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Biocatalytic Approach to Aldehydes Using Lyophilisates of *Bjerkandera adusta* Fungus

Abstract

An optimized biocatalytic oxidation protocol has been developed for the efficient conversion of benzylic and allylic alcohols into their corresponding aldehydes. The sustainable method uses lyophilized mycelia of *Bjerkandera adusta* white-rot fungus as a catalyst in the aqueous medium with 2-propanol (10 % *v*/*v*) as a co-solvent, and operates under mild conditions to give high yields for a wide range of substrates. On a preparative scale, the approach allowed the synthesis of important aldehydes, including benzaldehyde, piperonal, cinnamaldehyde, cuminaldehyde, methoxybenzaldehydes, and citral.

Keywords: biocatalytic oxidation; benzylic alcohols; allylic alcohols; fungal biocatalysis; mild oxidation

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Біокаталітичний підхід до синтезу альдегідів з використанням ліофілізатів гриба *Bjerkandera adusta*

Анотація

Розроблено оптимізований протокол біокаталітичного окислення для ефективного перетворення бензилових та алілових спиртів на відповідні альдегіди. Цей екологічно безпечний метод використовує ліофілізований міцелій гриба білої гнилі *Bjerkandera adusta* як каталізатор у водному середовищі з 2-пропанолом (10 % *v*/*v*) як розчинником і працює в м'яких умовах, забезпечуючи високий вихід продукту для широкого спектра субстратів. У препаративному масштабі цей підхід дозволив здійснити синтез важливих альдегідів, зокрема бензальдегіду, піпероналю, цинамальдегіду, кумінальдегіду, метоксибензальдегідів та цитралю.

Ключові слова: біокаталітичне окислення; бензилові спирти; алілові спирти; грибний біокаталіз; м'яке окислення

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Introduction

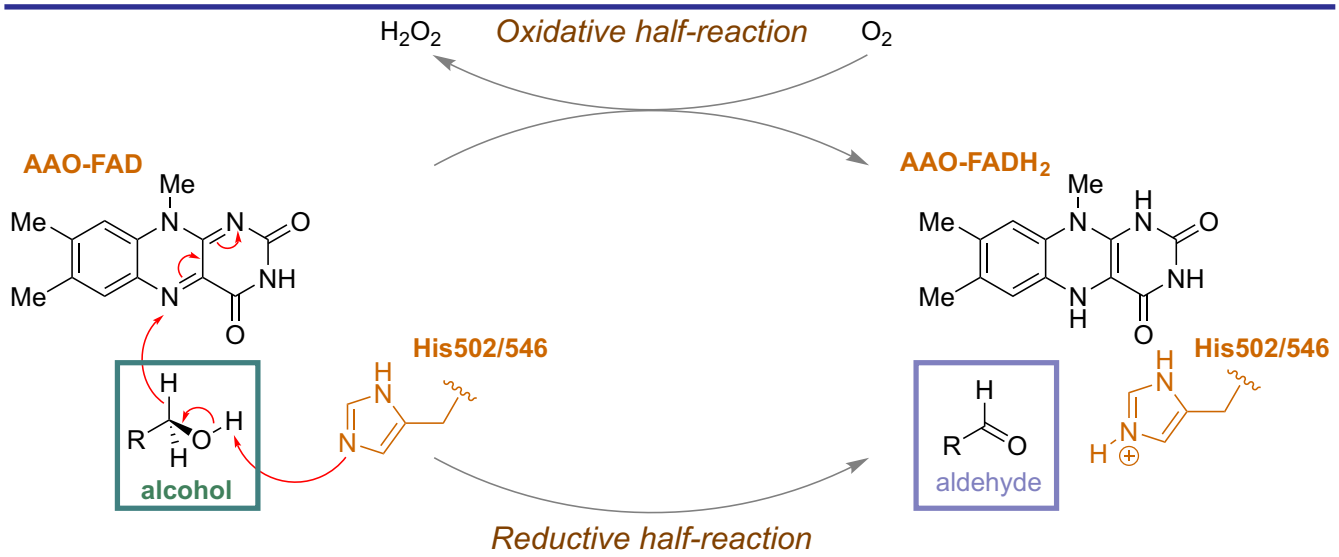
Flavor aldehydes represent the pivotal components in the food, fragrance, and pharmaceutical industries. However, their production remains constrained by factors, including their inherently low natural abundance and the limitations of the conventional chemical synthesis and biotechnological approaches. Aldehydes are most commonly produced by oxidizing primary alcohols using toxic and costly reagents, such as chromium(VI), manganese compounds, or other strong inorganic oxidants [1, 2]. Industrial aerobic oxidations typically employ transition-metal catalysts [3–6], while emerging photocatalytic methods also rely on transition metals [7–9]. Consequently, the food, beverage, and cosmetic industries are increasingly exploring microbial and enzymatic alternatives for aldehyde production [10, 11].

