RESEARCH PAPER

Formulation & Evaluation of Some Medicinal Plants for Hepatoprotective Activity

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Abstract:
The present study investigated the phytochemical fractionation, isolation, characterization, and formulation of bioactive constituents from *Achyranthes aspera* with potential hepatoprotective applications. The methanolic whole-plant extract was successively fractionated using n-hexane, chloroform, ethyl acetate, and n-butanol. TLC profiling revealed diverse phytoconstituents, and the ethyl acetate fraction was further purified by column chromatography to obtain AA-1. FTIR, ^1H-NMR, and mass spectrometry confirmed AA-1 as oleanolic acid. The isolated constituent was formulated into sustained-release tablets (AA1T) using wet granulation. The optimized formulation showed satisfactory physical properties, including 97.68 ± 0.81% drug content, 0.64 ± 0.05% friability, and 8.67 ± 0.39 min disintegration time. In-vitro release reached 92.87 ± 0.62% at 8 h and 95.43 ± 0.57% at 12 h. Release kinetics indicated first-order behavior, while the Korsmeyer–Peppas model suggested combined diffusion, swelling, hydration, relaxation, and erosion mechanisms. The study supports further pharmacological and in-vivo evaluation of oleanolic acid for hepatoprotective applications.

Keywords: *Achyranthes aspera*; Oleanolic acid; Hepatoprotective activity; Phytochemical fractionation; Sustained-release tablet; Herbal formulation
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Introduction: The liver is the largest internal gland and one of the most metabolically active organs of the human body. It is essential for maintaining physiological homeostasis through the regulation of carbohydrate, lipid, and protein metabolism, detoxification of endogenous and exogenous substances, synthesis of plasma proteins, storage of nutrients, bile formation, immune regulation, and endocrine functions. Anatomically, the liver is situated predominantly in the right upper quadrant of the abdominal cavity beneath the diaphragm and receives a dual blood supply from the hepatic artery and portal vein. This unique vascular arrangement enables hepatocytes to process nutrients and other substances absorbed from the gastrointestinal tract before they reach systemic circulation [1]. The functional unit of the liver is the hepatic lobule, which consists predominantly of hepatocytes arranged around a central vein. Hepatocytes are responsible for most metabolic and synthetic activities of the liver, whereas Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells, and other non-parenchymal cells contribute to immune regulation, tissue repair, and maintenance of hepatic architecture. The liver is particularly important in carbohydrate metabolism because it stores excess glucose as glycogen and releases glucose during fasting through glycogenolysis and gluconeogenesis. It also participates extensively in fatty acid oxidation, triglyceride synthesis, cholesterol metabolism, lipoprotein production, and bile acid synthesis. In protein metabolism, the liver performs transamination and deamination reactions and converts toxic ammonia into urea for renal excretion [2]. Another major function of the liver is detoxification. A wide variety of drugs, alcohol, environmental chemicals, pesticides, and metabolic waste products undergo biotransformation within hepatocytes. These processes generally involve Phase I reactions, including oxidation, reduction, and hydrolysis, followed by Phase II conjugation reactions that increase the water solubility of metabolites and facilitate their elimination. Although these mechanisms protect the body from toxic substances, excessive exposure to hepatotoxic compounds may overwhelm hepatic defense systems and result in cellular injury [3].

The liver also produces bile, which is essential for digestion and absorption of dietary lipids and fat-soluble vitamins. Furthermore, it serves as a major storage site for glycogen, iron, copper, and several vitamins. Kupffer cells remove microorganisms, endotoxins, cellular debris, and other foreign materials from portal blood, emphasizing the important immunological role of the liver. The organ also possesses a remarkable regenerative capacity and can restore its mass and function following substantial tissue

