

Synergistic Potential of Trikatu Essential Oil as an Aromatherapy Agent: A Chemical and Mechanistic Review

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Abstract: Combining *Piper nigrum*, *Piper retrofractum*, and *Zingiber officinale* var. *Rubrum*, Trikatu essential oil (TREO) exhibits promise as an inhaled aromatherapy agent with a range of biological activities. The antioxidant, anti-inflammatory, anxiolytic, and neuroprotective properties of the bioactive compounds in TREO are reviewed in this article along with scientific evidence supporting them. According to analysis, substances like β -Caryophyllene α -Pinene, linalool, D-Limonene, and Eucalyptol can interact with important molecular targets, such as GABA_A, CB2, and 5-HT_{1A}, 5-HT, 5-HT_{2A} receptors, also inhibits 5-HT₃ receptor, which are involved in the regulation of anxiety and the protection of brain. Furthermore, these substances work in concert to inhibit oxidative stress, lower the expression of pro-inflammatory cytokines like TNF- α and IL-1 β , and promote neuroplasticity through the BDNF pathway. The synergistic effects of TREO offer more potential than when used on its own, particularly when it comes to reducing inflammation, oxidative stress, and nervous system modulation. Essential oils in TREO are typically extracted through hydro-distillation with the Clevenger apparatus, which help preserve the integrity of volatile active constituents. The potential of TREO as an aromatherapy agent is assessed in this article along with its chemical composition, biological activities, and potential uses in promoting both physical and mental well-being.

Keywords: trikatu, aromatherapy, anxiolytic, antioxidant, anti-inflammatory, neuroprotective

Introduction

Traditional medicine has traditionally employed various plant combinations to treat a range of ailments and preserve health.¹ Trikatu (TR) is a traditional Ayurvedic formulation composed of black pepper fruit (*Piper nigrum* Linn.), Javanese long pepper (*Piper retrofractum* Vahl), and red ginger rhizome (*Zingiber officinale* var. *rubrum* Theilade).² TR is commonly used in herbal formulations due to its wide range of pharmacological effects, which include anti-inflammatory, digestive, and antioxidant properties.³ Its essential oil constituent show potential in aromatherapy. According to studies, the essential oils of black pepper (BP) and Javanese long pepper (JLP) contain important compounds like β -pinene, β -caryophyllene, and limonene⁴⁻⁶ that have been shown to possess antidepressant, cytotoxic,⁷ antioxidant, and anti-inflammatory effects.^{5,8,9} Notably, β -caryophyllene acts as a cannabinoid type-2 (CB2) receptor agonist, offering analgesic and anxiolytic effects.¹⁰ Dilrukshi claims that, BP's aroma can reduce acute physiological stress reactions, which makes it a potentially helpful aromatherapy agent in high-pressure situations.¹¹ Aromatherapy with *Zingiber officinale* has also been reported to improve the quality of life in women with breast cancer.¹²

Essential oils have long been utilized in aromatherapy as a holistic method to enhance both mental and physical well-being.^{13,14} These oils can exert local effects or enter systemic circulation through inhalation and skin absorption.¹⁵

Essential oils function through an inhalation mechanism in which their volatile compounds interact with the nose's olfactory receptors, which in turn transmit signals to the brain's limbic system, which controls emotions and stress reactions.^{16,17} As a complementary therapy for treating mental and physical illnesses and improving overall wellbeing, aromatherapy has become increasingly popular.^{18,19} Improved mood,²⁰ alleviation of chronic pain,²¹ depression,²² anxiety disorders,²³ migraine,²⁴ insomnia,²⁵ and improvements in physical symptoms like blood pressure and breathing rate¹⁹ are among the therapeutic advantages of aromatherapy.

Despite the growing evidence supporting the benefits of individual plant base essential oil, the synergistic potential of TR, a blend of BP, JLP, and red ginger (RG) in aromatherapy remains underexplored. Research that has already been done tends to concentrate on each plant separately rather than investigating the combination of TR essential oil (TREO), which may have more potent or unique effects. In order to guarantee the highest yield and ideal concentration of essential bioactive components for aromatherapy, such as β -caryophyllene, which has analgesic effects by cannabinoid type-2 (CB2) receptors,²⁶ the essential oil extraction process also merits discussion. Therefore, to support its use as an efficient aromatherapy agent, more research into the pharmacological potential of TREO is required, along with optimization of its extraction process.

However, one of the main challenges in harnessing the full potential of TREO lies in the standardization of its chemical composition. The variability in essential oil profiles is significantly influenced by factors such as the method of extraction (eg, hydro-distillation, steam distillation, or cold pressing), plant part used, harvest time, geographical origin, and post-harvest processing. These inconsistencies can lead to significant fluctuations in the concentration of key bioactive compounds, thereby affecting the efficacy, reproducibility, and safety of aromatherapeutic applications. Standardization efforts must therefore consider not only the qualitative identification but also the quantitative consistency of constituents like β -caryophyllene, sabinene, and zingiberene to ensure reliable therapeutic outcomes.

This review specifically aims to (1) analyze the chemical composition of TREO based on previously published studies; (2) explore the proposed pharmacological mechanisms relevant to its application in aromatherapy; and (3) evaluate the efficiency and appropriateness of various extraction method used in producing essential oils intended for therapeutic use. This study was carried out through a literature review method based on data in peer-reviewed journals (Google Scholar, PubMed, Springer, Scopus, and Science Direct). The search term is “Piper nigrum” or “Piper retrofractum” or “Zingiber officinale var. rubrum” or “Trikatu” or “Trikatu Essential oil” or “Aromatherapy” or “Biological effect” or “Therapeutic effects”. Relevant scientific research in the field of interest and important reference articles were used as inclusion criteria. Duplicate publications and irrelevant articles were excluded.

