Optimized low-cost isolation of α-amyrin acetate from *Alstonia boonei*: toward scalable production of a bioactive triterpenoid.

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Abstract

Alpha amyrin acetate is a pentacyclic triterpenoid with well-documented pharmacological activities, including anti-inflammatory, analgesic, antioxidant, hepatoprotective, antidiabetic, gastroprotective, and anticancer potentials. However, low yields and laborious purification steps from natural sources have limited its availability for advanced pharmacological studies. This study aimed to develop a simple, cost-effective, and efficient procedure by optimizing the isolation of α-amyrin acetate from *Alstonia boonei* stem bark with high yield and purity. Pulverized stem bark was extracted by maceration in absolute methanol and in methanol–dichloromethane (1:1) for 2–7 days. Extracts were filtered, and crude triterpenoids were precipitated via slow solvent evaporation. The crude precipitates were purified using Vacuum Liquid Chromatography (VLC) on silica gel with graded hexane–ethyl acetate solvent systems. The fractions were monitored by TLC. Methanol extracts yielded between 3.48–6.46% crude triterpenoids, with optimum yield from day 5. Methanol–dichloromethane extracts gave higher crude yields (6.46–8.16%), peaking at day 7. However, VLC purification revealed that methanol extracts produced significantly

higher yields of pure α-amyrin acetate, with the highest recovery (26.52% of crude triterpenoid) while the mixed solvent system yielded a maximum of 10.29%. Maceration in methanol for six days followed by precipitation and VLC purification provides a simple, low-cost, and efficient method for isolating α-amyrin acetate from *A. boonei*. The optimized protocol reduces the number of purification steps, improves selectivity, and enables the recovery of sufficient quantities for pharmacological and toxicological studies.

Key Word: *Alstonia boonei*, triterpenoids, alpha amyrin acetate, isolation technique, purification technique.

Introduction

Alpha-amyrin acetate is a pentacyclic triterpenoid ester, which has been isolated from a variety of medicinal plants, including *Alstonia*, *Ficus* and *Protium* species. Several pharmacological properties of this triterpenoid have been well documented. The compound has demonstrated strong anti-inflammatory (Okoye *et al.,* 2014; Abdelhameed *et al.,* 2021; Baburaj *et al.,* 2022), analgesic (Otuki *et al.,* 2005), antioxidant (Viet *et al.,* 2021), hepatoprotective (Donfack *et al.,* 2010; Singh *et al.,* 2015), antidiabetic (Singh *et al.,* 2009; Olaokun *et al.,* 2016), and gastroprotective activities (Anupama *et al.,* 2022). There are also recent studies suggesting the modulatation of oxidative stress and neuroinflammation, indicating possible benefits in neurodegenerative conditions (Baburaj *et al.,* 2024; Viet *et al.,* 2025), and studies on anticancer potentials (Neto *et al.,* 2021; Garcia-Pérez *et al.,* 2024; Silihe *et al.,* 2025).

At present, α-amyrin acetate is still an investigative *bioactive triterpenoid* in the laboratory, and has not yet reached approved clinical application as a drug on its own. All the available evidence so far sits mainly in preclinical pharmacology (cell culture and animal models), with a few exploratory studies in formulations (Neto *et al.,* 2021), but no large-scale human clinical trials have been published. Although the preclinical studies have shown great potentials for translation into clinical applications, deeper investigation viz. pharmacokinetics, toxicity, and controlled human trials will still be required. These later investigations will demand availability of larger quantities of the triterpenoid. One of the barriers for clinical translation of bioactive compounds has been low yields from natural products source like

medicinal plants (Ahmad *et al.,* 2025). There are also natural variations in plant material which makes consistent yield and purity a challenge (Chaachouay and Zidane, 2024). Large-scale extraction or synthesis is also usually costly and resource-intensive (Soldi *et al.,* 2008; Mungwari *et al.,* 2025). *Alstonia boonei* is one of the plant sources of alpha amyrin acetate, which has shown considerable yield of the bioactive compound from previous studies (Okoye *et al.,* 2014).

