RESEARCH ARTICLE

Organic Extraction, Purification and Evaluation of Long Chain Fatty Alcohol from *Saccharum officinarum* of Sugar Refinery Waste for Human Wellness

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ABSTRACT

The current study has explored an extraction and purification method for 1-octacosanol from sugarcane waste, commonly known as press mud, through the application of SCFE by the unique concept of solid-solid and liquid-liquid extraction which increases content and decreases impurity which is an extra advantage at purification stage by help of green solvent instead any carcinogenic solvent, which reduce process time, increase content and environment friendly. The isolated compounds were characterized by gas chromatography-flame ionization detection, which shows 50% content of octacosanol in the final product. Novel refinement technique through hot ethanolic reflux method has substantially increased the octacosanol assay to ≥ 50% with more than 67% purity from the crude wax assay of 26% obtained by the current method of extraction. The identity of said compound was further authenticated by X-ray diffraction (XRD), Diffraction scanning calorimetry (DSC) and FTIR studies. The purity of the octacosanol obtained was ≤70% and the conversion recovery of octacosanol was 80.04% by the reflux method, thereby representing the simplicity of the above process for industrial feasibility. The current process represents a value addition liquid-liquid supercritical extraction with a time-saving, cost-effective, reproducible, newer, green, safe, and sustainable technology valorizing sugarcane waste for a high-end nutraceutical.

Keywords: 1-Octacosanol, Supercritical fluid extraction, Press mud, *Saccharum officinarum*, Valorisation, Green extraction.

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INTRODUCTION

Plant-based bioactive compounds are gaining momentum in the current research scenario by virtue of the tremendous possibility for usage in pharmaceuticals, nutraceuticals, and functional food industries[1]. 1-Octacosanol is a major constituent of policosanols, which is the long-chain fatty alcohol aliphatic compound and found in plant epicuticular wax and insect wax. 1-Octacosanol an aliphatic fatty alcohol of 28-carbon straight-chain and be isolated from the epicuticular part of plants like *Saccharum officinarum*, insect wax and krill.[2] Many important impacts of these compounds such as cholesterol-lowering, antifatigue, anti-hypoxia, antiaging, cardioprotective, antioxidant, ergogenic properties, neurological and potential protective effects on parkinsonism were studied previously.[3] The toxicity profile of this compound exhibits lethal dose (LD_{50}) in rats was 18000 mg/kg orally, which is quite high compared to table salt (LD_{50}:3000 mg/kg).[4] Due to the nontoxic effect of octacosanol, it has been considered a secure and adequate functional food basic for function in food, medicine, cosmetics and dietary supplements/ wellness products.

Sugarcane (*S. officinarum*) is sweet[5] and the prime economic crop that has shared 31.9 million tonnes of production by the year 2021-22 in India. For every 100 tonnes of crushed sugarcane, three tonnes of press mud cake are produced as an industrial byproduct by sugar mills[6]. During cane harvesting and purification, each 1-ton sugarcane would produce 33 kg of filter press mud7 and crude wax, with octacosanol amounts up purportedly reported to 63%.[8] This press mud can be turned into a cost-effective and sustainable resource for extracting octacosanol, which can be further commercialized, thus valorizing the industrial waste into a high-value human wellness product.[9] The extraction of octacosanol was also attempted from molasses and vinasses, where the content would be 81% in a part of the wax extraction.[10] Recent research also justified the purity and found 3.13 g purified 1-Octacosanol

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against of 46.31 g gross sugarcane wax. Even the sugarcane bagasse was found to have more content of policosanol than other plant parts and a high and stable content of octacosanol.[11] Purification of a blend of fatty alcohols from press mud afforded 1-Octacosanol, accounting of 55–65% from blend.