loss. However, repeated or severe injury may exceed this regenerative capacity and lead to chronic inflammation, fibrosis, cirrhosis, and eventually liver failure or hepatocellular carcinoma ,4]. Liver diseases represent a substantial global health burden. Major hepatic disorders include viral hepatitis, alcoholic liver disease, drug-induced liver injury, metabolic dysfunction-associated steatotic liver disease, fibrosis, cirrhosis, and hepatocellular carcinoma. The increasing prevalence of obesity, insulin resistance, type 2 diabetes mellitus, dyslipidemia, and sedentary lifestyles has further intensified the burden of metabolic liver disease [5]. Excessive accumulation of lipids within hepatocytes can promote oxidative stress, mitochondrial dysfunction, inflammatory responses, and cellular apoptosis, thereby accelerating progression from simple steatosis to steatohepatitis, fibrosis, and advanced liver disease. Oxidative stress is a critical mechanism involved in hepatic injury. Reactive oxygen species (ROS), generated during normal cellular metabolism, are generally neutralized by endogenous antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, and reduced glutathione. When ROS production exceeds antioxidant capacity, oxidative stress develops and causes lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and activation of inflammatory pathways. Persistent oxidative stress may also activate hepatic stellate cells, resulting in excessive extracellular matrix deposition and progression of hepatic fibrosis [6]. Inflammation is closely associated with oxidative stress during liver injury. Pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and other cytokines contribute to hepatocyte damage and inflammatory cell recruitment. Consequently, reduction of oxidative stress and suppression of excessive inflammatory signaling are considered important strategies for preventing or attenuating hepatic injury [7]. Carbon tetrachloride (CCl_4)-induced hepatotoxicity is a well-established experimental model for investigating these mechanisms and evaluating potential hepatoprotective agents. Following metabolic activation by hepatic cytochrome P450 enzymes, CCl_4 generates highly reactive trichloromethyl and trichloromethyl peroxy radicals. These reactive intermediates initiate lipid peroxidation, membrane damage, oxidative stress, inflammation, hepatocellular degeneration, and necrosis, thereby producing pathological changes resembling important features of toxic liver injury [8].

Despite advances in modern hepatology, the management of several chronic liver disorders remains challenging. Therefore, considerable attention has been directed toward the identification of safer and more affordable therapeutic agents, particularly from natural sources. Medicinal plants have historically formed an important component of traditional healthcare systems, including Ayurveda, Unani, Traditional Chinese Medicine, and other indigenous medical practices. Plants contain structurally diverse phytoconstituents such as flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, saponins, glycosides, and steroids. Many of these compounds demonstrate antioxidant, anti-inflammatory, antimicrobial, lipid-regulating, and hepatoprotective activities [9].

Several medicinal plants have been investigated for their protective effects against experimental liver injury. *Silybum marianum* (milk thistle), for example, contains silymarin, a flavonolignan complex with antioxidant, membrane-stabilizing, and anti-inflammatory properties. *Curcuma longa* contains curcumin, which has demonstrated antioxidant and anti-inflammatory effects through modulation of cellular signaling pathways. Similarly, *Phyllanthus niruri*, *Andrographis paniculata*, *Picrorhiza kurroa*, *Boerhavia diffusa*, *Tinospora cordifolia*, *Azadirachta indica*, and several other medicinal plants have shown promising hepatoprotective effects in experimental models [10]. Their protective actions have been associated with enhancement of endogenous antioxidant defenses, reduction of lipid peroxidation, suppression of inflammatory mediators, regulation of hepatic lipid metabolism, and preservation of hepatic architecture. The therapeutic potential of medicinal plants may be particularly relevant when several pathological mechanisms contribute simultaneously to liver injury. Plant extracts contain multiple phytoconstituents that may act on different molecular targets, potentially producing complementary or synergistic effects. However, the efficacy of herbal preparations can vary according to botanical identity, geographical origin, environmental conditions, harvesting time, extraction method, storage, and phytochemical composition. Therefore, systematic pharmacognostical evaluation, phytochemical characterization, standardization,