The traditional method of isolation and purification of alpha amyrin acetate, and other triterpenoids generally involves an initial defatting with hexane to remove waxes that can cause tailing or clogging of the column (Sanusi *et al.,* 2020). This is followed by selective extraction using solvent mixtures, fractionation by polarity using VLC or flash column on silica and final purification with prep-TLC, flash, counter column chromatography, Sephadex LH-20 Gel chromatography or reversed-phase HPLC (Sanusi *et al.,* 2020). These processes are laborious and resource intensive. Newer extraction techniques, like supercritical CO_2 extraction, microwave-assisted extraction, ultrasound-assisted extraction and pressurized liquid extraction have also been developed for rapid extraction of alpha amyrin (Herzyk *et al.,* 2024). Although these techniques can yield α-amyrin acetate with high purity, they are also resource intensive and the facilities are not commonly available in resource constrained laboratories.

The present study is an attempt to develop and optimize a simple, low cost procedure for the isolation of α-amyrin acetate with high yield and purity. The procedure involves an initial maceration at room temperature in a polar solvent, which is followed by a slow precipitation of the crude triterpenoid and final purification with Vacuum Liquid Chromatography.

Materials and methods

Plant material

Alstonia boonei stem bark and root bark were collected from Nsukka, Enugu State, Nigeria. The plant materials were collected and authenticated by Mr. Felix Nwafor, a plant taxonomist from the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. The plant material was shade dried and pulverized using mechanical grinder.

Optimizing the extraction condition

The optimization of the extraction was carried out by adjusting only two parameters viz. the solvent type and the extraction time. Other parameters like concentration, temperature and solid-to-solvent ratio were kept constant. Two different solvent systems and different extraction times of 2, 3, 4, 5, 6 and 7 days were used.

Extraction with absolute methanol at different extraction times

Exactly 50 g each of the plant material was weighed and placed into 1 L extraction bottle labelled Day 2, 3, 4, 5, 6 and 7. To each of the extraction bottles containing the powdered plant material, 200 mL of methanol was added and stirred intermittently. The duration of the extraction was for 2 to 7 days respectively. At the end of every 24 h, the extract was decanted and filtered into beakers labelled Day 2, 3, 4, 5, 6 and 7 accordingly. About 100 mL of methanol was thereafter added into the extraction bottles and stirred intermittently. The process was repeated after every 24 hours and stopped at the end of the respective days labeled for each extraction bottle.

Extraction with methanol: dichloromethane (1:1) at different extraction times

The extraction procedure described above was repeated with methanol: dichloromethane (1:1).

Precipitation of the crude triterpenoids from each extract

Each of the beakers containing the filtered extract was covered with aluminum foil perforated at the top to allow for slow evaporation of the extracting solvent. The precipitate formed following slow evaporation of the solvent was harvested into a weighed and labelled beaker. This procedure was repeated for all the extracts for days 2 to 7. The precipitate was dried to constant weight and the weight obtained by difference. This represents the crude triterpenoid.

Purification of the crude triterpenoid from each extract.

The crude precipitate was purified by Vacuum Liquid Chromatography. The VLC was a column (3 x 15 cm) packed with silica gel (200-400 mesh size) to a bed size of 5 cm. The crude precipitate was adsorbed on about 30 g silica gel and placed on top of the column and thereafter covered with fresh

silica gel up to 1 cm layer and a ball of cotton wool was positioned to cover the layer. The column was eluted with 250 mL each of hexane and ethyl acetate mixture (10:0, 9:1, 8:2, 7:3 and 0:10). The eluate for each solvent was collected in a separate flask and the column was drawn dry between the collections of each solvent system. Each of the fractions were finally evaporated to dryness and collected in sample bottles. This process was carried out for Days 3, 5, 6 and 7 for methanol extracts and for Days 3, 5 and 7 for the methanol: dichloromethane (1:1) extracts. Alpha amyrin acetate was obtained from the fractions eluted with hexane: ethyl acetate 9:1 as white needles. The purity was confirmed by TLC on silica gel using hexane: ethyl acetate 9:1, 8:2 and 7:3 as solvent systems. Spots were detected with UV at 254 and 365 nm. The structure of alpha amyrin acetate was confirmed by EI-MS analysis (Okoye *et al.,* 2014).

