SHORT COMMUNICATION

EXTRACTION AND ISOLATION OF β-AMYRIN FROM *FICUS ELASTICA*

ABSTRACT

There is a huge interest in medicinally active constituents isolated from plants. β-Amyrin is a member of the class of pentacyclic triterpenoids and it is oleanane substituted at the 3 β-position by a hydroxyl group and containing a double bond between positions 12 and 13. These triterpenoids are generally found in many medicinal plants. The β-amyrin is generally extracted and isolated from leaves and oleo-resin (latex) exudates by bark incisions of the plant sources from many plant species. The β-amyrin isolation described in this article was by a novel method developed for isolation. Triterpenoids are constituents that have shown a great interest in recent years due to their pharmacological potential, with numerous therapeutic activities, such as anticancer, anti-inflammatory, antiviral, antibacterial, antifungal, anti-diuretic, and acetylcholinesterase inhibitory. Due to the wide range of activities of β-amyrin, research has been undertaken to isolate it in a simple way. The isolated β-amyrin was characterized, and confirmed by multiple analytical methods.

Keywords: Triterpenoids, β-amyrin isolation, TLC, IR, MASS, NMR

INTRODUCTION

Pentacyclic triterpenes are widely distributed as aglycones or combined forms throughout the plant kingdom and have been known to have various biological effects[1]. *Ficus elastica* is India's most significant, beautiful, evergreen, and important indigenous rubber tree. Many medicinal plants generally contain α-amyrin and β-amyrin; previously, these compounds were extracted from latex or oleo-resin of the bark incisions of several species[2]. Some research is available on the extraction of β-amyrin in many *ficus* species from different parts, including leaves[3], fruits[4], stem barks[5], root bark[6], oleo-resin exudates[7]. β- Amyrin has been reported for many pharmacological activities including anti-inflammatory[8], anti-diabetic, hypolipedemic[9], neurological activity[10], antioxidant activity[11] anti-proliferative activities[12], and antimicrobial actions[13]. Previously, some of the isolation techniques for β- amyrin have been reported, based on column chromatography[14,15,8] and also they had experimented with other *Ficus* species, but we focused on simple isolation methods of β- amyrin based on its multiple activities, as there is a need to isolate it from plants by simple methods.

MATERIALS AND METHODS

Plant materials and chemicals

Fully grown fresh leaves of *F. elastica* were collected in the ICT campus garden in Mumbai, Maharashtra. All the required solvents and chemicals used in this investigation were of analytical grade, purchased from Sigma Aldrich, Powai, Mumbai.

EXPERIMENTAL

Isolation of β-amyrin

The β-amyrin was isolated from *F. elastica* by a simple method developed in our laboratory. About 100 g of *F. elastica* leaves coarse powder was extracted with 300 mL of petroleum ether by a Soxhlet extractor for 2 h, then it was collected and concentrated to half of its volume, and afterwards it was precipitated out by adding methanol, because the plant leaves contained a more prominent quantity of β- amyrin than other constituents. The extract was filtered to obtain precipitation with 125 mm Whatman filter paper. The obtained crude precipitate was used for further purification by charcoal treatment using methanol. (Based on solubility, methanol had been chosen for recrystallization). In the isolation process shown below (Fig. 1), the isolated β- amyrin was confirmed by Melting Point, TLC, IR, NMR, and MASS spectrometry. The β- amyrin yield was 2.6% w/w.

RESULTS

Melting point

For the determination of melting point, the isolated β-amyrin was filled in one capillary tube which was sealed at one end. A similar procedure was used for the reference standard. The capillary tubes containing the samples were placed in the apparatus, and the temperature at which the sample get melted was noted.