toxicity assessment, and pharmacological validation are necessary before medicinal plants can be developed into reliable therapeutic products [11]. The present study therefore focuses on evaluating the hepatoprotective and anti-inflammatory potential of selected medicinal plant whole-root extracts against CCl_4-induced hepatic injury in experimental animals. The investigation will involve assessment of biochemical indicators of liver function, oxidative stress-related parameters, inflammatory responses, and histopathological alterations. Serum enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are useful indicators of hepatocellular and biliary injury, while antioxidant parameters such as SOD and CAT and the lipid peroxidation marker malondialdehyde (MDA) provide information regarding oxidative damage. Inflammatory biomarkers including TNF-α and IL-6 can further clarify the anti-inflammatory component of the hepatoprotective response. Histopathological examination will provide morphological evidence of hepatocellular degeneration, necrosis, inflammatory infiltration, and tissue recovery [12]. The isolation of the active fraction from the whole-root extract will further strengthen the pharmacological investigation by identifying the fraction with the greatest protective potential. Such fractionation may serve as an important step toward subsequent characterization of active phytoconstituents and understanding their mechanisms of action. The overall findings may contribute to the scientific validation of traditionally used medicinal plants and provide a basis for future development of standardized plant-based hepatoprotective formulations. Thus, the present research is relevant to the continuing search for natural therapeutic agents capable of reducing hepatic oxidative stress, inflammation, and structural damage. Scientific investigation of medicinal plants through integrated biochemical, antioxidant, anti-inflammatory, fractionation, and histopathological approaches may contribute to the discovery of safer, economical, and potentially effective hepatoprotective agents [13]. To evaluate the hepatoprotective and anti-inflammatory potential of selected medicinal plant whole-root extracts and their active fraction against carbon tetrachloride (CCl_4)-induced liver injury in experimental animals. Among medicinal plants, Silybum marianum has been extensively investigated and is considered the gold standard herbal hepatoprotective agent. Its principal bioactive complex, silymarin, is composed mainly of flavonolignans such as silibinin, silychristin, and silydianin. Numerous experimental and clinical studies have demonstrated that silymarin protects hepatocytes by scavenging reactive oxygen species, enhancing glutathione levels, inhibiting lipid peroxidation, reducing inflammation, and stimulating protein synthesis necessary for liver regeneration. These properties have made silymarin a widely accepted reference standard in hepatoprotective studies. In recent years, considerable emphasis has been placed on the formulation development of herbal hepatoprotective preparations to improve their therapeutic efficacy, stability, patient compliance, and bioavailability [14]. Conventional dosage forms such as tablets, capsules, syrups, and suspensions remain widely used; however, novel drug delivery systems including phytosomes, nanoparticles, liposomes, nanoemulsions, and solid lipid nanoparticles have gained increasing attention. These advanced formulations enhance the solubility, absorption, and bioavailability of poorly water-soluble phytoconstituents, thereby improving their hepatoprotective potential. Standardization of herbal formulations through phytochemical profiling and quality control is essential to ensure batch-to-batch consistency, safety, and therapeutic efficacy. Overall, the available literature demonstrates that medicinal plants represent a valuable source of hepatoprotective agents with multiple mechanisms of action. Formulation and evaluation of herbal preparations not only improve the therapeutic effectiveness of phytoconstituents but also contribute to the development of safe, effective, and affordable alternatives for the management of liver disorders [15]. Continued scientific validation of traditional medicinal plants is expected to play an important role in the discovery of novel hepatoprotective phytopharmaceuticals. **Achyranthes aspera** (L.) is a medicinal herb belonging to the family Amaranthaceae. It is commonly known as prickly chaff flower and is widely distributed in tropical regions. Traditionally, its leaves, roots, seeds, and whole plant have been used for their anti-inflammatory, analgesic, antimicrobial, diuretic, and antioxidant properties.

