A Novel Extraction and Chromatographic Investigation of Phytoconstituents in Sandal Seed

P. Rajavel [1*], S. Soundarrajan[2], S. Lavanya[3]

[1]Professor, Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, Tamil Nadu, India.
[2]Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, Tamil Nadu, India.
[3]Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, Tamil Nadu, India.
[1] velraj512@gmail.com, [2] soundarrajan1571999@gmail.com, [3]lavanyas5101@gmail.com

***Address for Correspondence**
P. Rajavel
Professor,
Department of Pharmaceutical Analysis,
Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, Tamil Nadu, India.
Email: velraj512@gmail.com; Contact No: +919581643612

Abstract:

One of the oldest and most precious sources of natural fragrance with significant therapeutic and economic value is Santalum album L. (Santalaceae), sometimes referred to as Indian sandalwood. The cosmetics sector may make use of the oil that is derived from the seeds. Three families' seed oils are the main source of natural ximenynic acid. Trans-11-octadecane-9-yonic acid is present in significant amounts in the seed oil of Santalum album L. Following extraction, separation, and characterization using gas chromatography, thin-layer chromatography, and high-performance liquid chromatography techniques, the identity of xymenynic acid was verified. When Santalum album L. seed oil was characterized and 12 fatty acids were identified, only the quantity of ximenynic acid was discovered to be 66.76%. Nitrogen was used as the gas to help move Ximenynic acid, flowing at a rate of 1.37 milliliters per minute, after adjusting the testing conditions. The ximenynic acid peak's retention time was 17.743 minutes for the standard and 17.753 minutes for the sample. Chromatographic development was performed on a TLC plate precoated with silica gel 60 F254 using a mobile phase consisting of a 9:1 v/v hexane: ethyl acetate combination. Xymenynic acid's Rf value was determined to be 0.72 ± 0.01. According to ICH criteria, this approach was verified and determined to be within the limit. HPLC investigation was conducted using Phenomenex (C18, 250 x 4.6mm, 5 µ) column was used to accomplish the chromatographic separation. UV detection at 229 nm was used .Temperature was maintained at 25°C using acetonitrile as a mobile phase for ximenynic acid, with a flow rate of 1.0 mL/min. The ximenynic acid sample and standard were found to have retention times of 6.10 and 6.12 minutes, respectively.

Keywords*: Sandal seed, ximenynic acid, characterization, separation, GC, HPLC, TLC.*

1. Introduction

The majority of plants are used in cosmetics and medicine to cure a variety of illnesses and enhance human health. The plant has a large number of active constituents that are classified as drugs and medicines [1]. Indian sandalwood, also known as Santalum album.L (Santalaceae), is one of the most ancient and valuable sources of natural fragrance with significant therapeutic and industrial uses. The cosmetics sector can find use for the oil that is derived from the seed. Ximenynic acid, sometimes referred to as santalbic acid (E-11-octalecen-9-ynoic acid), is an uncommon acetylenic fatty acid found in the Santalaceae family. Indian sandalwood is a genus of flowering plants, and it belongs to the family Santalaceae, the fourth largest family of angiosperms. Sandal seeds are obtained by removing the fleshy portion of the fruit. They are naked, lacking testa [2]. The percolation method was utilized to accomplish the extraction process with hexane [3]. The presence of ximenynic acid in the crude extract powder was determined by gas chromatography techniques using a flame ionization detector [4-5]. The powder extract is separated and identified by high-performance liquid chromatography with a UV/VIS detector and thin-layer chromatography using silica gel 60 F2S4 (20×20 cm) [6].

2. Materials and Methods

2.1. Plant collection

Sandal seed was purchased from the Madhavi Agencies, Guntur, and Andhra Pradesh.

2.2. Reference standard

Ximenynic acid was purchased from Sigma-Aldrich® Lab & Production material.

2.3. Chemicals and Solvent

Ethanol (AR grade, China make), Hexane (FINAR [HPLC Grade and spectroscopy]), Potassium hydroxide (SDFCL-S.D. fine chemical limited, AR Grade), Hydrochloric acid (RANKEM), HPLC Grade water (Milli-Q RO system), Acetonitrile (FINAR [HPLC Grade and spectroscopy]), Methanol (RANKEM), Anisaldehyde (SDFCL-S.D. fine chemical limited, AR Grade), Sulphuric acid (KANKEM).

