Terpenes in Cannabis: Review of therapeutic benefits and application to Distillate and Decarboxylated Rosin products.

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1 Introduction

Terpenes are a diverse class of volatile organic compounds produced by many plants, including *Cannabis sativa,* or cannabis, for interactions with other organisms [1] and contribute to the plant's characteristic aroma. While historically associated with fragrance and flavour, terpenes have gained increasing attention in pharmaceutical research for their medicinal properties and potential synergistic roles alongside cannabinoids [2]. The term "entourage effect" is frequently used to describe this synergy between cannabinoid efficacy and terpenes, and although a potential overlap in therapeutic benefits is suggested by research, it has not been validated through clinical trials and thus remains unproven [3].

This report aims to provide a scientific overview of terpenes, with a clear definition of their chemical nature and biological functions. A review on the pharmacological properties of the most abundant terpenes found in *Cannabis sativa*, drawing on peer-reviewed evidence, was done to establish their therapeutic relevance and a framework for potential applications. Finally, this framework is applied to selected in-house product cultivars, produced by GES Labs, to evaluate their potential use in targeted medical applications.

1.1 Classes and main terpenes

Terpenes and terpenoids are a class of secondary metabolites that are produced as a reaction to environmental stress or to aid in ecological functions such as attraction of pollinators or seed dispersion [4]. All terpenes are derived from the head-to-tail joining of isoprene, a C_5 carbon structure (Figure 1) and therefore the classification of terpenes is based in the number of isoprene units present in their structure. Most terpenes produced in *Cannabis sativa* (98%, [5]) are classified as either mono- or sesquiterpenes and larger compounds are generally not produced [6].

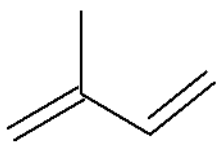

Figure 1: Chemical structure of an isoprene unit

Table 1: Classification of terpene structures commonly found in Cannabis

Class	Number of Isoprene units	Examples
Hemiterpenes	1	Isoprene
Monoterpenes	2	Pinene, Limonene, Terpinolene
Sesquiterpenes	3	Alpha-Humulene, Beta-Caryophyllene

The terms "terpenes" and "terpenoids" are often used interchangeably; however, they are distinct in that terpenes are simple hydrocarbons with general molecular structures of $C_NH_{N+N*3/5}$, whereas terpenoids (also known as isoprenoids) are modified terpenes with various functional groups and oxidised methyl groups [7]. Terpenoids are analogues of terpenes and therefore also follow the classification based on isoprenoid groups, i.e. monoterpenoids, sesquiterpenoids.

The pharmacological benefits of the common terpenes found in *C. sativa* include anti-inflammatory, analgesic and anti-anxiety effects, as outlined in Table 2. These effects have been demonstrated in preclinical studies which highlight the therapeutic potential of individual terpenes.

Table 2: Common terpenes/terpenoids found in C. sativa and their proven pharmacological effects.

Compound	Class	Effects	References
Alpha-Humulene	Sesquiterpene	Anti-inflammatory, anti-allergy	[8], [9], [10]
Alpha-Pinene	Monoterpene	Anti-inflammatory, analgesic, antinociceptive	[11], [12], [13]
Beta-Pinene	Monoterpene	Anti-inflammatory, antinociceptive	[14], [15]
Beta-Caryophyllene	Sesquiterpene	Anti-inflammatory, anti-anxiety, analgesic	[16], [17], [18], [19], [20], [21]
Beta-Myrcene	Monoterpene	Anti-inflammatory, analgesic, sedative	[22], [23], [24], [25]
Limonene	Monoterpene	Anti-inflammatory, anti-anxiety, anticonvulsant	[26], [27], [28], [29], [30]
Linalool	Monoterpenoid	Anti-inflammatory, anti-anxiety, analgesic	[31], [32], [33], [34]

Extensive research has been conducted on the anti-inflammatory properties of **beta-caryophyllene**. It has been observed that beta-caryophyllene interacts with the CB2 cannabinoid receptor from the human endocannabinoid system which is involved in regulating pain, mood and immune response [18], [19], [20], [35]. Studies have shown that doses of 50 - 200 mg/kg of beta-caryophyllene significantly reduced systemic inflammation in rodent models and prostate tissue in gerbils [16], [17]. **Alpha-pinene** was effective in suppressing inflammatory responses in immune cells and mouse models at 30 - 300 mg/kg doses [12], [13]. **Alpha-humulene**, with doses from 5 - 500 mg/kg, consistently demonstrated strong anti-inflammatory action in immune cell markers [8], [9], [10]. Among the three, α-humulene appeared the most potent, followed by β-caryophyllene, which also showed efficacy in broader inflammatory conditions. These results suggest that such terpenes could potentially be used as therapeutics in managing inflammatory conditions, with alpha-humulene standing out as particularly potent based on the literature reviewed.