In addition to its promising therapeutic potential, the safety and toxicity profiles of TREO and its individual components should be considered. Although essential oils are sourced from natural ingredients, they are 100 times more concentrated when distilled from plant materials and can cause side effects such as skin irritation, mucosal inflammation, or systemic toxicity if used improperly.¹⁴ However, research on the toxicity and safety of each component of TREO is still limited. Therefore, future research should include studies that focus on safety in preclinical and clinical settings to ensure the feasibility of TREO as a safe and effective aromatherapy agent. Standardized doses, routes of administration, and duration of treatment should also be established to minimize potential side effects and ensure reproducibility across studies and formulations.

Although preliminary evidence supports the therapeutic potential of TREO, particularly in the context of aromatherapy, the lack of clinical validation and variability in essential oil composition remain major challenges. To address these challenges, by identifying consistent bioactive profiles and optimizing extraction techniques, this study may contribute to the development of standardized, efficacious, and safe plant base therapies. It is hoped that this review will enhance the scientific understanding of the synergistic potential of TREO as a novel aromatherapy agent.

Trikatu Essential Oil: Composition and Extraction Methods

Botanical Sources and Traditional Uses

The formulation of TR consists of equal parts (1:1:1, w/w/w) of three pungent herbs: black pepper (*Piper nigrum* Linn.), long pepper (*Piper retrofractum* Vahl. or *Piper longum*), and red ginger rhizome (*Zingiber officinale* var. *rubrum*, a

variety of *Zingiber officinale*).^{27,28} TR's bronchodilator qualities combine to create a warmer and expectorant composition that effectively treats respiratory infections.²⁹ Using excipients and the wet granulation method, the tablet formulation was created.^{30,31} We have attempted to create a blend of TR in the form of massage oil²⁸ and essential oil.² Here, we list the traditional applications and botanical sources.

Black Pepper (*Piper nigrum* Linn)

The plant known as black pepper (*Piper nigrum* Linn.) belongs to the Piperaceae family. Many kinds of illnesses, such as cough, cold, dyspnea, throat disorders, intermittent fever, dysentery, stomach pain, worm infection, and hemorrhoids, have historically been treated with black pepper (BP).⁴⁶ Furthermore, BP has long been used as a gastro-protective, antidepressant, anti-inflammatory, antimutagenic, antimetastatic, hepatoprotective.³² According to a several of other studies (Table 1) BP essential oil (BPEO) has antioxidant,^{5,28,33} anti-inflammatory,³⁴ antimicrobial,³⁵ anticancer,³⁵ and anti-tumor properties.³⁶ Research has demonstrated that BPEO increases endogenous antioxidant activity, including glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), while decreasing levels of malondialdehyde (MDA), acetylcholinesterase (AChE),^{5,33} and pro-inflammatory cytokines (TNF- α , IFN- γ , IL-6 and IL-1 β).³⁴ This makes it promising candidate for investigation as an aromatherapy agent.

Piperine and essential oils constituent in the majority of BP.^{46,61} Using GC-MS techniques, several studies have identified the primary chemical constituents of BPEO (Table 2), which include β -caryophyllene, limonene, d-limonene, β -pinene, α -pinene, 3-carene, δ -3-carene, caryophyllene oxide, α -terpinene, α -curcumene, and others. In aromatherapy, β -caryophyllene, D-limonene, and β -pinene in BPEO have demonstrated beneficial effects, especially in decreasing anxiety and promote relaxation. It has been demonstrated that β -caryophyllene considerably lowers anxiety levels in human subjects. This substance improves the “happiness” ratio in facial expressions while reduce heart rate and systolic blood pressure.⁶² Its anxiolytic properties are attributed to its interaction with CB2 receptor and neuroprotective effects.^{63–67}

A monoterpene called D-limonene has been shown to increase alpha brain waves and decrease stress index values, suggesting that it may help people relax and manage about stress.⁶⁸ It is well-known for its ability to treat anxiety and depression.⁶⁹ β -Pinene, a monoterpene compound, has demonstrated neuroprotective, anti-inflammatory, and antioxidant properties in several studies, which may contribute to its potential role in alleviating depression and anxiety.^{68,69} BP is an attractive choice for inhalation therapy to promote mental and emotional well-being because of the combination of these compounds.

Javanese Long Pepper (*Piper retrofractum* Vahl)

The Javanese long pepper (*Piper retrofractum* Vahl). (JLP), also known as “tabia bun” in Bali, Indonesia, is used to treat several kinds of diseases, including stomach distension or flatulence, dyspepsia, colic, neurasthenia, rheumatism, bronchitis, neurotrophic, and anticancer.³² Furthermore, JLP is unique candidate for aromatherapy agents due to its capacity to inhibit the growth of *Mycobacterium tuberculosis*,³⁷ inhibit NF- κ B,⁴⁰ reduce NO, COX-2, IL-6, and IL-1,⁴¹ as well as blood pressure, pulse, and respiration (Table 1).³⁹

Based on of its different volatile compound composition, JLP essential oil JLP (JLPEO) could be an excellent candidate for aromatherapy. β -caryophyllene (0.98–27.7%),^{6,28,49,50,52,54,55} and germacrene D (7.78–24.20%)^{6,28,54,57} are among the primary compounds found in a several of studies (Table 2). γ -cadinene was the most abundant compound identified in JLP, accounting for 31.63%,⁴⁸ followed by 3-Methyl-1-butanol (30.24%).⁷⁰ Compounds like myrcene (14.4%),⁴⁹ limonene (0.35–18.51%)^{49,50} and another monoterpene and sesquiterpene are also present.