2.4. Extraction Process

500g of sandal seed paste like slurry weighed and transferred into round bottom flask. Four volumes (2Ltr) of hexane were added into round bottom flask. Kept the extract at reflux temperature for 2hrs under stirring. [Temp =60-65˚C]. Filtered through filter cloth and

collected the filtrate. Charged back the spent of 1st extraction for 2nd extraction into round bottom flask. The extraction was repeated with 4 volumes (2Ltr) of hexane .Clubbed both the filtrates and concentrated by distilling at 50-55˚C. Transferred 180g of concentrate extracted filtrate for the saponification process into the round bottom flask. Added 50ml of 98% ethanol, solution of 50g potassium hydroxide (KOH) dissolved in 250ml of RO water into a round bottom flask and Saponified at 90˚C for 4hrs under stirring. After that it was cooled for 1hrs in open condition so that ethanol evaporates. Transferred into a beaker and added 300ml of hexane and 1250ml of RO water into the beaker. Mixed well and transferred into a separating funnel and added 2Ltr of hexane into the separating funnel. Mixed well and kept for 30 minutes and separated the top layer and bottom layer. [Bottom layer = 750ml]. Added 750ml of hexane to the bottom layer obtained and mixed well and settled after 30 minutes and separated the top layer and bottom layer. [Bottom layer =600ml]. Took the bottom layer and added 600ml of hexane to the separating funnel. Mixed well and settled for overnight and separated the top layer and bottom layer. [Bottom layer = 1.5Ltr and pH of bottom layer = 14]. Took the bottom layer and adjusted the pH to 4.6 with dil.HCL, added RO water to the paste material. After that the paste was dried in a hot air oven at 60˚C for 16hrs. Crude extract powder was obtained [7].

2.5. Characterization of crude extracts powder by gas chromatography method

2.5.1. Preparation of internal standard solution

Weighed accurately 120mg of the nonadecane into a 10ml volumetric flask, 5 ml of hexane was added, and the mixture was sonicated for ten minutes. Then the volume was made with hexane.

2.5.2. Preparation of standard stock solution

The mixture of 5mg/ml solution of Methyl Oleate RS , 2mg/ml solution of Methyl Palmitate, 1 mg/ml solution of Methyl Linoleate ,0.4mg/ml solution of Methyl Caprote , 0.4mg/ml solution of Methyl Caprylate , 0.4mg/ml solution of Methyl Caprate , 0.4mg/ml solution of Methyl Palmitoleate ,0.4mg/ml solution of Methyl Stearate , 0.4mg/ml solution of Methyl Linolenate was prepared using hexane in equal proportions.

2.5.3. Preparation of working standard solution

1 ml of internal standard solution was mixed with 5 ml of standard stock solution.

2.5.4. Preparation of sample solution

Accurately weighed 100 mg of extract was transferred to pressure-proof, screw-capped vial, 30ml solution of sulphuric acid in methanol (5 in 100)was added then heated at 100˚C in oil bath for 2 hours by shaking. Allowed to cool, 1.0ml of the internal standard solution, 10.0ml

of water, 1g of sodium chloride, and 5 ml hexane were added. Then it was allowed to separate completely, the hexane layer was separated out and used for GC analysis [8].

2.6. Separation and identification of ximenynic acid from crude extract by thin layer chromatography method

2.6.1. Preparation of standard

10mg of ximenynic acid was weighed & transferred into a 10ml volumetric flask. 5 ml of hexane was added and sonicated for 10 minutes and made the final volume with hexane [9].

2.6.2. Preparation of sample

10mg of sandal seed extract was weighed accurately and transferred into a 10 ml volumetric flask, 5 ml of hexane was added and sonicated for 10 minutes. Then make the final volume with hexane [10].

2.6.3. Method

Purchased pre-made TLC plates coated on aluminum (TLC Silica gel 60 F 254) were cut to size (20×20 cm) for separation. The mobile phase was made by combining hexane and ethyl acetate in a 9:1 ratio. 20 ml of the mobile phase was taken into a TLC chamber and left to saturate. Next, the standard and sample solutions were applied onto a TLC plate and then placed in the TLC chamber. The TLC chamber was left to separate until the mobile phase had traveled 80% of the plate. Then the TLC plate was removed from the chamber and sprayed evenly with anisaldehyde reagent before being dried in a hot air oven at 150ºC for 2-3 minutes[11].