Results

Crude triterpenoid precipitates obtained from the initial solvent extraction

The yield of the crude triterpenoid precipitate from methanol extraction at different extraction times are given in Table 1. The lowest yield of 3.48% was obtained for the extraction that lasted for 2 days. From extraction time of 3 days, the yield peaked at 5.6% and formed an undulating plateau afterwards (Figure 1).

The yields of the crude triterpenoid precipitate from methanol:dichloromethane (1:1) extract for the different extraction times are given in Table 2. The yield did not show any obvious trend (Figure 2), although the highest yield was recorded after the extraction time of 7 days.

Generally, higher yield of crude precipitate was obtained for methanol:dichloromethane extract compared to the methanol extract.

Table 1. Yield of crude triterpenoid extracted with absolute methanol at different extraction time

Extraction time (days)	Weight of crude (g)	% Yield
2	1.81	3.48
3	2.80	5.60
4	2.90	5.80
5	3.23	6.46
6	2.79	5.58
7	2.85	5.70

Note: Yield expressed as percentage weight of crude precipitate relative to initial plant material (50 g).

Table 2. Yield of crude triterpenoid extracted with methanol–dichloromethane (1:1) at different extraction times.

Extraction time (days)	Weight of crude (g)	% Yield
2	3.76	7.52
3	3.46	6.92
4	4.07	8.14
5	3.55	7.10
6	3.23	6.46
7	4.08	8.16

Note: Yield expressed as percentage weight of crude precipitate relative to initial plant material (50 g).