Particularly in regions with warm tropical climates, compounds like β -caryophyllene are prevalent constituents of JLPEO. Several biological activities, including anti-inflammatory, analgesic, and antidepressant properties, are known to be exhibited by this compound.^{26,71} Given the high concentration of β -caryophyllene, JLP cultivated in tropical regions might have more medicinal benefits.

Germacrene D's presence aids to provide health benefits in a several of essential oils, particularly in antioxidant,^{72,73} anti-inflammatory,⁷² and skeletal wellness.⁷⁴ In addition, germacrene D shows promise as a component in aromatherapy due to its various biological activities, including antimicrobial,^{75–77} and antifungal properties.⁷⁵ Its potential to improve mood,⁷⁸ relieve stress,^{78,79} and relieve pain^{80,81} makes it a valuable candidate for further study and application in

Table I Botanical Sources of Trikatu and Its Pharmacological Mechanisms in Traditional Medicine

Plants	Part Used	Traditional Uses	Pharmacological and Biological Effect	Mechanisms of Action	Type of Extract	Study Type	Ref
Black Pepper (<i>Piper nigrum</i> Linn).	Fruit	Gastro-protective, antidepressant, Anti-inflammatory, antimutagenic, antimetastatic, hepatoprotective ³²	Antioxidant	↑ GSH, ↓ MDA, ↓ Pancreatic NO	Essential Oil	In vivo: Adult male Albino Wistar rats were induced pancreatic damage.	[33]
			Antioxidant	↑ CAT, ↑ SOD, ↓ MDA, ↓ AChE	Cold-pressed Oil	In vivo: Sprague Dawley male rats (were induced Alzheimer-type dementia.	[5]
			Antioxidant	Exhibited hydroxyl radical scavenging activity (IC ₅₀ 24.44 ± 0.11 mg/mL)	Essential Oil	In vitro: DPPH assay	[28]
			Anti-inflammatory	↓ TnF-α, ↓ IL-6, ↓ IL-1β, ↓ IFN-γ, ↓ TLR4/MAPK activity ↑ Expression of claudin-1, claudin-3, occludin, ZO-1, and mucin 2	Essential Oil	In vivo: Male C57BL/6 J mice weighing 18–22 g with DSS-induced colitis	[34]
			Antimicrobial	Exhibited significant activity against multiple microbial pathogens like <i>Salmonella enterica</i> serovar Typhi, <i>Escherichia coli</i> and <i>Staphylococcus saprophyticus</i>	Essential Oil	In vitro: disk diffusion test	[35]
			Anticancer	Selective cytotoxicity against cancerous cells	Essential Oil	In vitro: cancerous cells (HepG-2; PC-3 as compared to noncancerous 3T3L-1 cells)	[35]
			Anti-tumor	Inhibited the expressions of Wnt and β-catenin and significantly activated the expression of GSK-3β	Essential Oil nanoparticle	In vitro: MDA-MB-231 cells	[36]

Javanese Long Pepper (Pepper <i>retrofractum</i> Vahl).	Fruit	Stomach distension or flatulence, dyspepsia, colic, neurasthenia, rheumatism, bronchitis, neurotrophic, anticancer ³²	Antitubercular	Minimum inhibitory concentration (MIC) of 25.00 µg/mL	Crude Methanol Extract	In vitro: Mycobacterium tuberculosis	[37]
			Antimicrobial	Inhibit the majority of human and animal pathogens (Bacillus subtilis ATCC6633, Staphylococcus aureus ATCC25923, Enterococcus faecalis ATCC2921, Escherichia coli ATCC25922, Klebsiella pneumonia TISTR1843, Pseudomonas aeruginosa ATCC741, Salmonella typhi (clinical isolate), Vibrio parahaemolyticus (XN98 and 5HP), and Candida albicans ATCC90020)	Methanol extract	In vitro: Disk diffusion test	[38]
			Anticancer	The crude methanol extract showed moderate cytotoxic behaviour against lung cancer cells	Crude Methanol Extract	In vitro: Lung cancer cells (NCI -H187) and human gingival fibroblasts (HGF)	[37]
			Antioxidant	Exhibited hydroxyl radical scavenging activity (IC ₅₀ 20.97 ± 0.09 mg/mL)	Essential Oil	In vitro: DPPH essay	[28]
			Anti-anxiety	↓ Blood pressure, a pulse, respiration	Essential Oil	Clinic: patients of oral surgery aged 17–40 years of which teeth, either from upper or lower jaws, would be extracted.	[39]
			Antiphotaging effect	– Activated the peroxisome proliferator-activated receptor delta (PPARδ) and the adenosine monophosphate-activated protein kinase (AMPK). – Inhibited MMPs expression via suppressing mitogen-activated protein kinase (MAPK) and activator protein-1 (AP-1). – Inhibiting the nuclear factor-kappa B (NF-κB) activity – enhanced transforming growth factor-beta (TGF-β) and the Smad signaling pathway	Standardized extract	In vitro: Monkey kidney (COS-7) and human dermal fibroblasts (HS68) In vivo: Five-week-old female albino hairless mice were exposed to UVB and orally treatment	[40]
			Anti-inflammatory	Inhibitory effects on NF-κB, ↓LPS, ↓NO, ↓COX-2, ↓IL-6, ↓IL-1, and ↓NF-κB through the TLR4 axis	Extract	In vitro: RAW264.7 cells (ATCC, USA) and RAW264.7-NF κB-Luc2 cells In vivo: Balb/c mice (7–8 weeks old, Male, 18–19 g). Imiquimod-induced skin inflammation mice model.	[41]

(Continued)

Table 1 (Continued).