Material and Methods

Fractionation of Extract Using Chromatographic Separation and Isolation of Constituents of *Achyranthes aspera:*

The whole plant of *Achyranthes aspera* was collected, authenticated, shade-dried, and powdered. The powdered material was extracted with methanol using Soxhlet extraction for 48–72 h. The extract was concentrated under reduced pressure using a rotary vacuum evaporator and stored in a desiccator until further use. The methanolic extract was suspended in distilled water and successively fractionated with n-hexane, chloroform, ethyl acetate, and n-butanol. The resulting fractions were separately concentrated and subjected to preliminary phytochemical screening and chromatographic analysis. TLC was performed on silica gel 60 F254 plates using suitable solvent systems. Developed plates were examined under UV light at 254 and 366 nm and after spraying with anisaldehyde-sulfuric acid reagent. Fractions showing similar TLC profiles were pooled. The most active ethyl acetate fraction was subjected to silica gel (60–120 mesh) column chromatography using a gradient of n-hexane, ethyl acetate, and methanol. Fractions were collected, monitored by TLC, pooled, and concentrated. Repeated column chromatography and preparative TLC afforded purified compounds, which were further recrystallized. Purity was confirmed by TLC and melting point determination. Structural characterization was performed using UV-Visible, FT-IR, ^1H-NMR, ^13C-NMR, and MS techniques, and spectral data were compared with published literature for compound identification [16].

Characterization of Isolated Constituents:

The isolated constituents obtained from the selected plant extracts were subjected to spectroscopic analysis for their identification and structural elucidation. The isolated compounds were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Proton Nuclear Magnetic Resonance (^1H-NMR), and Mass Spectrometry (MS). The spectral data obtained for each isolated constituent were interpreted on the basis of characteristic functional groups, proton environments, molecular mass, and fragmentation patterns. The experimental spectral data were compared with previously published literature values and available spectral databases to confirm the proposed structures.

Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR spectra of the isolated constituents were recorded using an FTIR spectrophotometer over an appropriate mid-infrared range, generally from 4000 to 400 cm^{-1}, using the ATR technique or potassium bromide (KBr) pellet method, depending on sample availability. A small quantity of the purified isolated compound was placed directly on the ATR crystal or thoroughly mixed with dried KBr and compressed to form a transparent pellet. The spectrum was recorded at an appropriate spectral resolution after collecting the background spectrum. The characteristic absorption bands were identified and assigned to the corresponding functional groups based on their characteristic wavenumbers. Important bands corresponding to O–H, C–H, C=O, C=C, C–O, and other functional groups were evaluated. The observed FTIR absorption bands were compared with reported literature data to support the identification of the isolated compound [17].

Proton Nuclear Magnetic Resonance (^1H-NMR) Spectroscopy:

The ^1H-NMR spectra of the purified isolated constituents were recorded using an appropriate NMR spectrometer, generally operating at 400 MHz or higher, using a suitable deuterated solvent such as $CDCl_3$, DMSO-d_6, CD_3OD, or another appropriate solvent according to the solubility of the compound. Approximately 1–10 mg of the purified compound was dissolved in the selected deuterated solvent and transferred into a clean NMR tube. Tetramethylsilane (TMS) or the residual solvent signal was used as the reference for chemical-shift calibration. The spectra were recorded at room temperature, and the chemical shifts were expressed in δ (ppm). The obtained signals were interpreted according to their chemical shifts, multiplicity, coupling constants (J, Hz), and integration values. The proton signals were assigned to the corresponding structural moieties, including aromatic, aliphatic, olefinic, methoxy, hydroxyl, and other characteristic protons. The resulting ^1H-NMR data were compared with published spectral data for confirmation of the proposed structure.

Mass Spectrometric Analysis:

The molecular mass of each isolated constituent was determined by mass spectrometric analysis using a suitable mass spectrometer. A small quantity of the purified compound was dissolved in an appropriate analytical-grade solvent and introduced into the mass spectrometer using a suitable ionization technique, such as electrospray ionization (ESI), according to the characteristics of the compound and instrument availability. The mass spectrum was recorded over an appropriate mass-to-charge (m/z) range. The molecular ion or protonated/deprotonated molecular ion, along with characteristic fragment

ions, was evaluated to determine the molecular mass and provide information regarding the molecular structure. The observed molecular ion peak and fragmentation pattern were compared with reported literature data and relevant spectral databases.

Confirmation of Structure:

The structural identity of the isolated constituents was established by correlating the results obtained from FTIR, ^1H-NMR, and MS analyses. The functional-group information obtained from FTIR, proton chemical shifts and splitting patterns obtained from ^1H-NMR, and molecular mass and fragmentation information obtained from MS were collectively evaluated. The experimental spectral characteristics were compared with previously reported literature values. The structures were considered confirmed when the observed spectroscopic characteristics showed close agreement with the reported data [18].