Several studies have explored the pain-relieving (analgesic) potential of terpenes, showing encouraging results across different types of pain models. **Beta-myrcene**, at doses of 1 - 10 mg/kg, was effective in reducing joint pain and swelling in a rat arthritis model [22], [23]. **Beta-caryophyllene,** at 0.1 - 50 mg/kg, significantly reduced nerve pain in mice treated with antiretroviral drugs in other models of neuropathic pain [18], [19]. **Linalool** helped reduce pain sensitivity caused by typically non-painful stimuli (allodynia) in mice at <2 mg/kg [31]. These findings highlight the potential of these terpenes as alternative or complementary agents for managing various types of pain, with beta-caryophyllene standing out for its consistent effects across multiple models of nerve-related pain.

The calming, anti-anxiety effects of terpenes have also been investigated in various animals. **Linalool** consistently showed strong effective results in reducing anxiety-like behaviour in mice using doses of 1 - 10 mg/kg. The tests included measuring social interaction and avoidance behaviour from acute stress-induced models. The effects were seen whether linalool was inhaled or injected, suggesting its efficacy through multiple brain pathways [32], [34]. **Beta-caryophyllene,** tested at 5 - 50 mg/kg, also reduced anxiety in several models especially those involving chronic stress. It was proposed that this was through interaction with the CB2 receptor [20], [21]. **Limonene** was effective at 5 - 10 mg/kg and showed promising results mainly in stress-induced anxiety models [27], [30]. Among the three terpenes, beta-caryophyllene appears most effective in modulating immune responses associated with sustained anxiety whereas linalool excels in acute stress-induced scenarios, potentially offering immediate relief from anxiety trigger. Limonene was slightly less potent compared to both linalool and beta-caryophyllene in the studies reviewed, although it still demonstrated beneficial effects in stress reduction. The ranking considers not just potency, but also how well each terpene works across different types of anxiety models and brain mechanisms.

In addition to their shared pharmacological effects, several terpenes also have distinct individual properties. For instance, **alpha-humulene** has demonstrated anti-allergy effects in models of airway inflammation [9], **linalool** has indicated anti-depressant properties that could potentially be used for depression treatment [33], **limonene** has shown anticonvulsant activity relative to seizure treatment [29] and **alpha- and beta-pinene** were tested for antinociception, with pain response reduction comparable to those achieved with morphine [15] and diclofenac [11]. These findings highlight the diverse therapeutic potential of individual terpenes beyond their commonly studied roles.

1.2 In-house produced cultivars

Within GES Labs, two API sources are used to produce inhalation cartridges. The first source is a distillate, produced by distilling ethanol extracted decarboxylated crude oil and formulated by re-introducing the captured volatile fractions at a fixed mass percentage. The second source is a solventless extract, known as rosin, produced by pressing freeze-dried trichomes at elevated temperatures and high pressures. This procedure allows for the retainment of the terpenes through extraction and therefore does not require a formulation stage after decarboxylation.

The terpene profiles for two distillate cultivars and four rosin cultivars were analysed and the most abundant terpenes were determined as a mass percentage of the quantified terpene profile, as determined by GC-FID by an external validated testing laboratory. The distillate cultivars analysed were Black Cherry Punch (BCP) and Blueberry Kush (BBK), and the rosin cultivars were GMO cookies, East Coast Sour Diesel (ECSD), Dura x Nana and Gorilla cookies. The results of the terpene analysis are given in Figure 2.