Plants	Part Used	Traditional Uses	Pharmacological and Biological Effect	Mechanisms of Action	Type of Extract	Study Type	Ref
Red Ginger (<i>Zingiber officinale</i> var. <i>rubrum</i> Theilade)	Rhizome	Anti-inflammatory, anticancer, anti-proliferative, neuroprotective, rheumatoid, arthritis ³²	Antioxidant	Exhibited hydroxyl radical scavenging activity (IC_{50} 2.73 $\pm$ 0.09 mg/mL)	Essential oil	In vitro: DPPH essay	[28]
			Anti-inflammatory	$\downarrow$ NF- κ B and PGE-2 levels	Fractionated ethanol extract	In vivo: Male Wistar rats in periodontitis model	[42]
			Neuroprotective effect	$\downarrow$ MDA level $\uparrow$ Acetylcholinesterase (AChE) level	Ethanol extract	In vivo: Scopolamine-induced memory loss in mice. Male Balb/C mice (n = 30; 6–7 weeks old)	[43]
			Anti-hyperalgesia effect	$\downarrow$ Hyperalgesia Delaying spinal cord damage after painful diabetic neuropathy (PDN) $\downarrow$ Blood glucose level Islet pancreas cell breakage	Essential Oil	In vivo: Mice in diabetic	[44]
			Antibacterial	Moderate antibacterial activity against three Gram-positive (<i>B. licheniformis</i> , <i>B. spizizenii</i> , <i>S. aureus</i>) and three Gram-negative (<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. stutzeri</i>) bacteria	Essential Oil	In vitro: Micro-dilution technique	[45]

Table 2 Essential Oil Compounds of BP, PR, RG and TR

Compound	BP (%)	JLP (%)	RG (%)	TR (%)	Reference
α -Pinene	5.1–28.7	-	5.57	0.08	[4,28,34,47]
β -Pinene	9.1–48.0	-	-	0.15	[4,28,34,48]
Limonene	14.36–36.5	0.35–18.51	-	-	[4,48–51]
D-Limonene	6.67–25.83	-	-	1.0	[28,34,52,53]
δ -3-Carene	4.5–21.5	-	-	-	[4,52]
β -caryophyllene	4.79–62.23	0.98–27.7	-	11.92	[4,6,28,34,48–55]
Caryophyllene oxide	8.17–21.47	-	-	2.35	[28,34,53]
α -Terpinene	6.68–13.26	-	-	0.04	[28,48,51]
3-Carene	6.20–26.84	-	-	-	[34,53]
Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-(3R-trans)-	6.21	-	-	-	[53]
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	40.85	-	-	-	[53]
Copaene	5.04	4.67	-	3.60	[28,53]
(-)-Spathulenol	5.32	-	-	-	[53]
α -Curcumene	13.30	10.21	10.65–19.32	16.26	[28,47,56]
β -Gurjunene	5.79	-	-	0.61	[28]
Sesquiphellandrene	10.89	-	14.52	11.39	[28]
2- β -Pinene	14.3	10.05	-	-	[50,52]
α -Copaene	5.1	-	-	-	[52]
Isospathulenol	5.23	-	-	1.73	[28,34]
Sabinene	9.68	-	-	0.28	[28,48]
β -Longipinene	-	7.43	-	-	[48]
γ -Cadinene	-	31.63	-	-	[48]
(Z)-Muurolo-4(14),5-dieno	-	10.66	-	-	[48]
(Z)-Muurolo-4(15),5-diene	-	14.39	-	-	[48]
Myrcene	-	14.4	-	-	[49]
Bicycloelemene	-	9.9	-	-	[49]
Bicyclgermacrene	-	7.0	-	-	[49]
Benzyl benzoate	-	6.28–14.4	-	-	[49,57]
Germacrene D	-	7.15–24.20	-	-	[6,28,54,57]
α -Humulene	-	6.01–12.84	-	-	[6,28]
cis- α -Bisabolene	-	5.97	-	-	[28]
β -Bisabolene	-	10.82	5.21–10.01	5.14	[28,47,56]
8-Heptadecene	-	6.40	-	2.51	[28]
Heptadecane	-	6.71	-	2.51	[28]

(Continued)

Table 2 (Continued).