Biocatalysis is a powerful tool for a sustainable chemical synthesis, yet its broader industrial implementation is often constrained by economic and technical challenges. Current research in this field primarily focuses on the development of recombinant expression systems for the production of target enzymes. However, isolation and purification of these enzymes remain economically demanding, limiting their large-scale applicability. Recent analyses of manufacturing costs for biocatalysts revealed that industrial-grade (non-purified) enzymes are priced between 250 and 1000 € per kg, whereas whole-cell preparations are significantly more cost-effective, ranging from 35 to 100 € per kg [12]. Efforts in whole-cell biocatalysis, therefore, focus on engineering modified host organisms to enhance the productivity and stability of the enzymes they express and on using these organisms directly without further enzyme purification. Nevertheless, the use of genetically modified living systems can pose regulatory, safety, and operational challenges, making them less desirable for certain industrial applications. To address these limitations, we recently reported an alternative approach employing the *Bjerkandera adusta* white-rot fungus lyophilisate for the selective oxidation of alcohols to the corresponding aromatic aldehydes [13, 14]. This strategy avoids the recombinant expression and enzyme purification while preserving the principal advantages of whole-cell catalysts, namely, low cost, robustness, and cofactor independence. The high catalytic performance of the *B. adusta* lyophilisate is due to the presence of aryl-alcohol oxidases (AAO), which retain activity even after freeze-drying and extended storage, thereby offering a practical and scalable biocatalytic solution. AAOs are FAD (flavin adenine dinucleotide)-dependent oxidoreductases that use only molecular oxygen for the substrate oxidation, producing hydrogen peroxide as a by-product, without requiring additional cofactors (**Scheme 1**).

One of the main drawbacks of biocatalytic approaches is their limited scalability. The aim of this study was to evaluate the scalability of the *B. adusta* lyophilisate-based approach as a safe and sustainable strategy for the production of aldehydes with prospective applications in beverages, food, and cosmetics.

Results and discussion

The present work is based on our previous study, in which we demonstrated the catalytic



Scheme 1. The mechanism of the alcohol oxidation with aryl-alcohol oxidase (AAO) (FAD – Flavin Adenine Dinucleotide)

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activity of lyophilisates in relation to aromatic and allylic alcohols [13], extending this concept to a,b-unsaturated substrates. While our previous works [13, 14] are mainly method-oriented, this study is anchored in specific high-value compounds that define the aromas of almonds, cinnamon, cumin, and citrus, which directly addresses industrial relevance. It should also be noted that *Babkina et al.* [13] focused on aromatic substrates, whereas *Calza et al.* [14] investigated allylic substrates. In contrast, this study integrates both substrate classes within a single methodology, using the same lyophilisate preparation. This demonstrates that the catalyst system is not limited to one narrow substrate family.

The preparation of lyophilisates is summarized in **Figure 1**. Initially, *B. adusta* was cultivated as a surface culture, followed by the submerged cultivation to enhance mycelial the biomass production. The collected mycelia were subsequently frozen at –80 °C and freeze-dried. The resulting lyophilisates can be stored at –20 °C for extended periods without the loss of the catalytic activity.

Our previous studies demonstrated that 2-propanol, at 10% (*v*/*v*), was the most effective co-solvent for this procedure. This observation is notable given the presence of its hydroxyl functional group, which in principle could undergo oxidation to yield acetone. Nevertheless, under the reaction conditions developed, secondary alcohols, such as 2-propanol, were shown to be resistant to oxidation, thereby preserving their role as a benign co-solvent without introducing competing side reactions. In addition to solvent effects, the substrate concentration was identified as a decisive parameter influencing the reaction efficiency. At 20 mM, the product formation was consistently reduced, whereas 10 mM gave markedly higher yields, frequently exceeding 90%. Furthermore, the stirring intensity was found to be equally critical as the stirring rates below 500 rpm resulted in lower reproducibility and yields. Collectively, these findings highlight the intricate interplay between the solvent composition, the substrate concentration, and hydrodynamic conditions in determining the success of the procedure. Our study consolidates previously scattered optimization insights from earlier work [13, 14] and integrates them into a coherent and practical protocol, including the defined lyophilizate loading, buffer volume and pH, 2-propanol fraction, 10 mM substrate, stirring rate, reaction time, workup, and purification procedures. This level of detail and consolidation is new and directly useful for practitioners.