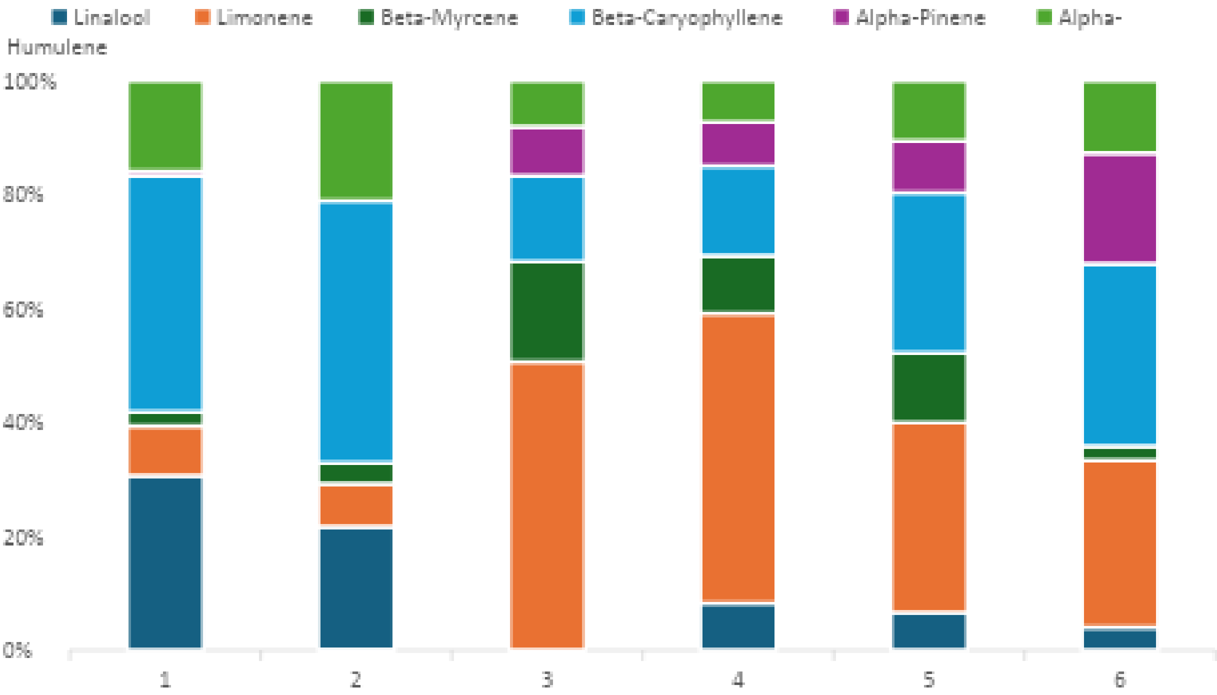

Figure 2: Relative terpene composition of our in-house produced inhalation products.

The average total terpene content for the inhalation products of the distillate and rosin cultivars were approximately 20 mg/$g_{product}$ and 68 mg/$g_{product}$, respectively. This was expected given the nature of the differences in extraction methods, where there is typically more terpene loss during distillation compared to solventless extraction. The most abundant terpene in the distillate products was beta-caryophyllene, which was also abundant in the rosin but in smaller amounts. A major difference between the API sources was the difference in the abundance of linalool and limonene. Although present in the distillate products, limonene was the most abundant terpene in all the rosin cultivars. Linalool was the second most abundant terpene in both distillate cultivars but had a 7% relative abundance in one rosin cultivar, namely East Coast Sour Diesel. Alpha-pinene was significantly present in only two rosin cultivars, whereas alpha-humulene was significant in all tested cultivars, albeit in lower relative abundance in the rosin cultivars.

A consolidated overview of the terpene content across each cultivar is presented in Table 3, along with the potential benefits and applications of the inhalation products based on their terpene profiles.

Table 3: Top 3 terpenes available in our in-house inhalation products and possible benefits.

Cultivar	Terpene	Relative Abundance (%)	Potential Treatment
Black Cherry Punch	Beta-Caryophyllene Linalool Alpha-Humulene	41 31 16	Inflammation, general pain, anxiety, allergies
Blueberry Kush	Beta-Caryophyllene Linalool Alpha-Humulene	46 22 21	Inflammation, general pain, anxiety, allergies
GMO Cookies	Limonene Beta-Myrcene Beta-Caryophyllene	51 18 15	Inflammation, general pain, anxiety, seizures
East Coast Sour Diesel	Limonene Beta-Caryophyllene Beta-Myrcene	51 16 10	Inflammation, general pain, anxiety, seizures
Dura x Nana	Limonene Beta-Caryophyllene Beta-Myrcene	33 28 12	Inflammation, pain, anxiety, seizures
Gorilla Cookies	Beta-Caryophyllene Limonene Alpha-Pinene	32 29 19	Inflammation, general and nociceptive pain, anxiety, seizures, allergies