2.7. Separation and identification of ximenynic acid from crude extract by HPLC

2.7.1. Preparation of standard stock solution

Accurately weighed 25mg of ximenynic acid was transferred into 100 ml volumetric flask; 50 ml of diluent was added to dissolve and sonicated for 10 minutes. Then cooled and the final volume made with diluent. The resulting solution was filtered by a 0.45µ membrane filter by discarding the first few ml of filtrate.

2.7.2. Preparation of sample

Accurately weighed 100mg of crude extract powder was transferred into 100 ml volumetric flask; 60ml of methanol was added to dissolve and sonicated for 10 minutes. Mixture was heated in a water bath at 60ºC for 30 minutes and cooled .The final volume was made with methanol. The resulting solution filtered by 0.45µ membrane filter by discarding the first few ml of filtrate.

2.7.3. Method

20µl of diluent was injected as a blank run in the chromatograph and discarded the peaks caused by the solvent in the sample and standard chromatograms. Then 20µl of standard solution was injected twice and ensured that the relative standard deviation for replicate injections is below 2.0%. Then, injected 20µl of the sample. The responses of both the standard and sample were recorded at 229 nm [12].

3. Result and Discussion

3.1. Extraction Process

Sandal seed was collected and grained using a mixture jar. The Phytochemicals constituents were extracted by percolation using hexane as solvent. By using 500g of seed, 130g of crude extract powder was obtained.

3.2. Gas Chromatography Method

Chromatographic conditions were optimized using nitrogen as the mobile phase at a flow rate of 1.37 ml/min. The chromatogram was run through a fused silica capillary column coated with a phase G16 film that was 0.25mm × 30m in size. Temperature was kept at 250˚C for the injector and 300˚C for the detector. The ximenynic acid peak was found to have a retention time of 17.753mins for standard and 17.743mins for sample. The chromatogram obtained from the GC analysis is shown in fig-1, 2. The GC analysis confirmed the presence of ximenynic acid in seed extract. The retention time and peak area observed in the chromatogram was consistent with those of the standard solution, indicating the reliability of the analysis method. The concentration of Ximenynic Acid in the sample was determined by comparing its peak area to that of the standard solution.