The optimized methodology was subsequently applied to a series of alcohol substrates serving as precursors to valuable flavor aldehydes and was tested at a preparative scale. Benzaldehyde (**1**), the principal compound responsible for the characteristic aroma of bitter almonds, was obtained in the yield of 88% (**Scheme 2**). Piperonal (**2**), a perfume agent with a sweet, floral, heliotrope-like odor, was isolated in the yield of 92%. Both 3- and 4-methoxybenzyl alcohols were subjected to the optimized procedure, giving the corresponding aldehydes **3** and **4**. 3-Methoxybenzaldehyde (**3**) is characterized by a sweet, floral, and slightly spicy aroma, whereas 4-methoxybenzaldehyde (**4**) possesses a similar but more intense fragrance, reminiscent of anise or hawthorn. Responsible for the characteristic warm, sweet, and spicy aroma of cinnamon, the cinnamic aldehyde (**5**) was produced in the yield of 95%. Similarly, cuminaldehyde (**6**) known for its spicy, green odor was produced in the yield of 92%. In addition, a mixture of isomeric geranial and neral known as citral (3,7-dimethyl-2,6-octadienal, **7**) [15] and distinguished by its fresh, lemon-like aroma was obtained as the oxidation product of geraniol in the yield of 96%. It should be noted that for all six target aldehydes and a mixture of two additional aldehydes, we report isolated yields close to the quantitative ones. In contrast, *Calza et al.* [14] reported similarly high yields for model allylic substrates, but not specifically for this flavor portfolio.

No traces of carboxylic acids were detected in the reaction mixtures for any of the flavor aldehydes,

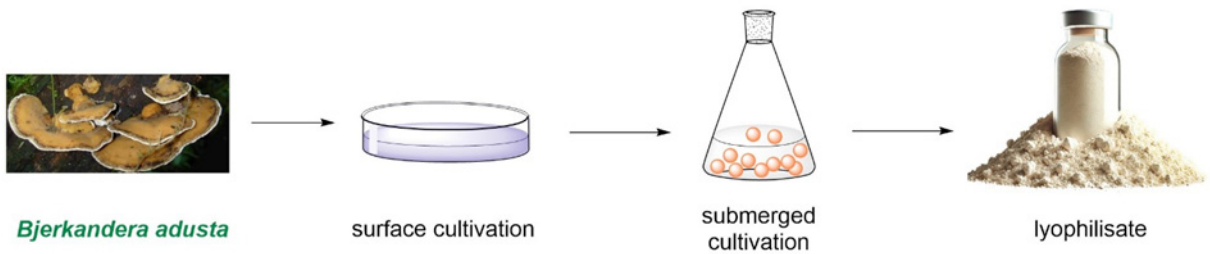
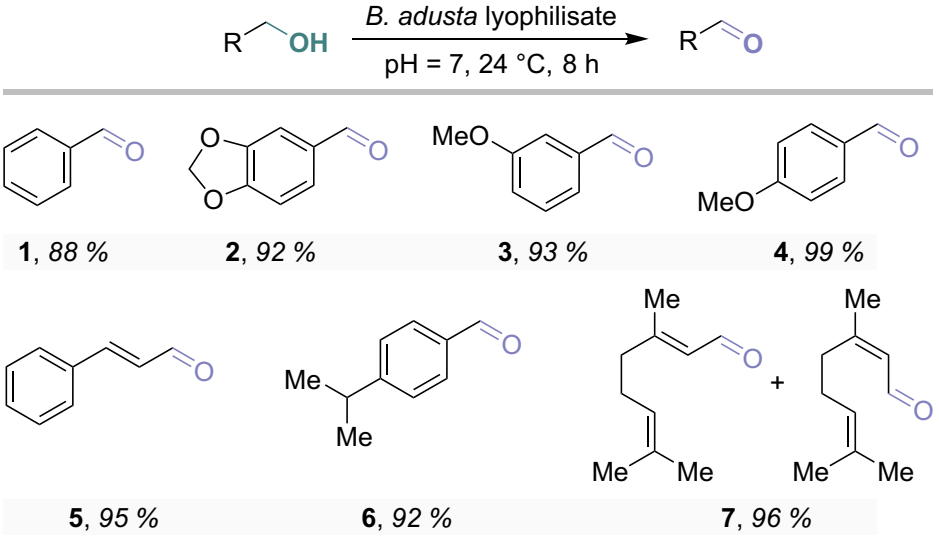