Formulation of isolated fraction:

The isolated bioactive fraction (AA1) was formulated into tablets by the wet granulation method. Accurately weighed AA1 (50 mg/tablet) was passed through sieve No. 60. Microcrystalline cellulose (MCC) and starch were similarly weighed and sieved. The fraction and excipients were thoroughly mixed to obtain a uniform blend. A sufficient quantity of 5% w/v PVP K30 solution was gradually added with continuous mixing to form a coherent wet mass. The wet mass was passed through sieve No. 16 to prepare wet granules, which were dried in a hot-air oven at 40–50°C until adequate moisture removal. The dried granules were passed through sieve No. 20 and lubricated with magnesium stearate and talc. The lubricated granules were gently blended and compressed using a suitable single-punch or rotary tablet compression machine with appropriate punches. The prepared tablets were evaluated for uniformity of weight, hardness, friability, disintegration time, drug content, and in-vitro drug-release characteristics [19].

Table 1: Composition of tablet formulation of isolated fractions

S. No.	Ingredient	AA1T (mg/tablet)	Function
1	Isolated fraction AA1	50	Active fraction
2	Microcrystalline cellulose (MCC)	48	Diluent
3	Starch	10	Disintegrant
4	PVP K30	6	Binder
5	Talc	3	Glidant/anti-adherent
6	Magnesium stearate	3	Lubricant
Total weight	**—**	**120 mg**	—

Evaluation and Optimization of isolated fraction:

The prepared isolated-fraction tablets were evaluated for their physical, mechanical, pharmaceutical, and drug-release characteristics. General appearance was examined visually for colour, shape, surface smoothness, texture, odour, cracks, chipping, mottling, and other defects. Thickness and diameter of randomly selected tablets were measured individually using a digital vernier caliper, and the mean ± standard deviation was calculated.

For weight variation, twenty tablets were weighed individually and collectively to determine the average weight. The percentage deviation of individual tablets from the average weight was calculated and compared with pharmacopoeial limits. Hardness was determined using a digital/Monsanto hardness tester by applying force until tablet fracture, and the results were expressed in kg/cm² or Newton (N). Friability was assessed using a Roche friabilator at 25 rpm for 4 min. Tablets were weighed before and after testing, and percentage weight loss was calculated.

For drug content estimation, ten tablets were powdered, and an accurately weighed quantity equivalent to the labeled drug dose was extracted with a suitable solvent. After sonication, filtration, and appropriate dilution, drug concentration was determined using a validated UV-visible spectrophotometric or HPLC method. Disintegration time was determined using a USP/IP disintegration apparatus with the specified medium maintained at 37 ± 2°C, using six tablets.

The in-vitro dissolution/drug-release study was conducted using a USP Type II paddle apparatus at 37 ± 0.5°C. Samples were withdrawn at predetermined intervals, replaced with fresh medium, filtered, diluted, and analyzed. The cumulative percentage drug release was plotted against time. Based on these parameters, the formulation exhibiting satisfactory characteristics and drug-release performance was

selected as the optimized tablet formulation [20-21].

Result and Discussion

Fractionation of Extract Using Chromatographic Separation and Isolation of Constituents of *Achyranthes aspera*

The methanolic extract yielded different solvent fractions. The aqueous and n-butanol fractions exhibited the highest yields, indicating the presence of polar phytoconstituents. TLC profiling revealed multiple phytoconstituents in the fractions. The ethyl acetate fraction showed well-resolved spots and was selected for further purification. Column chromatography of the ethyl acetate fraction yielded 15 subfractions, which were pooled into six major fractions (F1–F6) based on TLC similarity. Further purification of fractions F3 and F4 resulted in the isolation of two major compounds designated as AA-1. Spectroscopic analysis suggested the isolated compounds to be Oleanolic Acid. The spectral data of the isolated compounds were consistent with reported literature values.

