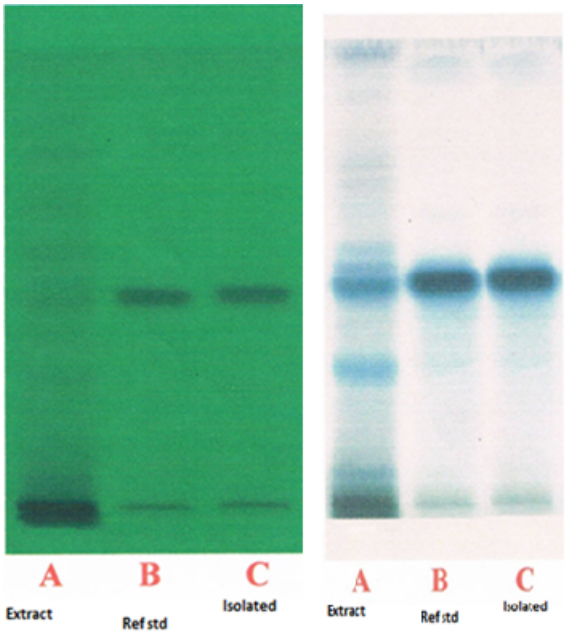

Fig. 2: HPTLC of A. aspera extract showing separation patterns under UV light (254 nm) and post-spraying with vanillin sulfuric acid reagent

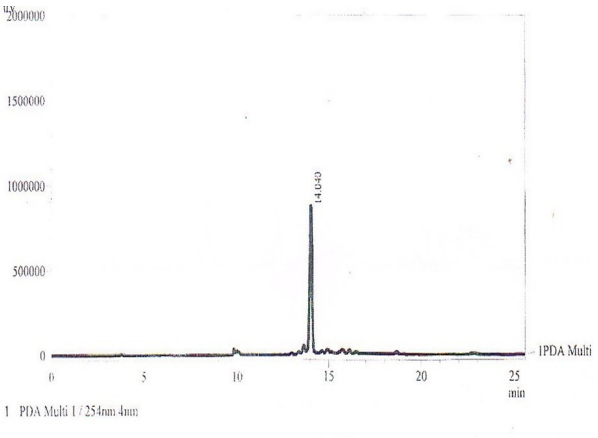

Fig. 3: HPLC chromatogram of isolated (AA1) compound

Spectroscopic analysis was performed to determine the structure of the AA1. The IR spectrum had absorption bands due to hydroxyl groups at 3397.97 and 3373.6 cm^{-1}, aliphatic C–H stretching at 2940.7 and 2867.9 cm^{-1}, and a C=C stretch at 1641.6 cm^{-1}. Additional peaks were due to CH_2 bending (1457.3 cm^{-1}), OH deformation (1381.6 cm^{-1}), cycloalkane signals (1038.7 cm^{-1}), and an absorption at 881.6 cm^{-1} (Figure 4).

1H NMR ($CDCl_3$, 400 MHz) of AA1 revealed multiplet signals at δ 5.25 (H-6), 5.14 (H-22), and 5.03, and methyl signals at δ 1.01 (Me-19) and 0.93 (Me-27). Other prominent peaks were δ 0.76–0.89 (9H), 0.91–1.05 (5H), 1.35–1.42 (4H), 0.69–0.73 (3H), 1.80–2.00 (5H), 1.07–1.13 (3H),

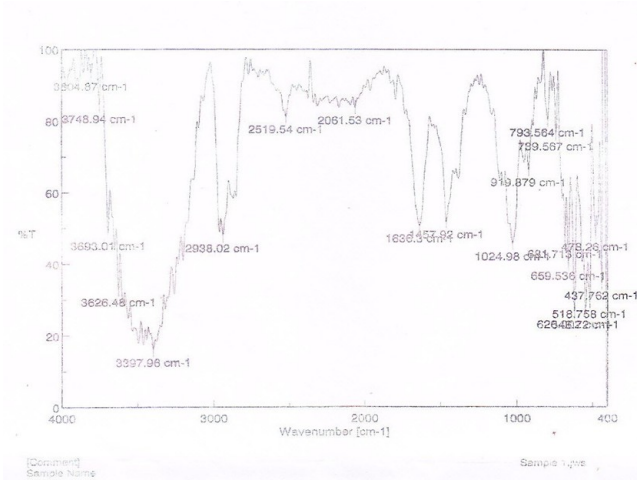

Fig. 4: R Spectrum of compound AA1 showing major absorption peaks at 3397.97, 2940.7, 1641.6, 1457.3, and 1038.7 cm^{-1}

and 1.35–1.6 (9H), representing a complicated aliphatic structure.

Mass spectrometry scanning yielded a molecular ion peak at m/z 414, according to the formula $C_{29}H_{50}O$. Peaks resulting from fragmentation at m/z were found at 367, 271, 255, 229, 189, 175, 161, 133, 121, 107, 105, 95, 81, 69, 55, and 41.