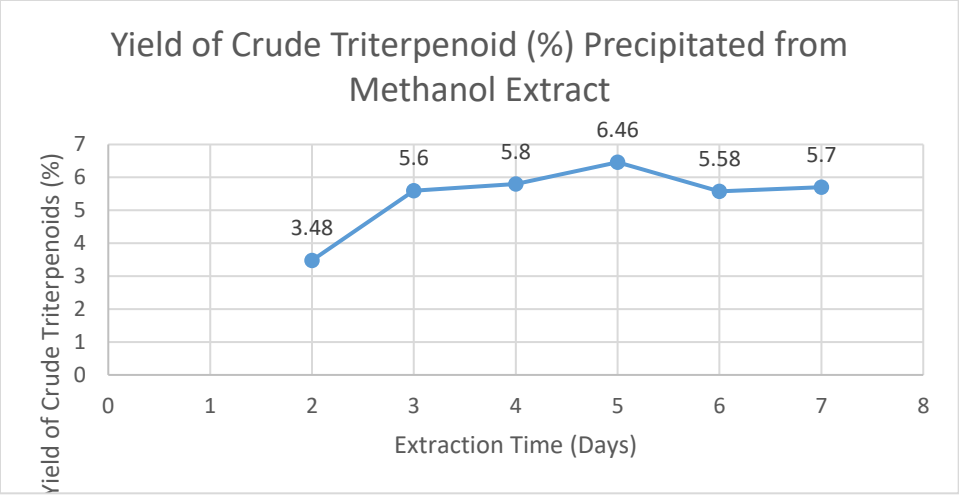

Figure 1: Yield of Crude Triterpenoid (%) Precipitated from Methanol Extract

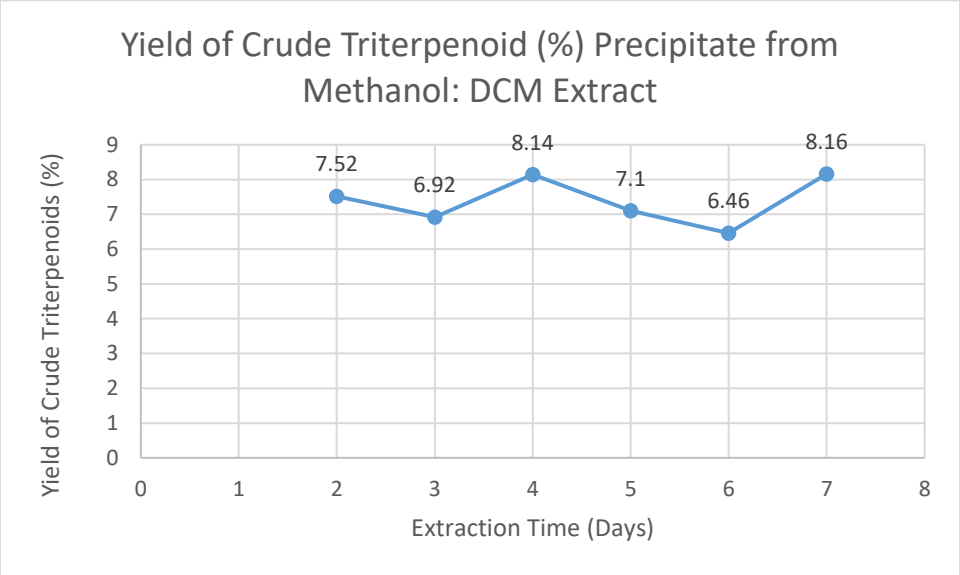

Figure 2: Yield of Crude Triterpenoid (%) Precipitated from Methanol: Dichloromethane Extract

Vacuum Liquid Chromatography Isolation of alpha amyrin from the crude triterpenoid precipitate

The fractional yields of the VLC purification of the precipitates from methanol extraction at the extraction times are shown in Table 3. From the result, it was observed that the highest percentage yield (26.52% of crude triterpenoid precipitate) of pure alpha amyrin acetate was obtained from the extraction time of 6 days. This was followed by 7 days and 5 days.

The results of the purification showed that, although higher yields of crude precipitate was obtained for methanol: dichloromethane extract compared to the methanol extract, the precipitate from the methanol extract yielded more pure alpha amyrin acetate compared to the methanol: dichloromethane extract

Table 3. Vacuum Liquid Chromatography (VLC) fractions of crude triterpenoid precipitate from methanol extract.

Solvent system (Hexane : EtOAc)	3 days	5 days	6 days	7 days
10:0	0.00 g	0.00 g	0.00 g	0.00 g
9:1	0.45 g (16.07%)	0.56 g (17.33%)	0.74 g (26.52%)	0.67 g (23.51%)
8:2	0.20 g	0.62 g	0.27 g	0.31 g
7:3	0.00 g	0.03 g	0.00 g	0.01 g
0:10	0.03 g	0.14 g	0.12 g	0.10 g

Note: Values in parentheses represent % yield of α-amyrin acetate relative to crude triterpenoid.

Table 4. Vacuum Liquid Chromatography (VLC) fractions of crude triterpenoid precipitate from methanol–dichloromethane (1:1) extract.

Solvent system (Hexane : EtOAc)	3 days	5 days	7 days
10:0	0.00 g	0.00 g	0.00 g
9:1	0.32 g (9.25%)	0.24 g (6.76%)	0.42 g (10.29%)
8:2	0.21 g	0.14 g	0.01 g
7:3	0.00 g	0.02 g	0.00 g
0:10	0.04 g	0.08 g	0.01 g

Note: Values in parentheses represent % yield of α-amyrin acetate relative to crude triterpenoid.