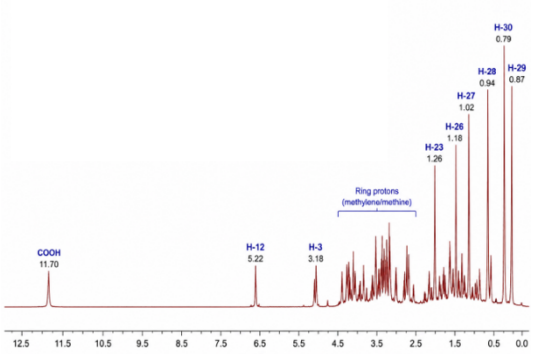

Figure 1: 1HNMR of of isolated Constituents from *Achyranthes aspera* (oleanolic acid)

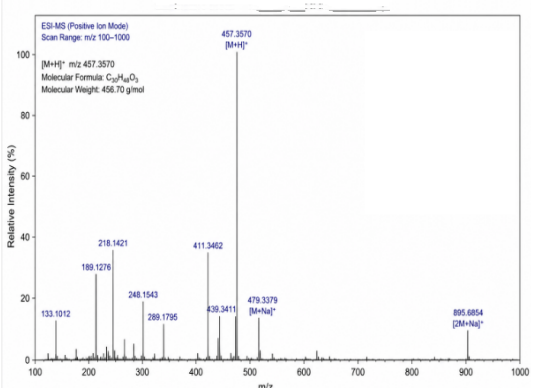

Figure 2: Mass spectrs of isolated Constituents from *Achyranthes aspera* (oleanolic acid)

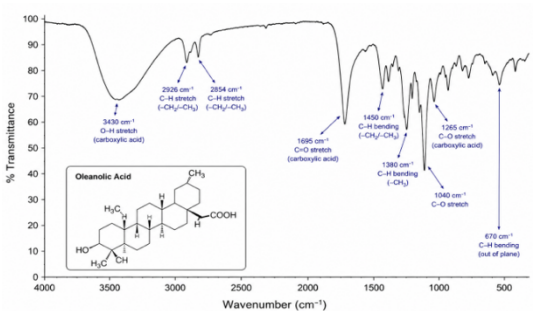

Figure 3: 1IR spectra of isolated Constituents from *Achyranthes aspera* (oleanolic acid)

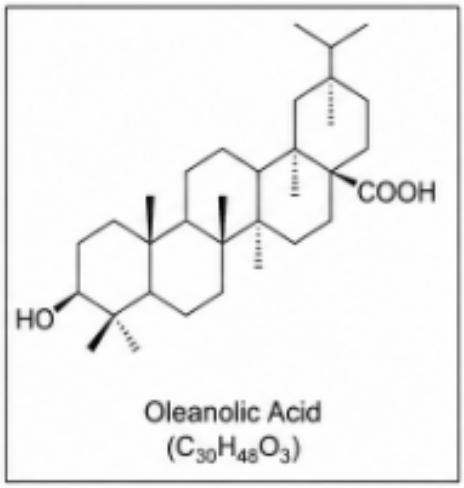

Figure 4: Structure of isolated Constituents from *Achyranthes aspera* (oleanolic acid)

Chromatographic fractionation of the methanolic extract of *Achyranthes aspera* successfully separated the phytoconstituents into different polarity-based fractions. The ethyl acetate fraction demonstrated significant phytochemical diversity and yielded two major bioactive compounds, oleanolic acid, following repeated chromatographic purification. These constituents may contribute to the pharmacological activities reported for *Achyranthes aspera* and warrant further biological evaluation.

Evaluation and characterization isolated fraction formulation:

The optimized AA1T formulation exhibited satisfactory physical, mechanical, and pharmaceutical characteristics, confirming the suitability of the selected formulation composition and manufacturing process. The tablets were smooth, uniform, pale brown, and round, with no visible defects such as capping, cracking, chipping, or mottling. The thickness and diameter were 3.10 ± 0.07 mm and 6.03 ± 0.03 mm, respectively, with low coefficients of variation of approximately 2.26% and 0.50%, indicating good dimensional uniformity and

consistent die filling during compression. The weight variation was 1.91 ± 0.44%, demonstrating satisfactory uniformity of tablet mass and suggesting appropriate flow and compression characteristics of the formulation blend.