Supercritical carbon dioxide is crucial for selectivity and potential for isolation of heat-sensitive compounds due to their gas-like and liquid-like properties and, low critical temperature and pressure. Further, the combination of a few co-solvents like ethanol, water, and methanol also makes it more feasible to extract large and polar compounds. The CO_2 supercritical extraction process was utilized to separate 1-octacosanol from sugarcane.[12]

Our goal of current study is to add value to the currently used procedure for extracting octacosanol from sugarcane press mud. The study has aimed to utilize green solvents and a cost-effective method for the isolation of octacosanol with the potential to be implemented at industrial scale. Further, the method is environmentally friendly due to the effective route for using the sugarcane industry waste into higher yield of octacosanol than existing methods.

MATERIAL AND METHODS

Sugarcane press mud was collected from local sugar refining industries in Uttarakhand (Shakumbari Sugar Mill) and was procured as raw material. The sugarcane press mud was dried by help of vacuum at 60°C at least 9 hours and grinded and sifted through 40 ASTM sieve after drying. 1-Octacosanol standard (97%) was purchased from Sigma-Aldrich and Ethanol (99% purity) was provided by India Glycols Limited, Kashipur Unit. The rest of the other chemical reagents were LR (laboratory grade) and used as received.

Supercritical Fluid Extraction of Press Mud Wax

Extractions were carried out in an extractor (Thar Process, Inc. PA 15238, USA) in which 1 kg of filter sugarcane press mud was blended with 4 g powdered sodium hydroxide filled into a 2 L extraction vessel and extracted with 99.99% pure carbon dioxide with flow rate of 1.1 kg/min. The extract was collected in cool separator at room temperature. Solid extractions and liquid-liquid extraction were performed three times. The impact of process temperature, time, and pressure during isolation to check impact on efficiency were checked.

Purification of 1-Octacosanol Extracts from Sugarcane Press Mud Wax through Reflux Extraction and Rotary Evaporator Distillation

In 100 gm of sugarcane press mud extracted wax were poured in 8 L of pure ethanol along with 5% (w/w) NaOH in a 20 L reactor to extract chlorophyll, fat and then saponified under refluxing at 120 rpm under 80°C for 6 hours. After isolation, the slurry was passed through 300 ASTM mesh muslin cloth, a filtered solution dried with help of a rotary evaporator at optimized process parameters like temperature, vacuum, and rotation then obtained semi-dried octacosanol which was dried in a vacuum tray dryer at 40°C to obtained free-flow white powder and content of 1-Octacosanol in the obtained powder were quantified by GC-FID.

GC-FID analysis of policosanols for 1-Octacosanol assay determination

GC-FID quantified octacosanol as per the defined method[10] with slight modification. The GC-FID (Agilent GC-7890A) system uses the DB5 (30 mm × 0.25 mm I.D, layer thickness 0.25 μm) capillary column. using the 1:20 split ratio of nitrogen gas as an inert vehicle at flow rate 3.0 mL/min. The detector and injector temperatures was operated at 280˚C. An injected volume were performed at 0.1 μL. The column temperature of were set for 260˚C. The amount of 1-octacosanol was calculated through the comparison of the area covered.

Determination of XRD

The structural characteristics of octacosanol was checked through XRD (Brook Technology Co. Ltd., Germany), by some modifications.[13] The analysis performed at ambient temperature at a scanning rate 1.2°/min, the diffraction angle ranges 2 to 50° and the output results were checked through (Materials Data, Inc., USA) MDI Jade 6 software. The Cu-Ka radiation and current of 15 mA and output voltage 40 kV.

Determination of DSC

The enthalpy of octacosanol was determined with a SHIMADZU- DSC-60 differential scanning calorimetry. For this, octacosanol (4 ± 0.3 mg) was taken. Instrument temperature parameters gradually increased from 40 to 300°C with 20°C/min.[14] By an open empty aluminum pan sample baseline were obtained.