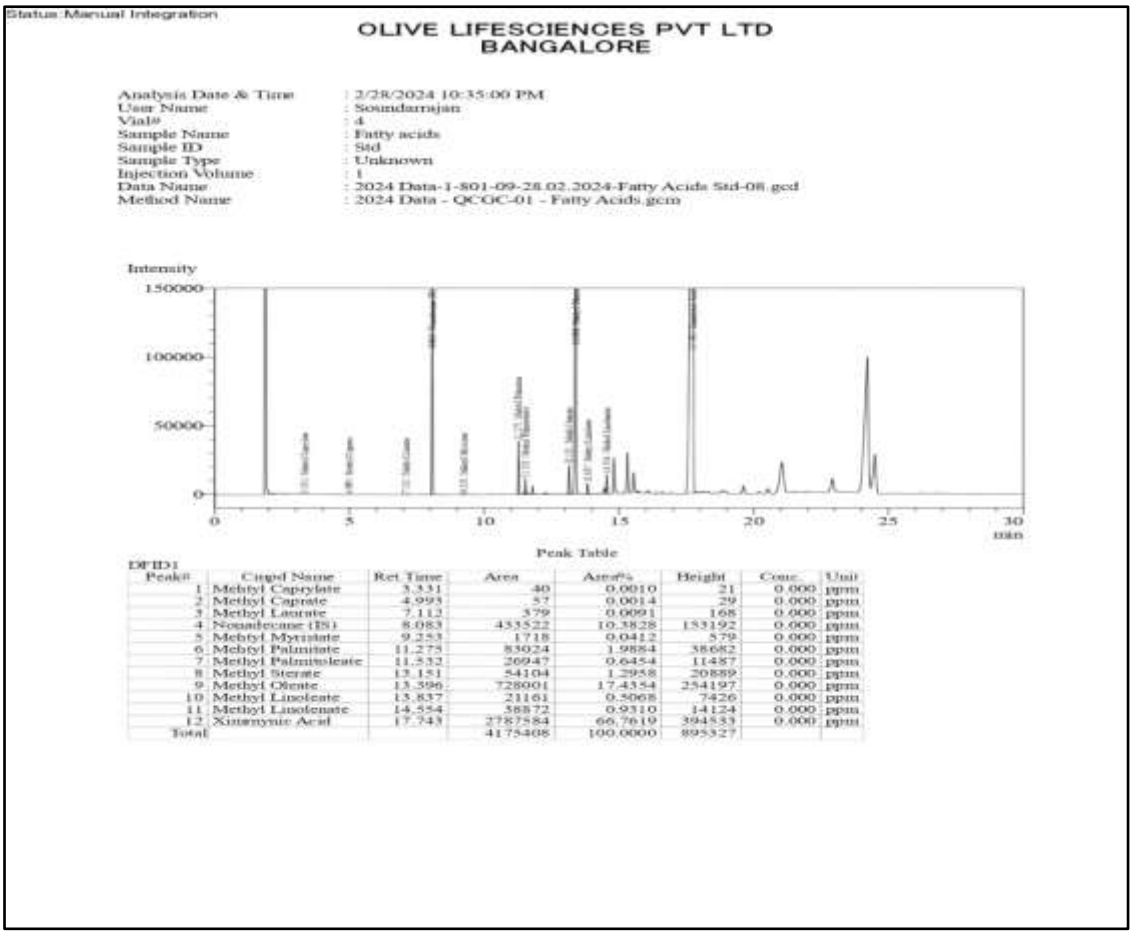

Figure 1. GC Chromatogram of Standard

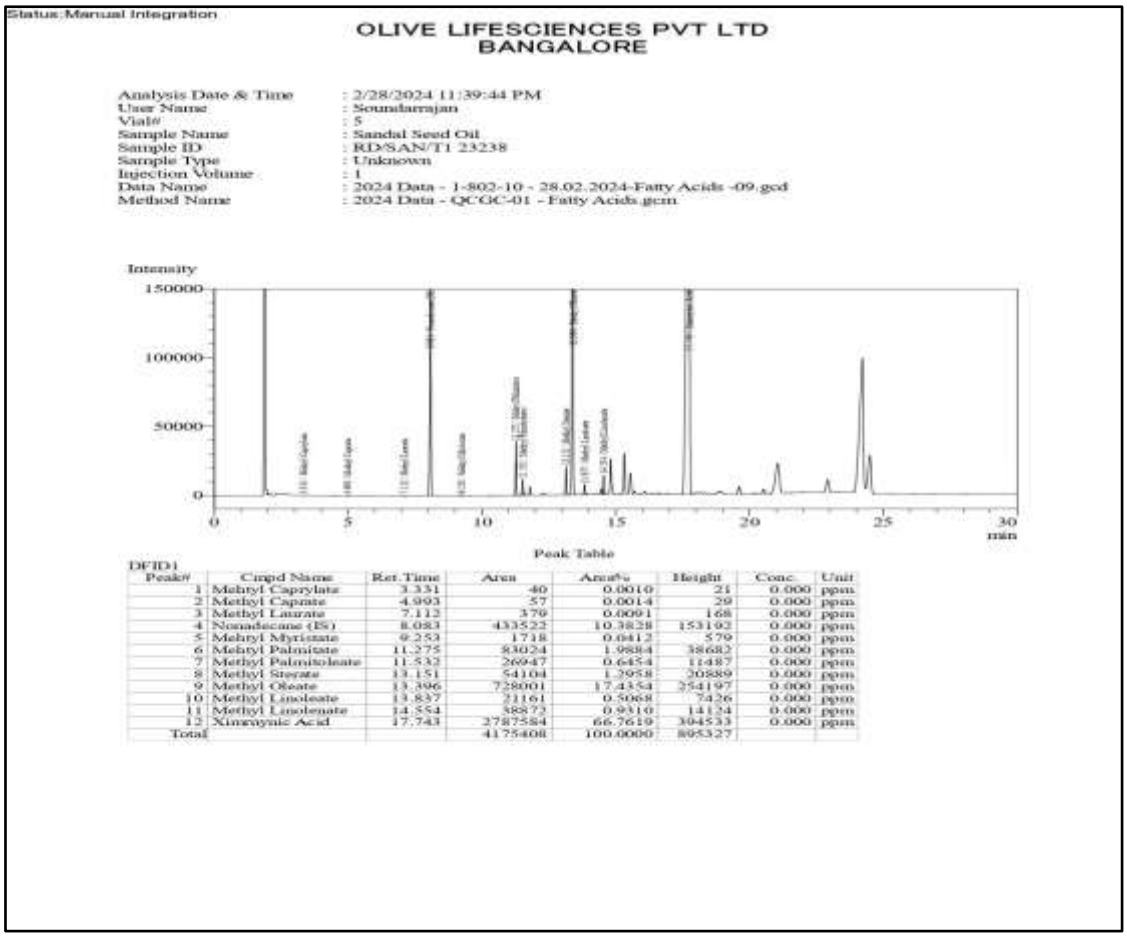

Figure 2. GC Chromatogram of Sample

3.3. HPLC Method

The optimization of chromatographic condition was done and the mobile phase used was Acetonitrile with flow rate of 1.0ml/min. The detection wavelength was 229 nm. Chromatogram was run through the phenomenex (C18, 250 × 4.6mm, 5µ) column. Temperature was maintained at 25˚C. The injection volume fixed was 20µl. The retention time was found to be 6.10 min & 6.12 min for ximenynic acid in sample and standard chromatogram respectively. A typical HPLC Chromatogram was shown in fig-3, 4. The retention time of Ximenynic Acid in sample RT (6.10mins) observed in chromatogram matched with that of the standard RT (6.12mins), indicating the presence of Ximenynic Acid in the collected fraction.

Figure 3. HPLC Chromatogram of Standard

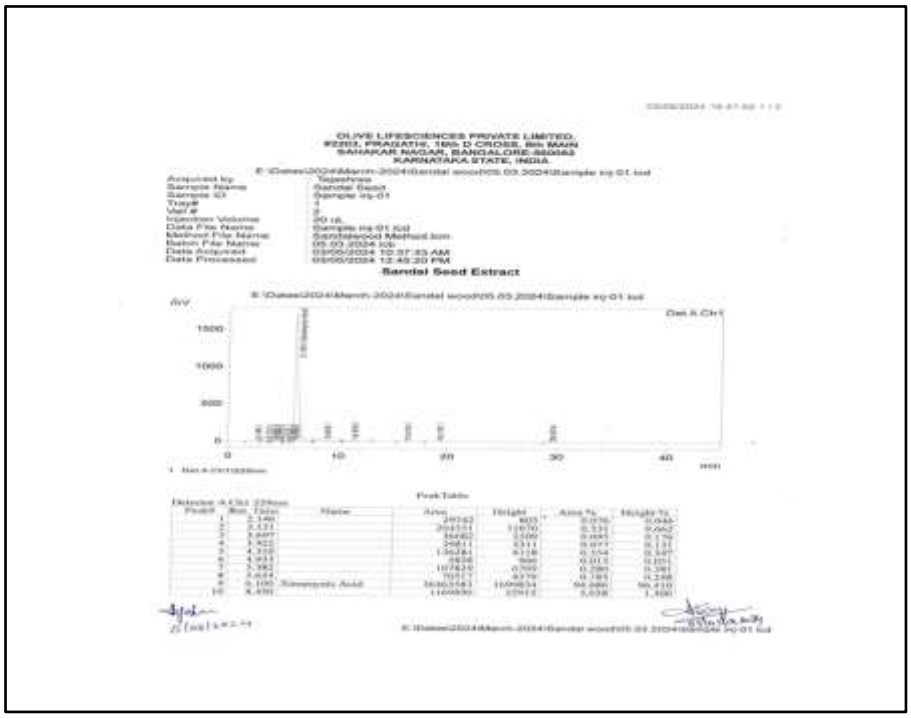

Figure 3. HPLC Chromatogram of Sample

3.4. TLC method

Pre-coated plate(silica gel 60F2S4) and the mobile phase containing Hexane: Ethylacetate in the ratio of 9:1v/v used for the TLC separation. Violet colored spots in TLC Plate was observed using Anisaldehyde as a detecting agent. The Rf value of sample (0.72) was close to

the Rf value of standard (0.70). The TLC method identified the presence of ximenynic acid in crude extract powder.

4. Conclusion

In the present research, the ximenynic acid was successfully isolated and purified from *Santalum album* seed. Chemical characterization,isolation and identification of ximenynic acid was done by Gas Chromatography, High Performance Liquid Chromatography and Thin Layer Chromatography techniques. Ximenynic acid has the potential as a novel antiaging in functional food. This method is new, simple and economical for routine isolation and characterization of ximenynic Acid in sandal seed extract. In future research, the detailed structure of ximenynic acid needs further study.

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