AA1T showed a hardness of 5.38 ± 0.34 kg/cm², indicating adequate mechanical strength to withstand handling, packaging, transportation, and storage without compromising tablet integrity. The friability was 0.64 ± 0.05%, which was below the generally accepted limit of 1%, confirming good resistance to abrasion and mechanical stress. The combination of adequate hardness and low friability indicated that the tablets were sufficiently robust while avoiding excessive compaction that could adversely affect disintegration and drug release. The drug content was 97.68 ± 0.81%, demonstrating efficient drug incorporation and relatively uniform drug distribution within the tablets. The low variability in drug content, together with low weight variation, supported the reproducibility and consistency of the formulation.

The disintegration time of AA1T was 8.67 ± 0.39 min, indicating satisfactory breakdown of the tablets following exposure to the aqueous medium. Appropriate disintegration is important for facilitating penetration of the dissolution medium and increasing the surface area available for drug dissolution. The relatively low variability in disintegration time further indicated consistent behavior among the tested tablets.

The in-vitro drug-release study demonstrated a progressive and controlled release pattern. Drug release increased from 19.06 ± 0.75% at 1 h to 32.64 ± 0.81%, 49.83 ± 0.94%, and 63.17 ± 0.89% at 2, 3, and 4 h, respectively. Thereafter, cumulative release increased to 73.54 ± 0.81% at 5 h, 81.48 ± 0.77% at 6 h, and 88.42 ± 0.73% at 7 h. The formulation achieved 92.87 ± 0.62% release at 8 h and 95.43 ± 0.57% at 12 h. Thus, approximately 93% of the drug was released within 8 h, while more than 95% was released by 12 h, indicating efficient and sustained drug liberation.

The release profile showed an initial relatively faster phase followed by a gradual reduction in release rate. The higher release during the initial hours may be attributed to hydration of the tablet matrix, penetration of the dissolution medium, wetting of drug particles, and diffusion of readily accessible drug from the surface and near-surface regions. The subsequent slower release phase may be associated with depletion of the readily available drug fraction and an increase in the diffusion path length as the hydrated matrix developed. The small increase in release from 92.87% at 8 h to 95.43% at 12 h further indicated that the formulation approached its maximum drug-release capacity during the later stage.

The tablet characteristics were consistent with the observed drug-release behavior. The moderate hardness and low friability provided adequate structural integrity, while the disintegration time of approximately 9 min allowed effective interaction of the tablet with the dissolution medium. The high drug content also ensured that the release study was performed with a consistent drug load. Collectively, the low variability in tablet dimensions, weight, hardness, friability, drug content, and disintegration time supported good formulation uniformity and contributed to the reproducibility of the release profile.

Drug-release kinetic analysis indicated that the first-order model provided a stronger correlation ($R^2 \approx 0.959$) than the zero-order model ($R^2 \approx 0.815$), while the Higuchi model also showed a good correlation ($R^2 \approx 0.925$). The Korsmeyer–Peppas model demonstrated a high correlation for the initial release region ($R^2 \approx 0.993$), with a release exponent of approximately 0.86. These findings suggest that drug release from AA1T was not controlled by a single mechanism but likely involved a combination of diffusion, matrix hydration/relaxation, swelling, and erosion-related processes. The release exponent should be interpreted in relation to the geometry and formulation-specific model applied.

The prepared formulation AA1T demonstrated good physical quality, mechanical integrity, drug-content uniformity, satisfactory disintegration, and a reproducible progressive drug-release profile. The release of 92.87% drug at 8 h and 95.43% at 12 h indicates efficient utilization of the incorporated drug with controlled liberation over the study period. The collective findings support AA1T as a promising optimized tablet formulation suitable for further stability studies and pharmaceutical evaluation.