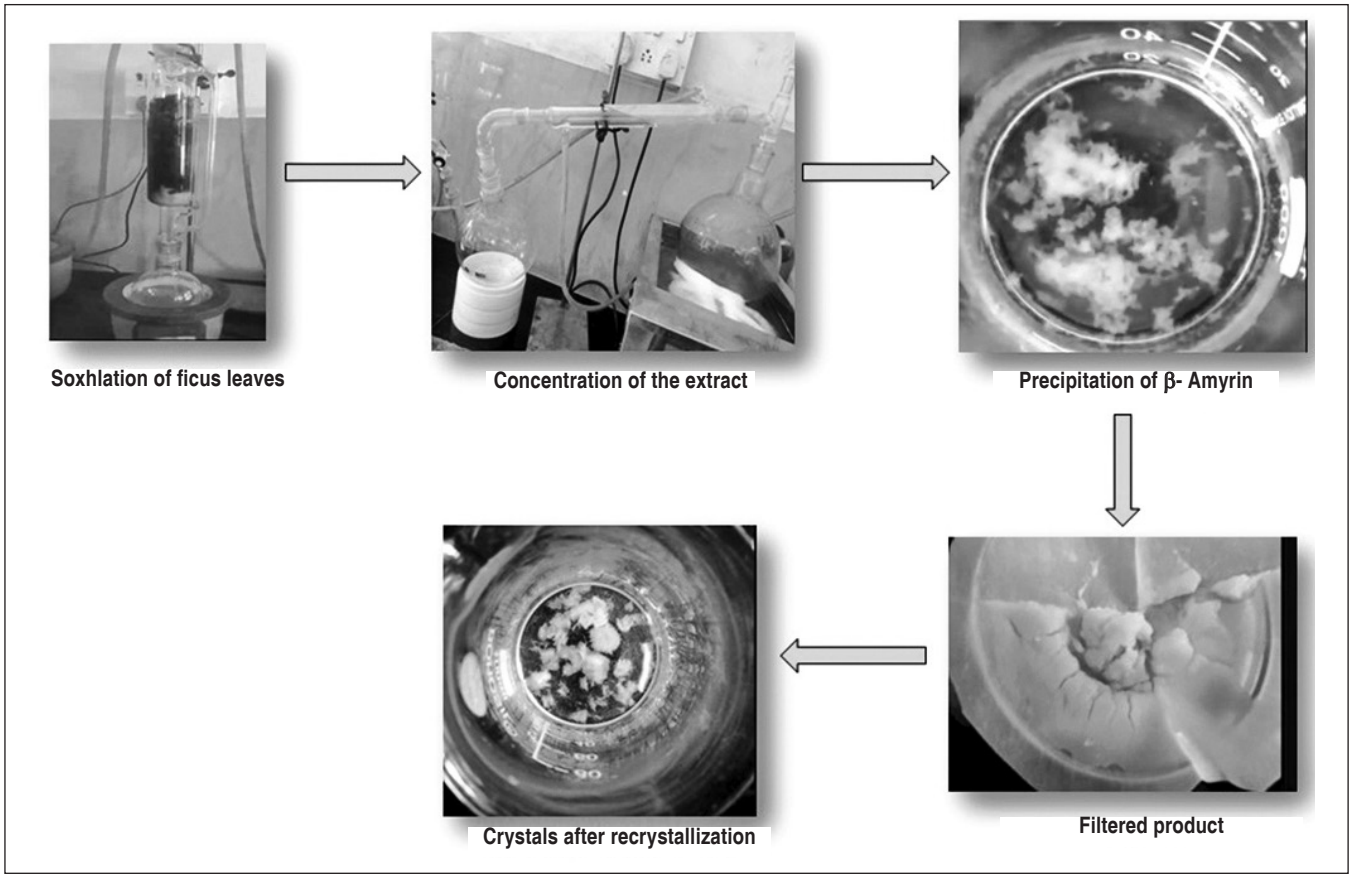

Fig. 1: β-Amyrin extraction and isolation process step by step

TLC profile of extract and isolated compound

F. elastica powder was transferred into the test tube, and 5 mL of methanol was added to it. Then it was shaken for 2-3 mins and the contents were allowed to settle down. Afterwards, the methanol solution was separated from undissolved solids using a pipette or by decantation. This solution was used for the development of TLC, the mobile phase toluene: ethyl acetate (95: 5 V/V) was used, to run the TLC and derivatized with anisaldehyde sulfuric acid (Fig. 2); the R_f value found to be 0.42.