Compound	BP (%)	JLP (%)	RG (%)	TR (%)	Reference
γ -Murolene	-	10.14	-	0.41	[28,55]
3-Methyl-1-butanol	-	30.24	-	-	[55]
Linalool	-	8.85–12.9	-	0.50	[28,54,55]
α -Caryophyllene	-	6.80–9.61	-	-	[54,55]
n-Pentadecane	-	4.94	-	-	[55]
Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	-	17.73	-	-	[57]
Ar-turmerone	-	11.55	-	-	[57]
Undecane	-	14.63	-	-	[50]
α -bergamotene	-	15.61	-	-	[50]
β -farnesene	-	5.46	-	-	[50]
5-octadecene	-	10.7	-	-	[50]
Naphthalene	-	15.4	-	-	[50]
Cyclohexene	-	20.62	-	-	[50]
l-phellandrene	-	6.55	-	-	[50]
Trans-ocimene	-	6.26	-	-	[50]
Trans- β -ocimene	-	4.67	-	0.02	[28,54]
Ocimen	-	15.6	-	-	[54]
Camphene	-	-	8.77–27.3	0.08	[28,45,47,58–60]
Geranial	-	-	12.0–14.3	-	[45,56]
Geranyl acetate	-	-	13.7	-	[45]
Neral	-	-	7.1–10.12	0.31	[28,45,56,58]
Geraniol	-	-	7.3	0.57	[28,45]
l,8-Cineole	-	-	5.0–15.1	-	[45,56,60]
(-)-Zingiberene	-	-	19.21	9.57	[28]
Zingiberene	-	-	15.4–28.50	-	[47,59]
Z- γ -bisabolene	-	-	8.05	-	[28]
Citral	-	-	12.7–18.83	0.43	[28,58,60]
Z-citral	-	-	14.21	-	[60]
Eucalyptol	-	-	12.59	0.19	[28,58]
Sesquisabinene	-	-	7.07	-	[47]
β -Sesquiphellandrene	-	-	6.8–8.4	-	[47,56]
Borneol	-	-	6.4	-	[56]
Trans- α -Bergamotene	-	-	-	5.29	[28]

Note: BP, Black Pepper (*Piper nigrum* Vahl); JLP, Javanese Long Pepper (*Piper retrofractum* Linn); RG, Red Ginger (*Zingiberene officinale* var. *Rubrum* Theilade); TR, Trikatu.

aromatherapy. However, more research is needed to standardize its use and determine the most effective formulations and dosages.

All things considered, the diversity of JLPEO composition offers a great deal of potential for the creation of inhalation-based aromatherapy products that promote energy and concentrations as well as relaxation and stress reduction. Therefore, to optimize the formulation of aromatherapy products based on JLPEO, more research on the inhalation effects of this essential oil as well as clinical trials on the physiological responses of its use are required.

Red Ginger (*Zingiber officinale* var. *rubrum* Theilade)

The Zingiberaceae family includes ginger (*Zingiber officinale*), which was initially grown in Asia, including Indonesia. *Zingiber officinale* var. *rubrum* Theilade (red ginger) is one of three varieties of chemical composition.⁵⁸ Red Ginger (RG) has long been used for its anti-inflammatory, antioxidant, antidiabetic anti-cancer, anti-proliferative, neuroprotective, rheumatoid, and arthritis properties.^{32,58} RG can inhibit Gram-positive as well as Gram-negative bacteria,⁴⁵ and decrease MDA levels,⁴³ NF- κ B,⁴² PGE-2,⁴² AChE,⁴³ hyperalgesia, blood glucose, and delaying spinal cord damage after painful diabetic neuropathy (PDN).⁴⁴ It is therefore a special candidate for an aromatherapy agent and requires further investigation.

The essential oil contained in RG is higher than other types of ginger, which makes it have a strong spicy smell and taste.⁵⁸ RG essential oil (RGEO) contains various volatile compounds that provide a distinctive aroma and therapeutic benefits. Based on the data (Table 1), there are several major components of the essential oil *Zingiber officinale* var. *rubrum* Theilade includes zingiberene (15.4–28.50%),^{47,59} (-)-zingiberene (19.21%),²⁸ camphene (8.77–27.3%),^{45,47,58–60} α -curcumene (10.65–19.32%),^{28,47,56} geranial (12.0–14.3%),^{45,56} sesquiphellandrene (14.52%),²⁸ and others which show the potential of RG as an aromatherapy agent.

The composition of essential oils contained in RG shows that RG has strong potential for use in aromatherapy. The presence of zingiberene and camphene dominates with high levels. Although direct studies on the specific effects of zingiberene in aromatherapy are limited, its presence in RG essential oil (RGEO) has an important role in improving mood,⁸² reducing stress,⁸³ and providing anti-inflammatory,⁸⁴ and also antioxidant.²⁸ These properties make zingiberene a valuable component in aromatherapy applications aimed at improving overall well-being. Camphene also provides various benefits as aromatherapy, especially in improving respiratory health,^{85,86} reducing stress,⁸⁷ and supporting the immune system.^{86,87} Its various biological properties make camphene a valuable component in essential oils used for therapeutic purposes. Additionally, α -curcumene, citral, geranial, and eucalyptol each have unique and beneficial effects in aromatherapy: anti-anxiety,^{62,88–90} respiratory benefits,^{88,91} antimicrobial,⁹² anti-inflammatory,⁸⁸ and antioxidant.^{88,92,93} These compounds can be used effectively in a variety of therapeutic and health enhancing applications.

RGEO's compound composition makes it a promising ingredient for inhalation aromatherapy, therapeutic massage oil, and formulations aimed at enhancing respiratory health and body immunity. Its development as a natural essential oil-based aromatherapy product can be supported by additional research on its composition and efficacy.