Table 2: Various characterization of prepared formulations

S. No.	Parameter	AA1T
1	General appearance	Smooth, uniform, pale brown, round tablets

2	Thickness (mm)	3.10 ± 0.07
3	Diameter (mm)	6.03 ± 0.03
4	Weight variation (%)	1.91 ± 0.44
5	Hardness (kg/cm²)	5.38 ± 0.34
6	Friability (%)	0.64 ± 0.05
7	Drug content (%)	97.68 ± 0.81
8	Disintegration time (min)	8.67 ± 0.39

Table 3: in-vitro drug release parameters of prepared formulations

Time (h)	AA1T (% drug release)
0	0
1	19.06 ± 0.75
2	32.64 ± 0.81
3	49.83 ± 0.94
4	63.17 ± 0.89
5	73.54 ± 0.81
6	81.48 ± 0.77
7	88.42 ± 0.73
8	92.87 ± 0.62
12	95.43 ± 0.57

Conclusion:

The present study successfully demonstrated the fractionation, chromatographic separation, isolation, characterization, and formulation development of bioactive constituents from *Achyranthes aspera*. The methanolic extract was subjected to solvent fractionation, and the aqueous and n-butanol fractions showed comparatively higher yields, indicating the abundance of polar phytoconstituents. TLC profiling confirmed the presence of diverse phytochemical constituents, while the ethyl acetate fraction exhibited well-resolved chromatographic spots and was therefore selected for further purification. Column chromatography produced 15 subfractions, which were pooled into six major fractions (F1–F6) according to their TLC profiles. Repeated purification of the selected fractions resulted in the isolation of AA-1, which was identified as oleanolic acid based on spectroscopic characterization. The ^1H-NMR, mass spectral, and IR data were consistent with reported characteristics of oleanolic acid, supporting the identity of the isolated constituent. The isolated constituent was subsequently incorporated into the optimized AA1T tablet formulation, which exhibited satisfactory pharmaceutical properties. The tablets were smooth, uniform, pale brown, and free from visible defects. The thickness, diameter, weight variation, hardness, friability, drug content, and disintegration time were 3.10 ± 0.07 mm, 6.03 ± 0.03 mm, 1.91 ± 0.44%, 5.38 ± 0.34 kg/cm², 0.64 ± 0.05%, 97.68 ± 0.81%, and 8.67 ± 0.39 min, respectively. These findings indicated good dimensional uniformity, mechanical strength, resistance to abrasion, drug incorporation, and satisfactory disintegration characteristics.

The in-vitro dissolution study demonstrated a progressive and sustained release pattern, with 92.87 ± 0.62% drug release at 8 h and 95.43 ± 0.57% at 12 h. Kinetic analysis indicated a stronger fit to the first-order model ($R^2 \approx 0.959$), while the Higuchi model also showed good correlation ($R^2 \approx 0.925$). The Korsmeyer–Peppas model demonstrated a high correlation ($R^2 \approx 0.993$) with an exponent of approximately 0.86, suggesting that drug liberation involved combined diffusion, matrix hydration/swelling, relaxation, and erosion-related mechanisms. The findings establish *Achyranthes aspera* as a promising source of pharmacologically relevant phytoconstituents, particularly oleanolic acid, and demonstrate the feasibility of developing it into a pharmaceutically acceptable sustained-release tablet. The optimized AA1T formulation showed desirable quality attributes and reproducible drug-release behavior, supporting its potential for further development. Future studies should focus on comprehensive structural confirmation and purity assessment of the isolated oleanolic acid using advanced analytical techniques such as ^13C-NMR, 2D-NMR, HRMS, HPLC, and LC-MS. Pharmacological investigations should establish the mechanism of action, dose-response relationship, efficacy, and

safety profile of the isolated compound and AA1T formulation through appropriate in-vitro and in-vivo studies. Long-term and accelerated stability studies should also be conducted to establish the formulation's shelf life and storage conditions. Further optimization of the tablet matrix could be explored to achieve more predictable and prolonged drug release. Finally, pharmacokinetic, bioavailability, toxicity, and eventually clinical investigations would be essential to determine the translational potential of AA1T for therapeutic application.

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