Liebermann-Burchard test

In a dried test tube, β-amyrin extract was dissolved in chloroform and a few drops of acetic anhydride were added to it. Afterwards, 2-3 drops concentrated sulphuric acid was added from the side of the test tube, and then it was kept aside for a few minutes; the development of pink color indicated the presence of triterpenoids (Fig. 2).

Infra-red spectrometry

A Fourier-transform infrared (FTIR Shimadzu model: IRAffinity-1S) was used for the IR spectrum of the sample. The spectrum of the isolated compound was obtained by scanning in an FTIR spectrophotometer at room temperature and the KBr pellet method was used for the analysis. The isolated white crystalline solid and powdered sample was mixed with KBr pellets to obtain a thin pellet and was used in the FT-IR instrument. The sample was scanned in the 4000 to 400 cm^{-1} range (Fig. 2).

NMR spectrophotometer

Nuclear Magnetic Resonance (Bruker NMR at 500 MHz Germany with 100 scans for better resolution) spectrophotometer was used to measure the spectrum of the isolated sample. NMR spectrum is given below (Fig. 2). The proton NMR of the isolated compound matched with the previously available data. It was analyzed with Bruker software.

Mass spectrometry

The mass spectrum of the isolated compound was performed by mass spectrometer (MS - EI) obtained with a Finnigan MAT 8430 mass spectrometer) to confirm the identity of the compound. The reported molecular weight of the β-amyrin was found to be 426 Daltons[16].