Chemical Composition of Trikatu Essential Oil

To understand each component of TREO and its contribution to its synergistic effects, we can analyze the data provided for relevant information. TREO is derived from the Ayurvedic formulation of TR, which includes BP, JLP and RG which provide some synergistic effects due to the combined nature of its elements. Our research indicates that the composition of significant compounds that were identified by gas chromatography-mass spectrometry (GC-MS), such as α -curcumene (16.26%), caryophyllene (11.92%), sesquiphellandrene (11.39%), (-)-zingiberene (9.57%), trans- α -bergamotene (5.29%), and β -bisabolene (5.14%). Furthermore, linalool, caryophyllene, and β -bisabolene are among the eleven compounds present in TREO that are jointly owned by the three plants that comprise TR.²⁸ When compared into using individual essential oils from each component, the complex interactions resulting from the diversity of these compounds can improve the efficacy of TREO.

The combined antioxidant,²⁸ anti-inflammatory,⁹⁴ and neuroprotective properties of TREO's different bioactive are primarily responsible for its synergistic effects.⁵ As demonstrated by several aromatherapy studies, linalool has a calming and stress-reducing effect,^{62,94} and β -caryophyllene interacts with CB2 receptors, which are involved in regulating

inflammation and the immune system.⁷¹ The potent antioxidant effect of α -curcumene helps shield cells from oxidative stress-induced damage.⁹⁵ TREO is a promising option for aromatherapy to lower stress and promote emotional balance because of this synergistic interaction, which also increases antioxidant activity and improves the effects of relaxation and mental well-being.⁹⁶

In addition, the composition of TREO rich in sesquiterpenes and monoterpenes provides a complex aromatic profile,²⁸ which contributes to beneficial psychological and physiological effects. The use of this essential oil in aromatherapy can help lower cortisol levels, reduce anxiety, and improve sleep quality.^{97–99} In broader therapeutic applications, TREO has the potential to be used in inhalation or diffusion formulations to support mental health and hormonal balance naturally. However, despite its promising synergistic effects, further research is needed to understand the molecular mechanisms underlying the interactions of compounds in TREO as well as to optimize its dosage and application methods for maximum therapeutic benefits.

Extraction Methods and Yield from Trikatu Components

In the Clevenger apparatus, the hydro-distillation method is typically used to extract essential oils from TR components (BP, JLP, and RG) (Table 3). This technique is authorized as a reference for oil quantification and is frequently used to extract essential oils from aromatic plants.^{102,103} Aromatic compounds are generally said to be thermolabile, and their half-life may be impacted by hydro-distillation.¹⁰² The hydro-distillation method in the Clevenger apparatus is used to reduce the loss of essential oils due to dissolution in hydrosols. The Clevenger-type apparatus has a unique system, which can return the hydrosol to the distillation device directly during the distillation process. So that oil and water will be continuously separated during the process which can improve the efficiency of essential oil extraction. Thus, this method does not require a lot of water.¹⁰⁴

Table 3 Extraction Method of Essential Oil from Trikatu Botanical Source

Plants	Part Used	Method	Yield	Reference
Black Pepper (<i>Piper nigrum</i> Linn).	Fresh fruit	• Steam distillation method • Solid-liquid ratio (1:6 g/mL) • For 5,4 hours • Grinding time 79s	3,342mL/100g	[34]
	50kg of Fresh fruit	• Steam distillation method • For 6 hours at 100°C	0.96% (v/w)	[100]
	300g of Dried fruits	• Hydro-distillation using a modified Clevenger-type apparatus • For 3 hours at (97 ± 3)°C	-	[101]
	150g of Dried fruits	• Hydro-distillation in the Clevenger apparatus • The temperature of the heating mantle was adjusted to 100 °C	0.01% v/w	[5]
	90g of Dried fruits	• Ground into 40-mesh powder • Stahl hydro-distillation in the Clevenger apparatus • Solid-liquid ratio (1:8 g/mL) • For 6 hours	(1.186 ± 0.155)% w/w	[28]
Javanese Long Pepper (<i>Pepper retrofractum</i> Vahl).	Dried fruits	• Ground into 40-mesh powder • Steam distillation • Solid-liquid ratio (1:10 g/mL) • For 4 hours	-	[6]
	90g of Dried fruits	• Ground into 40-mesh powder • Stahl hydro-distillation method in the Clevenger apparatus • Solid-liquid ratio (1:8 g/mL) • For 6 hours	(1.058 ± 0.036) % w/w	[28]
	0.5kg of Dried fruits	• Hydro-distillation method • For 4 hours	0.2% v/w	[49]

(Continued)

Table 3 (Continued).

Plants	Part Used	Method	Yield	Reference
Red Ginger (<i>Zingiber officinale</i> var. <i>rubrum</i> Theilade)	Rhizomes	- Hydro-distillation method - For 8 hours	1.43%	[58]
	125g of homogenised rhizomes	- Hydro-distillation method - For 4 hours	0.02% w/w	[45]
	90g of Dried rhizomes	- Ground into 40-mesh powder - Stahl hydro-distillation method in the Clevenger apparatus - Solid-liquid ratio (1:8 g/mL) - For 6 hours	(1.549 ± 0.020)% w/w	[28]
	Rhizomes	- Hydro-distillation method - Solid-liquid ratio (1:2 g/mL) - For 5–6 hours	0.36%	[44]
Trikatu	90g of mix dried fruits and rhizome	- Stahl hydro-distillation method in the Clevenger apparatus - Solid-liquid ratio (1:8 g/mL) - For 6 hours	(1.256 ± 0.020)% w/w	[28]