A deeper analysis into the distillate cultivars BCP and BBK shows that the top 3 terpenes were beta-caryophyllene, linalool and alpha-humulene, in the order of most to least abundant. Based on the studies reviewed (Table 1), either cultivar may be suitable for inhalation products targeting inflammation, pain, anxiety and allergies. Linalool showed better results in acute stress-induced scenarios and has potentially better effectiveness in immediate stress relief than limonene and beta-caryophyllene, therefore it can be suggested that distillate products might prove better for stress relief, especially for fast-acting relief. For the rosin cultivars, GMO cookies, ECSD and Dura x Nana both had the same dominant terpene profile: limonene, beta-caryophyllene and beta-myrcene; though Dura x Nana had a slightly different terpene composition with 10% more beta-caryophyllene and 18% less limonene compared to the other two (Table 3). Nevertheless, this terpene combination could potentially be used in treating inflammation, pain, anxiety and seizures. The other rosin cultivar investigated, Gorilla cookies, had a different top 3 terpene profile consisting of beta-caryophyllene, limonene and alpha-pinene.

Therefore, an inhalation product based on this cultivar could be beneficial in the treatment of inflammation, general and nociceptive pain, anxiety, seizures and allergies. These findings highlight the value of terpene profiling in developing inhalation products tailored to consumer needs and desired therapeutic outcomes.

1.3 Conclusions

Although the synergistic effect with cannabinoids is yet to be validated, the therapeutic benefits of various terpenes are well documented in scientific literature and have been found to be effective as anti-inflammatory, analgesic, and anxiolytic agents. Extending this to the products developed by GESLabs, the terpene profiles were used to propose treatment applications of the different production methods and cultivars. It was seen that the higher presence of limonene in the rosin cultivars may suggest application for relief of seizure symptoms, although this is based on the results obtained from the use of isolated limonene as an anticonvulsant. The more abundant presence of linalool in distillate products, along with the abundance of beta-caryophyllene suggests that these products might be effective for fast-acting stress relief based on what was found in literature. The substitution of limonene for linalool in rosin products in relative abundance suggests that these products would also have good anti-stress effectiveness, but this would be more effective for treating other symptoms of stress such as immune responses from sustained anxiety.

Further reports are set to be focused on olfactory properties of terpenes and will include more cultivars that are produced by GESLabs. Combined with the therapeutic properties, blends of products will be proposed to target either sensory or therapeutic goals. This will allow for more specificity in production and will further aid in the standardising of *Cannabis* as medicine for targeting specific patient symptoms. Additionally, the energising or sedative effects of various terpenes is set to be investigated with the goal of standardising the classification of “Day” or “Night” cultivars based on their terpene profiles.

1.4 References

[1] J. Gershenzon and N. Dudareva, 'The function of terpene natural products in the natural world', *Nat Chem Biol*, vol. 3, no. 7, pp. 408–414, Jul. 2007, doi: 10.1038/nchembio.2007.5.

[2] E. B. Russo, 'Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects LINKED ARTICLES', *Br J Pharmacol*, vol. 163, pp. 1344–1364, Jan. 2011, doi: 10.1111/bph.2011.163.issue-7.

[3] R. André *et al.*, 'The Entourage Effect in Cannabis Medicinal Products: A Comprehensive Review', Nov. 01, 2024, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/ph17111543.

[4] J. Schwachtje and I. T. Baldwin, 'Why Does Herbivore Attack Reconfigure Primary Metabolism?', *Plant Physiol*, vol. 146, no. 3, pp. 845–851, Mar. 2008, doi: 10.1104/pp.107.112490.

[5] T. Nuutinen, 'Medicinal properties of terpenes found in Cannabis sativa and Humulus lupulus', Sep. 05, 2018, *Elsevier Masson SAS*. doi: 10.1016/j.ejmech.2018.07.076.

[6] S. R. Sommano, C. Chittasupho, W. Ruksiriwanich, and P. Jantrawut, 'The Cannabis Terpenes', Dec. 01, 2020, *MDPI*. doi: 10.3390/MOLECULES25245792.

[7] S. Perveen, 'Introductory Chapter: Terpenes and Terpenoids', in *Terpenes and Terpenoids*, IntechOpen, 2018, ch. 1, pp. 1–12. doi: 10.5772/intechopen.79683.

[8] L. Becker and D. Holtmann, 'Anti-inflammatory effects of α-humulene on the release of pro-inflammatory cytokines in lipopolysaccharide-induced THP-1 cells', *Cell Biochem Biophys*, vol. 82, no. 2, pp. 839–847, Jun. 2024, doi: 10.1007/s12013-024-01235-7.