RESULTS AND DISCUSSION

Octacosanol Yield and their Characteristics

The sugarcane filter press mud contained 0.62/100 g wax based on the dry matter while the octacosanol content in the wax was found to be 2.32/100 g. The GC-FID (Figure 1) results revealed the presence of a major policosanol,[15] the octacosanol (RT: 28.9 minutes) in the filter mud with the eicosanol at (RT: 15.5 minutes) taken as internal standard.

The octacosanol extracted by the successive hot solvent extraction of the crude wax obtained by supercritical enrichment led to an achievement of overall purity of octacosanol by 67 to 70% with minimum contributions of other policosanols in trace amounts and an overall assay enrichment of more than 50%. The octacosanol obtained by the above process represents a fast and recoverable method of easy conversion of high-purity octacosanol from the crude wax in rapid and easy achievable steps thus creating a potentiality for commercialization.

The melting point of octacosanol was 85.3°C and loss on drying at 70°C was 0.88% w/w. The result of the melting point was corroborated with purified 1-Octacosanol and powder-free flow due to low loss on drying value.

The XRD patterns showed these diffraction peaks where peaks of octacosanol were found in respect of angles 21.27°

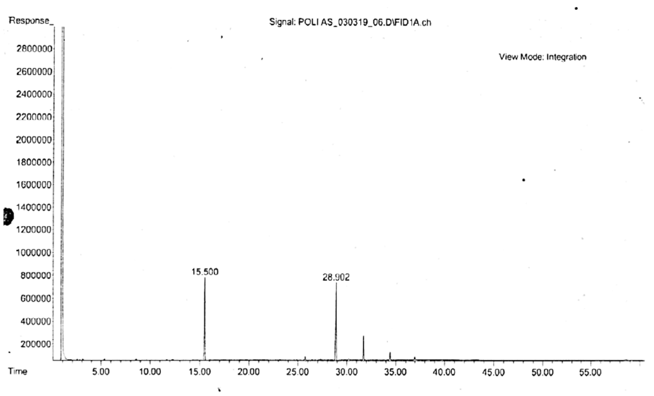

Figure 1: GC-FID chromatogram of octacosanol obtained by extraction with hot ethanol reflux. (By GC-FID Octacosanol purity quantified after extraction and purification by supercritical CO_2, Liquid-liquid CO_2 extraction and hot ethanol reflux extraction, respectively).

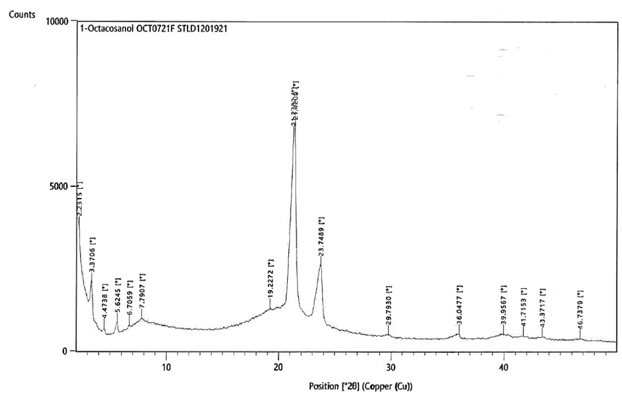

Figure 2: XRD Graph of Octacosanol obtained by extraction with hot ethanol reflux. (By XRD of octacosanol powder crystalline nature quantified after extraction and purification by supercritical CO_2, Liquid-liquid CO_2 extraction and hot ethanol reflux extraction, respectively).

and 23.74° at a °2θ scale, in which we conclude the crystalline nature of 1-Octacosanol (Figure 2). Thus, the results were confirming the supercritical fluid extraction followed by different extraction procedures was safe and productive and able to extract the desired compounds.

However, the temperature, pressure, saponification treatment, and complexation have not influenced the 1-Octacosanol structure and purity during the extraction, thus establishing the stability of the molecule. DSC graph of octacosanol powder (Figure 3) shows onset value start from 59.17 to 79.02°C and peak value from 62.37 to 85.09°C.