Figure 1. The process of the lyophilisate production



Scheme 2. The general scheme of the biocatalytic oxidation of aromatic and allylic alcohols to the corresponding aldehydes and the structures of the products and their respective yields

indicating the selective formation of aldehydes under mild conditions. Compared to our results, *Calza et al.* [14] demonstrated that lyophilisates gave a selective oxidation of primary benzylic and allylic alcohols to aldehydes, with the overoxidation suppression under optimized conditions. Also, these researchers upscaled a model allylic substrate to about ten millimolar in 90 mL buffer with 1.6 g of lyophilisate, mainly to confirm that the reaction remained selective. In our study, the general protocol was applied to hundreds of milligrams of structurally diverse substrates without the loss of selectivity or formation of detectable carboxylic acids.

The biocatalytic transformations were performed on a 300–500 mg scale, and the results clearly demonstrated the potential for upscaling of the approach. This shows that the protocol works on a scale and with solvents acceptable for the flavor and fragrance development. *Babkina et al.* [13] mostly reported smaller-scale experiments in the DMSO-containing medium, which was less attractive for food applications. *Calza et al.* [14] later demonstrated that 10% (*v*/*v*) 2-propanol gave very good performance for α,β-unsaturated alcohols. Our study adopts 2-propanol universally, confirms its chemical inertness under the oxidative conditions, and demonstrates that it remains effective at a preparative scale for a realistic set of flavor precursors. This is a genuine improvement in terms of safety.

Compared to conventional oxidation protocols, the *B. adusta*-lyophilisate method offers significant improvements in both efficiency and mildness of conditions. Traditional chemical oxidations of benzylic or allylic alcohols typically rely on stoichiometric inorganic oxidants (e.g. chromates, permanganates or nitric acid) and elevated temperatures, which often lead to poor selectivity, overoxidation to carboxylic acids, and hazardous waste generation [16]. For example, the benzyl alcohol oxidation by ferric nitrate in aqueous nitric acid reaches ~85% yield of benzaldehyde [17], but requires careful control of corrosive reagents. In stark contrast, our biocatalytic process employs a freeze-dried fungal mycelium in a simple H_2O / 2-propanol medium under ambient air. This benign setup cleanly converts primary aromatic and allylic alcohols to the corresponding aldehydes with no detectable overoxidation (no acids formed) and without additional co-oxidants [14]. For instance, 3,4-dimethozybenzyl alcohol (veratryl alcohol) was oxidized to veratraldehyde in the isolated yield of 84% under our conditions, and geraniol/nerol were selectively oxidized to citral (a mixture of (*E*)- and (*Z*)-isomers) in a high yield, with no side-products from further oxidation or double-bond reduction [14]. These outcomes illustrate the excellent chemoselectivity of the *B. adusta* catalyst, which contrasts with many chemical oxidants and even some enzymatic systems (e.g. the aryl-alcohol oxidase from *P. ostreatus* can overoxidize alcohols beyond the aldehyde stage to acids [18]). Notably, fungal laccases are also known to oxidize benzylic alcohols to aldehydes, but laccase reactions typically require artificial mediators and can nonselectively oxidize a broad range of phenolics [18], making them less ideal for the preparative flavor synthesis.

Equally important are the practical and green engineering advantages of our approach.