[9] A. P. Rogerio, E. L. Andrade, D. F. P. Leite, C. P. Figueiredo, and J. B. Calixto, 'Preventive and therapeutic anti-inflammatory properties of the sesquiterpene α-humulene in experimental airways allergic inflammation', *Br J Pharmacol*, vol. 158, no. 4, pp. 1074–1087, Oct. 2009, doi: 10.1111/j.1476-5381.2009.00177.x.

[10] E. S. Fernandes *et al.*, 'Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of Cordia verbenacea', *Eur J Pharmacol*, vol. 569, no. 3, pp. 228–236, Aug. 2007, doi: 10.1016/j.ejphar.2007.04.059.

[11] S. Fakhri, S. Jafarian, M. B. Majnooni, M. H. Farzaei, E. Mohammadi-Noori, and H. Khan, 'Anti-nociceptive and anti-inflammatory activities of the essential oil isolated from Cupressus arizonica Greene fruits', *Korean Journal of Pain*, vol. 35, no. 1, pp. 33–42, Jan. 2022, doi: 10.3344/kjp.2022.35.1.33.

[12] D. S. Kim *et al.*, 'Alpha-Pinene Exhibits Anti-Inflammatory Activity Through the Suppression of MAPKs and the NF-κB Pathway in Mouse Peritoneal Macrophages', *American Journal of Chinese Medicine*, vol. 43, no. 4, pp. 731–742, Aug. 2015, doi: 10.1142/S0192415X15500457.

[13] K. Rahimi, M. Zalaghi, E. G. Shehnizad, G. Salari, F. Baghdezfoli, and A. Ebrahimifar, 'The effects of alpha-pinene on inflammatory responses and oxidative stress in the formalin test', *Brain Res Bull*, vol. 203, Oct. 2023, doi: 10.1016/j.brainresbull.2023.110774.

[14] E. S. Santos *et al.*, 'Hypoglycemic, Hypolipidemic, and Anti-Inflammatory Effects of Beta-Pinene in Diabetic Rats', *Evidence-based Complementary and Alternative Medicine*, vol. 2022, 2022, doi: 10.1155/2022/8173307.

[15] C. Liapi, G. Anifantis, I. Chinou, A. P. Kourounakis, S. Theodosopoulos, and P. Galanopoulou, 'Antinociceptive Properties of 1,8-Cineole and β-Pinene, from the Essential Oil of Eucalyptus camaldulensis Leaves, in Rodents', *Planta Med*, vol. 73, no. 12, pp. 1247–1254, 2007, doi: 10.1055/s-2007-990224.

[16] S. S. Dahham, Y. M. Tabana, M. B. Khadeer Ahamed, and A. M. S. Abdul Majid, 'In vivo anti-inflammatory activity of β-caryophyllene, evaluated by molecular imaging', *Molecules & Medicinal Chemistry*, Jun. 2016, doi: 10.14800/mmc.1001.

[17] N. F. da C. Castro, F. C. Jubilato, L. H. A. Guerra, F. C. A. dos Santos, S. R. Taboga, and P. S. L. Vilamaior, 'Therapeutic effects of β-caryophyllene on proliferative disorders and inflammation of the gerbil prostate', *Prostate*, vol. 81, no. 12, pp. 812–824, Sep. 2021, doi: 10.1002/pros.24177.

[18] E. Aly, M. A. Khajah, and W. Masocha, 'β-caryophyllene, a CB2-receptor-selective phytocannabinoid, suppresses mechanical allodynia in a mouse model of antiretroviral-induced neuropathic pain', *Molecules*, vol. 25, no. 1, 2020, doi: 10.3390/molecules25010106.

[19] A. L. Klauke *et al.*, 'The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain', *European Neuropsychopharmacology*, vol. 24, no. 4, pp. 608–620, 2014, doi: 10.1016/j.euroneuro.2013.10.008.

[20] A. Bahi, S. Al Mansouri, E. Al Memari, M. Al Ameri, S. M. Nurulain, and S. Ojha, 'β-Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice', *Physiol Behav*, vol. 135, pp. 119–124, Aug. 2014, doi: 10.1016/j.physbeh.2014.06.003.

[21] M. Rabbani, M. Rabbani, S. Ebrahim Sajjadi, and A. Vaezi, 'Evaluation of anxiolytic and sedative effect of essential oil and hydroalcoholic extract of Ocimum basilicum L. and chemical composition of its essential oil', 2015. [Online]. Available: http://journals.lww.com/rips

[22] J. J. McDougall and M. K. McKenna, 'Anti-Inflammatory and Analgesic Properties of the Cannabis Terpene Myrcene in Rat Adjuvant Monoarthritis', *Int J Mol Sci*, vol. 23, no. 14, Jul. 2022, doi: 10.3390/ijms23147891.