Impact of Process Time, Process Pressure and Process Temperature against Extract Output in Supercritical CO_2 Fluid Isolation

The experiments were performed to optimize process conditions for the extraction of extract in sugarcane press mud by supercritical fluid extraction. Process pressure and temperature showed a convincing effect on the extraction efficiency of extract. The rising temperature from 45 to 75°C showed a rise in the yield; a similar pattern was also detected with the rise of pressure. The prolonged increase in time

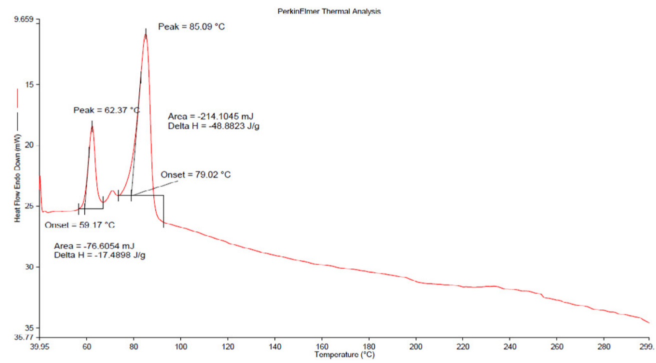

Figure 3: DSC Graph of Octacosanol obtained by extraction with hot ethanol reflux. (DSC graph of octacosanol powder show onset value start from 59.17 to 79.02°C and peak value from 62.37 to 85.09°C).

Table 1: Correlation of extraction efficiency by isolation through CO_2 supercritical, liquid-liquid CO_2 extraction and reflux extraction

Stages	*Extract (per/100 g)*	*1-Octacosanol in extract (per/100 g)*
Super critical fluid extraction	5.15	26.25
Liquid-liquid CO_2 extraction	4.66	34.46
Hot ethanol reflux extraction	2.13	50.80

has also promoted a rise in yield. Increasing isolation time marginally improve the yield of extract.

Effect of Purification of Octacosanol Extracts from Sugarcane Press Mud Wax

The outcome of the extraction efficiency of the Supercritical fluid extraction has more efficiency for the extraction of waxes. The hot ethanol method extracted significantly lower waxes amount (Table 1). Due to taking away esters of 9,12-octadecadienoic acid ethyl ester and n- n-hexadecenoic ethyl ester due to the application of sodium hydroxide during saponification in supercritical fluid method. The yield of 1-Octacosanol after a hot ethanol extraction were significantly above after the supercritical fluid extraction method. The recovery of octacosanol increases by 80.04% after the hot ethanol reflux compared to the supercritical fluid extraction method.

However, the extracted compound's color turned from light greenish to off-white powder during the extraction.

CONCLUSION

In X-ray diffraction, the major peaks at 21.2321 and 23.6226 Position [°2θ] (Cu) confirmed the crystalline nature of powder. In fourier-transform infrared spectroscopy, the following bands 3294.9665 cm^{-1} represented O-H stretching for the alcohol group with intramolecular bond and characteristic frequencies at 719.37620 to 2914.77819 cm^{-1} show medium peak for C-H bending and stretching for alkane skeleton thus confirming the identity of octacosanol. Through differential scanning calorimetry we observed enthalpy (normalized): 139.89 J/g, Onset x: 78.49°C and Peak temperature: 81.27°C which shows the melting point of extract range 78.49 to 81.27°C exhibiting the purity of the final extract. Thus, it can be considered a secure and environment-friendly approach for reconstructing the important composite from the filter mud. However, the step used for additional extraction and purification by ethanol reflux to decolorizing treatment and saponification showed similarity in the wax content. However, octacosanol content was enhanced from other previously described procedures. Thus, the current study was able to present a newer green, safe and sustainable concept of inclusion of liquid-liquid extraction during supercritical fluid extraction which was adopting green solvents. Overall the study has also provided valuable input for the recovery of sugarcane industry waste thus protecting the environment by offering a sustainable, cost-effective, and green method.

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