Looking at Table 3, the amount we got from each TR component we tested using the hydro-distillation method in the Clevenger apparatus was greater than what we got from other methods.²⁸ The research that we have conducted using dried fruits BP, JLP, and rhizomes RG powder (40 mesh) in obtaining essential oils. The essential oil distillation process was carried out for 6 hours with a solid-liquid ratio (1:8 g/mL). The yield obtained for each essential oil, respectively, was (1.186 ± 0.155), (1.058 ± 0.036), and (1.549 ± 0.020)% w/w.²⁸ Previous studies have also tried to make TREO using 40 mesh dry powder from each TR component (BP, JLP, and RG) with a ratio of 1:1:1 w/w/w. The yield we obtained was (1.256 ± 0.020)% w/w; this shows that the combination of the three ingredients in balanced amounts still produces high yields.²⁸ So, this method is very effective and efficient for use in obtaining essential oils.

Table 3 shows that a several of parameters, such as the sample's condition (wet or dry), sample size, which influences surface area, water content, solid-liquid ratio, and distillation time, can affect the yield differences. The effectiveness of oil release and the consistency of the extraction process are probably enhanced by the use of dry materials and consistent size, like 40 mesh.²⁸ Furthermore, the length of the distillation period must be considered. Since the length of distillation period typically does not always result in a substantial improvement in yield and actually runs the risk of inducing heat degradation of bioactive chemicals, it appears that 6 hours is a fairly time for all three types of materials.¹⁰⁴

Overall, the hydro-distillation method in the Clevenger apparatus, with proper optimization of conditions, can be used effectively to obtain essential oils from TR constituents with competitive yields. This knowledge is important as a basis for the development of aromatherapy formulations or other functional applications of TREO.

Mechanistic Insights into the Aromatherapeutic Potential of Trikatu Essential Oil

Aromatherapy is a complementary, holistic, and versatile therapy that utilizes the natural properties of essential oils to improve health and well-being.^{78,105,106} It can be used by inhalation, topically applied, or diffused into the air.^{107–110} The use of aromatherapy is commonly used for physical and mental health care to improve quality of life in various health care settings.^{16,110–113}

TR is traditionally used to enhance digestion, reduce inflammation, and relieve respiratory ailments. Current research shows that its essential oil components have potent pharmacological activities, making it an attractive candidate for inhalation-based therapy. TREO, which consists of sesquiterpenes and monoterpenes, provides diverse effects such as antioxidant, anti-inflammatory, anxiolytic, and neuroprotective (Table 4). To explore the aromatherapy potential of TR and its individual components, we discuss them in this section.

Antioxidant

Essential oils from each TR component, namely BP, JLP, and RG, have been known to collectively exhibit antioxidant activity (Table 1) in scavenging free radicals. We found that the combination of BP, JLP, and RG contributed to the antioxidant activity of TREO in vitro, with an IC_{50} against DPPH radicals of (13.74 ± 0.46) mg/mL.²⁸ Although the antioxidant activity of TREO, and each of its components individually, is relatively low (>1 mg/mL), BPEO has been reported to indirectly increase endogenous antioxidant defenses (GSH, SOD, CAT) and reduce MDA levels.^{5,33}

The compounds β -caryophyllene, (-)-zingiberene, α -pinene, linalool, camphene, eucalyptol contribute to providing antioxidant effects in TREO (Table 4) through mechanisms similar to BPEO, namely increasing SOD, CAT, GSH, GSPx, Nrf2, HO-1, improving the GSH/GSSG ratio, and reducing lipid peroxidase and MDA levels.^{83,85,114,122,131,145,146} The molecular pathways involved include activation of Nrf-2/HO-1 which triggers increased cell defense against oxidative stress, inhibition of pro-oxidant enzymes such as iNOS and H_2O_2 .^{83,85,122}

These compounds can work synergistically as antioxidants (Figure 1). For example, there is the fact that eucalyptol specifically targets Sirt1 and supports direct interaction between Sirt1 and Nrf2 which can suppress oxidative stress.¹⁴⁶ Synergy occurs between eucalyptol and β -caryophyllene which can increase and activate Nrf2. They can work synergistically in providing antioxidant effects against chronic obstructive pulmonary disease (COPD) caused by cigarette smoke. Cigarette smoke can activate Nrf2 by increasing ROS levels. So with the activation of Nrf2, HO-1 is also activated which is able to neutralize ROS from cigarette smoke as an antioxidant. However, on the other hand, ROS caused by cigarette smoke causes another effect, namely inflammation which causes increased neutrophils (NE). This can cut Sirt1, so that it can suppress the Nrf2/HO-1 pathway.^{148,149} This is where the role of eucalyptol and β -caryophyllene contained in TREO works together. Eucalyptol will interact with Sirt1 so that NE cannot cut Sirt1, and β -caryophyllene activates the Nrf2/HO-1 pathway. Thus, TREO is not only promising as an antioxidant, but also has the potential to be developed as an inhalation aromatherapy oil to ward off chronic oxidative stress in various degenerative conditions.