Discussion

In our earlier report (Okoye *et al.,* 2014) α-amyrin acetate which co-precipitated with a small amount of ß-amyrin was isolated and purified using Vacuum Liquid Chromatography (VLC). Its structure was fully characterized using EIMS and NMR. In

this present study, a simplified and optimized isolation and purification process was developed using different solvent systems (methanol and dichloromethane) and varying extraction time in an attempt to achieve a more efficient yield for both crude triterpenoid and α-amyrin acetate with a higher purity. This was closely monitored using Thin Layer Chromatography (TLC).

It is evident from the findings that both methanol and methanol–dichloromethane (1:1) solvent systems were effective for this extraction, however, the methanol extract yielded a higher amount of α-amyrin acetate upon purification. After the third day of maceration, the extraction yield using absolute methanol had reached an average peak, although day 5 recorded the highest yield (6.46%). The Vacuum Liquid Chromatography (VLC) fractionation further revealed that methanol extraction at 6 days was optimal, yielding 26.52% α-amyrin acetate from the crude precipitate. Interestingly, methanol-Dichloromethane (1:1) which consistently recorded a higher yield of crude triterpenoid precipitate gave a defimitely lower yield of pure α-amyrin acetate. This suggests that the addition of dichloromethane merely enhanced the solubility of diverse non-polar secondary metabolites, possibly other triterpenoids or unrelated metabolites (Sabaragamuwa and Perera, 2023, Sharma *et al.,* 2023) thereby increasing the yield of the crude (Park *et al.,* 2025).

This implies that while mixed solvents provided higher crude extraction yields, methanol alone facilitated more selective precipitation of α-amyrin acetate. This difference is likely due to the polarity balance of methanol, which favors the extraction of intermediate-polar triterpenoids, while dichloromethane extracts more lipophilic compounds in addition, thus reducing the purity of the crude extract (Chatepa *et al.,* 2024; Lee *et al.,* 2024)

Unlike conventional multistep triterpenoid isolations from other plant sources involving sequential defatting, partitioning, multiple chromatographic steps like flash column, Sephadex LH-20, prep-TLC, or HPLC (Sanusi *et al.,* 2020), this approach uses slow precipitation followed by a single VLC purification step to obtain analytically pure α-amyrin acetate. This reduction in steps minimizes solvent use, labor, and time, making the method cost-effective and adaptable to laboratories with limited infrastructure.

These findings are consistent with our earlier reports that α-amyrin acetate can be isolated in appreciable amounts from *Alstonia boonei* (Okoye *et al.,* 2014), but the current protocol further demonstrates that the process can be simplified without compromising yield or purity. Importantly, the availability of an optimized procedure for α-amyrin acetate isolation addresses one of the major barriers to advancing preclinical studies on this promising triterpenoid, namely, the difficulty in obtaining sufficient quantities for pharmacological and toxicological investigations (Chaachouay and Zidane, 2024; Ahmad *et al.,* 2025; Mungwari *et al.,* 2025).

Overall, the study establishes that maceration in methanol for six days, followed by slow precipitation and VLC purification, is the most efficient and practical procedure for high-yield isolation of α-amyrin acetate from the stem bark of *Alstonia boonei*.

Conclusion

This study has demonstrated a simple and efficient procedure for the isolation of α-amyrin acetate, a pharmacologically relevant triterpenoid, from the stem bark of *Alstonia boonei*. Among the different extraction conditions evaluated, maceration in methanol for six days followed by slow precipitation and purification by Vacuum Liquid Chromatography provided the highest yield and purity of α-amyrin acetate. While mixed solvent systems produced higher crude yields, methanol alone was more selective for α-amyrin acetate.

The optimized method reduces the number of purification steps traditionally employed, thereby lowering cost, solvent consumption, and labor, while maintaining efficiency. This streamlined protocol is suitable for laboratories with limited infrastructure and provides a practical approach to obtaining sufficient quantities of α-amyrin acetate for further pharmacological, toxicological, and formulation studies.

Future studies should focus on scaling up this procedure, as well as exploring its application in the isolation of structurally related triterpenoids from other medicinal plants.

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Conflict of interest

The authors declare no conflict of interests

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