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The freeze-dried *B. adusta* mycelium serves as a readily accessible, whole-cell oxidase catalyst that obviates the need for the extensive enzyme purification or cofactor addition. *In situ*, the fungus own aryl-alcohol oxidase (AAO) uses molecular oxygen (from air) as the terminal oxidant, producing only water (*via* H_2O_2) as a benign by-product [16]. This differs from many isolated enzyme systems, which may require expensive cofactors and regeneration systems. For example, while a recombinant AAO from *Pleurotus eryngii* showed a high catalytic turnover (over 2 million for trans-2-hexen-1-ol oxidation), it had to be obtained via the inclusion body refolding and could not be used in whole-cell form. Engineered microbial approaches achieved complete conversion of allylic alcohols to aldehydes, but only through complex cascade designs: *Qiao et al.* [16] co-expressed an NAD^+-dependent alcohol dehydrogenase and an NADPH oxidase in *E. coli*, and further introduced a hemoglobin for oxygen uptake, to boost the yield of 3-methyl-2-butenal from 21% to 51%. Even then, pure oxygen and a fused multi-enzyme construct were required to attain >80% yield within 8 hours. In contrast, our single-organism, freeze-dried catalyst achieves high yields in similar or shorter timeframes under air without genetic modifications or intensive process controls. Typical reactions in this study went to completion within hours to a day, whereas traditional submerged fungal fermentations for flavor aldehydes often require several days to weeks. For instance, *B. adusta* cultures produce benzaldehyde from *L*-phenylalanine or benzyl alcohol over multiple days of growth, reaching titers of only a few hundred mg L^{-1} [19] and necessitating tricks like the product adsorption to mitigate toxicity [20, 21]. By using the non-growing (lyophilized) mycelium, we circumvent such limitations: the enzymatic activity is harnessed directly in a controlled batch reaction, decoupled from the slower kinetics and complexities of the microbial growth. Additionally, the stability of the freeze-dried catalyst is an asset – we have found that *B. adusta* lyophilisate retains full activity for at least 7 months when stored dry [18], which is advantageous for the consistent performance and potential reusability in a process setting.

We also studied alternative fungal lyophilisates to ensure that the efficiency observed was unique to *B. adusta*. Lyophilized mycelia of *Pleurotus sapidus* and *P. eryngii* (basidiomycetes known to produce oxidative enzymes) were tested in the model oxidation of cinnamyl alcohol. These preparations yielded significantly less aldehyde than *B. adusta* under identical conditions. According to the literature, *B. adusta* is an especially potent source of aryl-alcohol oxidase [14]. The choice of *B. adusta*, a non-toxic white-rot fungus, is particularly relevant for the flavor synthesis. *Babkina et al.* [13] have highlighted that *B. adusta* lyophilisate is a safe, metal-free oxidation catalyst suitable for food, beverage, and cosmetic applications. In our process, the fungal biomass is removed by a simple filtration after the reaction, and the resulting aldehyde product can be classified as nature-derived. This has important regulatory implications: flavors produced via biocatalysis from natural precursors may be labeled as "natural flavorings", whereas the same compounds made by synthetic chemical routes would be considered artificial [22]. The ability to market benzaldehyde, cinnamaldehyde, and other aroma aldehydes as natural products adds significant value in the flavor and fragrance industry. Moreover, the mild aqueous reaction conditions and avoidance of toxic reagents mean that the process complies with the principles of green chemistry and sustainability. Scalability is also favorable as the fungus can be cultivated on inexpensive media (including agro-industrial residues) and easily preserved by lyophilization, enabling a robust supply of a biocatalyst. In summary, this innovative method distinguishes itself by combining high yields and selectivity (no overoxidation) with milder, greener conditions and a straightforward, scalable setup. These advances underscore the novelty and applicability of using *B. adusta* lyophilisates for the synthesis of flavor aldehydes, offering a sustainable alternative to both traditional chemical oxidation and other biocatalytic systems [16].

■ Conclusions

This sustainability-focused study incorporates renewable feedstocks and minimizing environmental impact in accordance with the principles of green chemistry. The results underscore the versatility and scalability of the protocol, highlighting its potential for applications in the fragrance and flavor production. An optimized biocatalytic oxidation protocol was developed using 2-propanol (10% *v*/*v*) as a "green" co-solvent, with the reaction efficiency strongly dependent on the substrate concentration (10 mM optimal) and the stirring intensity (>500 rpm). The method was applied to a variety of alcohols serving as

precursors to valuable flavor aldehydes. It still provides high isolated yields and clean product mixtures without overoxidation. High yields were obtained for benzaldehyde (*88%*), piperonal (*92%*), methoxybenzaldehydes (*96%*), cinnamaldehyde (*95%*), cuminaldehyde (*92%*), and citral (*96%*). The results highlight the broad applicability of the protocol and its potential for the preparative-scale and industrial upscaling.