[23] L. I. G. Paula-Freire, G. R. Molska, M. L. Andersen, and E. L. D. A. Carlini, ‘Ocimum gratissimum Essential Oil and Its Isolated Compounds (Eugenol and Myrcene) Reduce Neuropathic Pain in Mice’, *Planta Med*, vol. 82, no. 3, pp. 211–216, Nov. 2015, doi: 10.1055/s-0035-1558165.

[24] A. T. Rufino *et al.*, ‘Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis’, *Eur J Pharmacol*, vol. 750, pp. 141–150, Mar. 2015, doi: 10.1016/j.ejphar.2015.01.018.

[25] L. Chen *et al.*, ‘Beta-Myrcene as a Sedative–Hypnotic Component from Lavender Essential Oil in DL-4-Chlorophenylalanine-Induced-Insomnia Mice’, *Pharmaceuticals*, vol. 17, no. 9, p. 1161, Sep. 2024, doi: 10.3390/ph17091161.

[26] N. El Hachlafi *et al.*, ‘Chemical Composition and Assessment of the Anti-Inflammatory, Antioxidant, Cytotoxic and Skin Enzyme Inhibitory Activities of Citrus sinensis (L.) Osbeck Essential Oil and Its Major Compound Limonene’, *Pharmaceuticals*, vol. 17, no. 12, Dec. 2024, doi: 10.3390/ph17121652.

[27] P. A. D’Alessio, J. F. Bisson, and M. C. Béné, ‘Anti-Stress Effects of d-Limonene and Its Metabolite Perillyl Alcohol’, *Rejuvenation Res*, vol. 17, no. 2, pp. 145–149, Apr. 2014, doi: 10.1089/rej.2013.1515.

[28] C. Zhao, Z. Zhang, D. Nie, and Y. Li, ‘Protective Effect of Lemon Essential Oil and Its Major Active Component, D-Limonene, on Intestinal Injury and Inflammation of E. coli-Challenged Mice’, *Front Nutr*, vol. 9, Jun. 2022, doi: 10.3389/fnut.2022.843096.

[29] S. Seo *et al.*, ‘D-limonene inhibits pentylenetetrazole-induced seizure via adenosine a2a receptor modulation on gabaergic neuronal activity’, *Int J Mol Sci*, vol. 21, no. 23, pp. 1–11, Dec. 2020, doi: 10.3390/ijms21239277.

[30] Y. Song *et al.*, ‘Limonene has anti-anxiety activity via adenosine A2A receptor-mediated regulation of dopaminergic and GABAergic neuronal function in the striatum’, *Phytomedicine*, vol. 83, Mar. 2021, doi: 10.1016/j.phymed.2021.153474.

[31] H. Kashiwadani, Y. Higa, M. Sugimura, and T. Kuwaki, ‘Linalool odor-induced analgesia is triggered by TRPA1-independent pathway in mice’, *Behavioral and Brain Functions*, vol. 17, no. 1, Dec. 2021, doi: 10.1186/s12993-021-00176-y.

[32] V. M. Linck, A. L. da Silva, M. Figueiró, E. B. Caramão, P. R. H. Moreno, and E. Elisabetsky, ‘Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice’, *Phytomedicine*, vol. 17, no. 8–9, pp. 679–683, Jul. 2010, doi: 10.1016/j.phymed.2009.10.002.

[33] É. R. Q. dos Santos, J. G. S. Maia, E. A. Fontes-Júnior, and C. do Socorro Ferraz Maia, 'Linalool as a Therapeutic and Medicinal Tool in Depression Treatment: A Review', *Curr Neuropharmacol*, vol. 20, no. 6, pp. 1073–1092, Sep. 2021, doi: 10.2174/1570159x19666210920094504.

[34] J. R. Bi, H. W. Zha, Q. L. Gao, H. Wu, Z. J. Liu, and D. Sun, 'Pleasant Odor Decreases Mouse Anxiety-like Behaviors by Regulating Hippocampal Endocannabinoid Signaling', *Int J Mol Sci*, vol. 25, no. 19, Oct. 2024, doi: 10.3390/ijms251910699.

[35] F. Francomano *et al.*, 'β-caryophyllene: A sesquiterpene with countless biological properties', Dec. 01, 2019, *MDPI AG*. doi: 10.3390/app9245420.