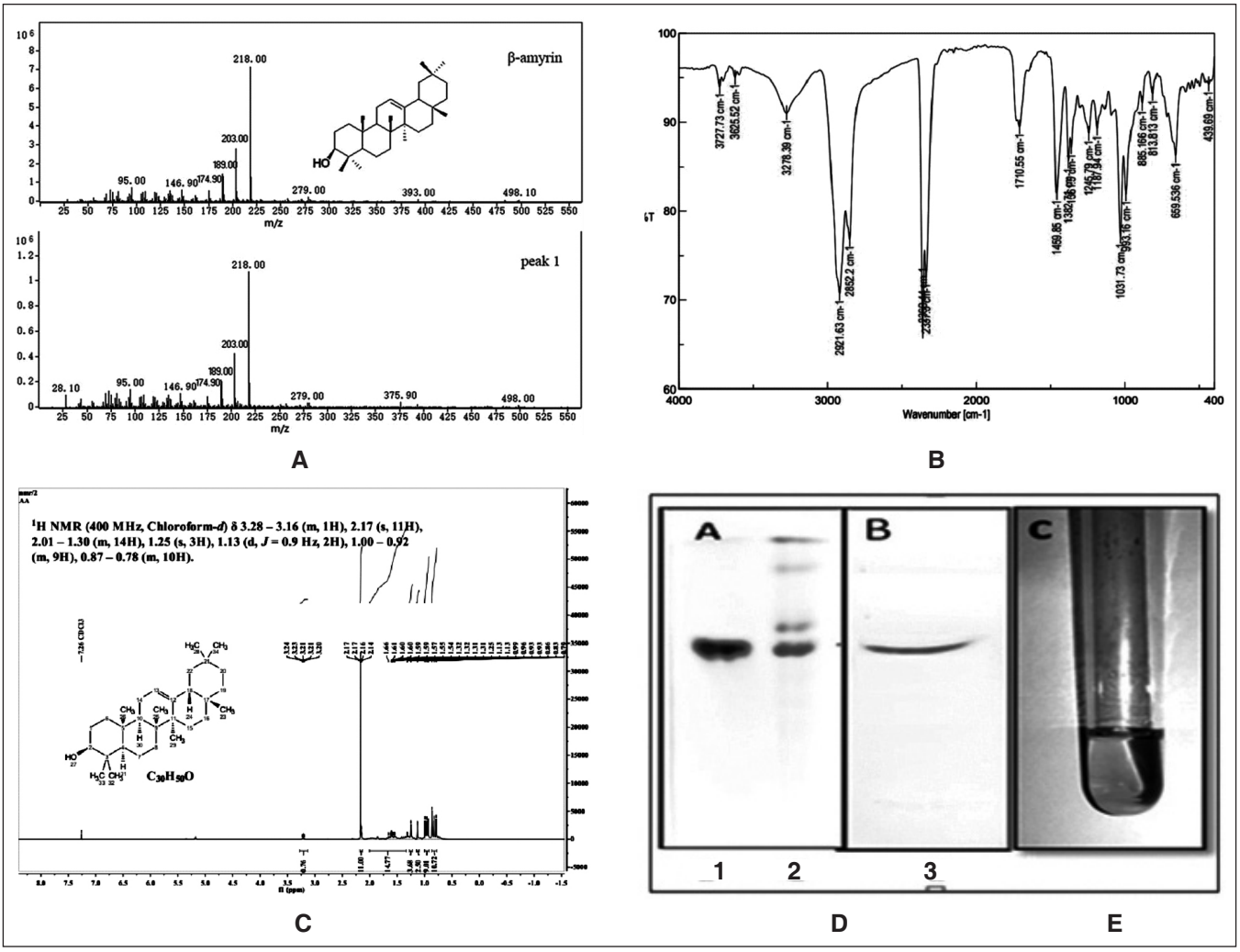

Fig. 2: A- Mass, B- IR, C- NMR, D- TLC and E- Liebermann-Burchard test

Note: TLC profile: Isolated β-amyrin (track 1) *Ficus* extract (track 2) and standard β-amyrin (track-3)

DISCUSSION

The research objective was to isolate the essential triterpenoids, β-amyrin, from the *F. elastica* leaves with minimum of time and solvent. Various parameters such as solubility, temperature and stability have been considered. The powdered sample was initially (previously isolated by researchers) extracted with methanol, and then isolated using column chromatography[16]. This method was time-consuming, and the yield was poor. Another process used for isolation includes defatting, and further extraction with chloroform. The chloroform layer was concentrated, and charcoal treatment was done. The isolated compound was also impure, having a low yield, and the process was thus rejected. The β-amyrin was also extracted and isolated by column chromatography in its acetate form[17-18] (β-amyrin acetate), but the yield was poor, time-consuming, and with solvent wastage. Finally, as β-amyrin had good solubility in non-polar solvents, it was extracted with *n*-hexane, and then concentrated, recrystallized with petroleum ether, white color crystals of β-amyrin were observed. For the TLC, also the system was optimized as toluene: ethyl acetate (95:5 V/V). The developed method has advantages such as simplicity and ease. Then isolated compound was analyzed by different spectroscopic methods[19-20]. The isolated β-amyrin has been clearly shown in the picture (Fig. 2): of FT-IR data (Fig. 2) (KBr, cm^{-1}) ν: 3274 (OH), 2913 (CH); 236 (C=O); 1600(C=C), the NMR data (Fig. 2)^{1}H-NMR: (500MHz, $CDCl_3$-d6): δ 1.910-1.982 (m, 14H, aliphatic), δ 2.847-3.084 (m, 8H, Aliphatic), δ 4.689 (s, 1H, amine NH), δ 7.370-7.408 (t, J=7.2Hz, 1H, Ar-H), δ 7.567-7.607(t, J=7.6Hz 1H, Ar-H), δ 7.703-7.724 (d, δ 8.4Hz, 2H, Ar-H), δ 7.927 (s, 1H, amide NH), mass spectrum of this compound (Fig. 2) showed $[M+H]^+$ peak

at 427.4. The analytical results $[M+H]^+$ confirmed that the isolated compound was β-amyrin.

CONCLUSION

β-Amyrin was isolated from the dried leaves of *F. elastica* from Moraceae family. It was extracted, isolated, and confirmed as β-amyrin by analytical parameters such as melting point, TLC, IR, NMR, and mass spectrometry. This research was used to determine the qualitative and quantitative analysis of the various marketed formulations which contain β-amyrin as medicament. The isolation method was simple, hence it can be used for future research and serves as a biochemical marker compound for standardization of β-amyrin in the pharmaceutical industry and also to make it more available for synthesis of many new analogues of β-amyrin.

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