■ Experimental part

This section contains protocols for preparing the compounds described in the paper. All starting compounds were obtained from commercial sources and used without additional purification. All solvents were purified according to the standard procedures. Experimental data comply with the referenced samples. ^{1}H NMR and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance II 400 MHz WB instrument (Billerica, Massachusetts, USA) at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. The NMR chemical shifts are referenced using the solvent signals at 7.26 and 77.1 ppm for ^{1}H and ^{13}C nuclei, respectively, in $CDCl_3$.

Lyophilisates

Lyophilisates from submerged cultures of *Bjerkandera adusta* fungus were produced following the procedure described in [14].

The general procedure for biotransformation

The *B. adusta* lyophilisate (4.5 g) was rehydrated in 270 mL of the potassium phosphate buffer (100 mM, pH 7) while stirring at 800 rpm and 24 °C for 20 min. The corresponding alcohol dissolved in 30 mL 2-propanol was added to the final concentration of 10 mM. The reaction mixture was stirred at 24 °C and 800 rpm for 8 h. Subsequently, sodium chloride (7 g) was added, and the mixing continued for 10 min. The mixture was extracted with diethyl ether (3 × 60 mL), the suspension was centrifuged, and the combined organic phases were dried over Na_2SO_4. After evaporation of the organic phase to dryness, the products were purified by column chromatography on silica gel using a gradually changing pentane/diethyl ether eluent (10:1 → 7:3 → 1:1). The identity of the products was confirmed by comparing their ^{1}H and ^{13}C NMR spectra with those of an authentic reference compound.

Benzaldehyde (1)

A colorless liquid. Yield – 279 mg (88%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 10.02 (1H, s), 7.87 (2H, m), 7.64 (1H, m), 7.56 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 192.6, 136.4, 134.6, 129.9, 129.1.

Piperonal (2)

A colorless solid. Yield – 414 mg (92%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 9.79 (1H, s), 7.37 (1H, dd, J = 7.9, 1.8 Hz), 7.28 (1H, d, J = 1.8 Hz), 6.89 (1H, d, J = 7.8 Hz), 6.05 (2H, s). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 190.31, 153.14, 148.81, 131.92, 128.54, 108.40, 107.18, 102.32.

3-Methoxybenzaldehyde (3)

A colorless liquid. Yield – 380 mg (93%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 9.93 (1H, s), 7.46–7.39 (2H, m), 7.36–7.33 (1H, m), 7.16–7.10 (1H, m), 3.83 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 192.11, 160.09, 137.61, 129.95, 123.65, 121.60, 112.07, 55.52.

4-Methoxybenzaldehyde (4)

A colorless liquid. Yield – 405 mg (99%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 9.89 (1H, s), 7.84 (2H, d, J = 8.8 Hz), 7.01 (2H, d, J = 8.7 Hz), 3.89 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 191.10, 164.71, 132.05, 130.00, 114.25, 55.72.

Cinnamic aldehyde (5)

A pale-yellow liquid. Yield – 377 mg (95%). ^{1}H NMR (400 MHz, $CDCl_3$), ppm: 9.68 (1H, d, J = 8.0 Hz), 7.63–7.54 (2H, m), 7.51–7.42 (4H, m), 6.73 (1H, dd, J = 16.0, 8.0 Hz,). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 193.78, 152.90, 134.07, 131.33, 129.14, 128.67, 128.53.

Cuminic aldehyde (6)

A pale-yellow liquid. Yield – 409 mg (92%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 10.00 (1H, s), 7.82 (2H, d, J = 8.0 Hz), 7.39 (2H, d, J = 8.0 Hz), 3.03–2.96 (1H, m), 1.29 (6H, s, J = 7.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 192.05, 156.25, 134.56, 130.02, 127.15, 34.49, 23.64.

Citral (7)

A pale-yellow liquid. Yield – 444 mg (96%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 9.96-9.87 (1H, m), 5.85 (1H, m), 5.06 (1H, m), 2.56 (1H, t, J = 7.5 Hz), 2.21 (3H, m), 2.14 (d, J = 1.4 Hz, 2H), 1.96 (1H, d, J = 1.4 Hz), 1.66 (3H, s), 1.57 (3H, d, J = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm: 191.22, 190.68, 163.77, 133.61, 132.83, 128.61, 127.38, 122.59, 122.28, 40.55, 32.57, 27.05, 25.71, 25.59, 25.03, 17.65, 17.53.

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