The 13C NMR ($CDCl_3$, 100 MHz) of AA1 compound gave explicit carbon assignments with peaks at δ 141.98, 137.2 (C-5), 129.8 (C-22), 121, 118.89 (C-6), 79.03 (C-3), and others between δ 15.6 and 56.3, which matched a sterol framework (Figure 5).

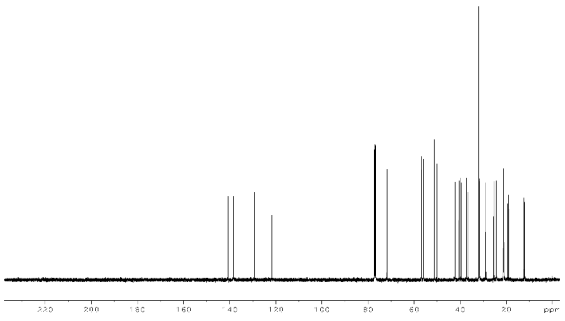

Fig. 5: 13C NMR Spectrum ($CDCl_3$, 100 MHz) of isolated compound aa1 showing characteristic peaks

The results as a whole established the isolation and structural identification of a phytosterol (AA1) compound from A. aspera (Figure 6).

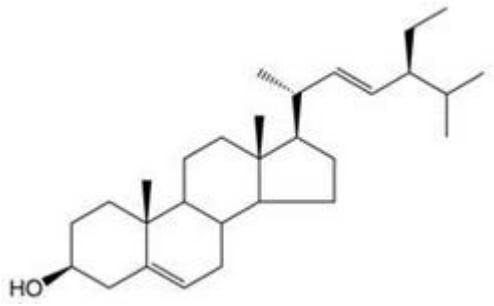

Fig. 6: Spectral characterization of compound AA1 confirming phytosterol structure

DISCUSSION

Krishnaveni and Thaakur, in 2006, carried out an exhaustive pharmacognostical and initial phytochemical study of A. aspera Linn., a plant well known in traditional medicine for the treatment of a variety of diseases like dropsy, strangury, cough, kidney stone, dysentery, and bowel disorders[16]. The researchers established standardised parameters for the identification and quality determination of plants. Preliminary phytochemical screening indicated the occurrence of various bioactive constituents[16]. Interestingly, these experiments verified the presence of sterols, flavonoids, tannins, and carbohydrates in the plant. These results were consistent with the historical uses of A. aspera, attesting to its medicinal uses in different cultures. The presence of sterols in the plant is especially important because these molecules possess known health-enhancing properties, such as cholesterol-lowering and anti-inflammatory activity. Krishnaveni and Thaakur's research established a baseline method for future work on A. aspera[16]. Having determined key pharmacognostical characteristics and ascertaining the occurrence of significant phytochemicals, their investigation set the foundation for essential guidelines in the standardisation and quality assessment of herbal medicine preparations based on this species.

In the current work, an attempt was made to isolate and characterise certain constituents of A. aspera to identify chemically defined markers that would be useful as reliable tools for the standardisation of plant extracts or formulations. The petroleum ether extract of the plant was treated with silica gel column chromatography (120–200 mesh), and different elution solvents were used, resulting in the isolation of a single compound, which was termed AA1. The first analysis included the determination of the melting point and solubility profile of the compound. In addition, AA1 was subjected to elemental analysis and purification by preparative HPLC, which ensured that its purity was 96.8%. Extensive spectroscopic analyses were performed using IR, ^13C-NMR, ^1H NMR, and mass spectrometry. The IR spectrum of AA1 had major peaks at 3397.97 cm^{-1}, 1636.4 cm^{-1}, and 1457 cm^{-1}. The ^13C NMR spectrum

showed δ values of 141.6, 137.2, 129.0, 121.6, and 56.7, whereas the ^1H NMR spectrum showed peaks at δ 5.25, 5.14, 5.03, and 4.55. Mass spectrometry analysis revealed a molecular ion peak at m/z 413 and base peak at m/z 365. Together, spectroscopic data, such as integral values from NMR, typical IR absorptions, and molecular ion data from mass spectrometry, established that AA1 was a phytosterol.

CONCLUSION

In conclusion, this study was able to isolate and identify a significant phytosterol, a phytosterol (AA1), from the aerial parts of A. aspera through column chromatography and recrystallisation methods. The compound was analysed extensively using different methods, such as HPTLC, HPLC, IR, NMR, and mass spectrometry, to confirm its structure and purity. This study demonstrates the potential of A. aspera as a plant source of bioactive compounds with medicinal properties, especially for the development of natural products for healthcare purposes. These results contribute to the continued search for plant-based remedies and the discovery of new therapeutic agents.

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