Anti-Inflammatory

TREO, which is a mixture of BP, JLP, and RG, has a synergistic effect as a promising anti-inflammatory agent, especially in the form of aromatherapy. This potential is supported by the activity of each TREO component individually (Table 1) which is known to be an anti-inflammatory agent with various inflammatory signaling pathways.^{34,41,42} In addition, the presence of various active compounds in TREO also supports this potential (Table 4), especially from the monoterpene and sesquiterpene groups such as β -caryophyllene,^{65,114} D-limonene,¹¹⁶ (-)-zingiberene,^{119,120} α -pinene,^{122,123} linalool,^{132,133} camphene,⁸⁵ citral,^{140,141} and eucalyptol.^{146,147}

One of the main compounds of TREO, β -caryophyllene shows its ability to reduce pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), so that it can improve chronic pathologies characterized by inflammation and oxidative stress.⁶⁵ It seems that β -caryophyllene synergistically works with other compounds (D-limonene, (-)-zingiberene, α -pinene, linalool, camphene, citral, and eucalyptol) in reducing pro-inflammatory mediators. Figure 2 explains the working mechanism of the synergy of these compounds.^{85,114,116,119,120,122,123,132,133,140,146,147}

For example, the synergistic anti-inflammatory effects between β -caryophyllene and (-)-zingiberene are shown by a decrease in the levels of inflammatory cytokines such as TNF- α , IL-6, NF- κ B, and IL-1 β .^{65,119} This seems to be what causes (-)-zingiberene and linalool to be able to suppress phosphorylated (p)-p38 mitogen-activated protein kinase (MAPK), p-c-Jun N-terminal kinase (JNK), and p-extracellular signal-regulated kinase $\frac{1}{2}$ (p-ERK1/2).¹²⁰ In addition to its ability as an anti-inflammatory agent, through the mechanism of suppressing p38-MAPK, JNK, and p-ERK1/2, (-)-zingiberene can also be antiapoptotic through this mechanism which plays a major role in regulating the expression of apoptosis proteins and degradation of the extracellular matrix, including reducing the expression of Bax, caspase-3, MMP-2, MMP-9, and suppressing Bcl-2.¹¹⁹ The p38-MAPK is activated by inflammatory cytokines such as TNF- α , IL-6, NF- κ B, and IL-1 β and environmental stress, and can contribute to diseases such as asthma and autoimmunity.¹⁵⁰ In addition to (-)-zingiberene, α -pinene, citral and linalool also have almost similar mechanisms in providing anti-inflammatory effects, namely by

Table 4 Mechanisms of Action Trikatu Compounds as Aromatherapy

Component	Group Compound	Mechanism of Action	Effect	Reference
β -Caryophyllene	Sesquiterpene	● Agonist CB2 receptors ● Inhibit adenylyl cyclase ● Activate MAPK ● Modulation of PPARs ● β-endorphin release ● $\downarrow$ TNF-α and IL-1β expression ● $\uparrow$ Nrf2 and HO-1 ● $\downarrow$ MDA, NO, LPO ● $\uparrow$ SOD, CAT	● Anti-inflammatory effect ● Anti-anxiety ● Reduces heart rate ● Influences EEG activities ● Neuroprotective in cerebral I-R injury ● Antioxidant	[62,65–67,114,115]
D-Limonene	Monoterpene	● $\uparrow$ BDNF ● $\downarrow$ IL-1β and caspase-1 levels ● $\downarrow$ Neuroinflammation and stress ● $\downarrow$ Anhedonic and defensive behaviours and the impairing effects of stress ● $\downarrow$ The heart rate ● $\uparrow$ The high-frequency (HF) value of HRV ● $\uparrow$ Feeling Comfortable	● Anti-neuroinflammatory ● Antidepressant ● Physiological and psychological relaxation	[116,117]
α -Curcumene	Sesquiterpene	Stronger binding to GluN1, GluN2B, and GluA2	Antidepressant	[17]
Sesquiphellandrene	Sesquiterpene	-	-	-
β -Bisabolene	Sesquiterpene	$\downarrow$ the growth of transplanted 4T1 mammary tumours	Anti-tumor	[118]
(-)-Zingiberene	Sesquiterpene	● $\downarrow$ Hyperlipidemia and oxidative stress ● $\downarrow$ TNF-α, IL-6, NF-κB, and IL-1β ● $\downarrow$ MDA ● $\uparrow$ CAT, SOD, GSPx, GSH/GSSG ● $\downarrow$ Macrophage ● $\downarrow$ MMP-2 and MMP-9 ● $\downarrow$ Bax and caspase-3 ● $\uparrow$ The Bcl-2 expression ● Suppresses proteins of phosphorylated (p)-p38 mitogen-activated protein kinase (MAPK), p-c-Jun N-terminal kinase (JNK), and p-extracellular signal-regulated kinase $\frac{1}{2}$ (p-ERK1/2)	● Anti-inflammatory ● Antioxidant ● Antiapoptotic	[83,84,119–121]
trans- α -Bergamotene	Sesquiterpene	-	-	-

(Continued)

Table 4 (Continued).

Component	Group Compound	Mechanism of Action	Effect	Reference
α -Pinene	Monoterpene	• ↑The Bcl-2 expression • ↑D2R and tyrosine hydroxylase in the striatum • ↓Iba-1, Bax • ↑Mn-SOD and GCS via activation of Nrf-2 • ↓Lipid peroxidase • Restoring the BDNF/TrkB/CREB signaling pathway in the rat hippocampus • ↓Activations of MAPKs and NF-κB • ↓The LPS-induced production of TNF-α, IL-6, and NO • Inhibited iNOS and COX-2 in macrophage • Displayed sleep-increasing behaviour by direct binding to GABAA-Benzodiazepine receptors (GABAA-BZD) • ↑BDNF mRNA expression in olfactory bulb and hippocampus • ↑TH mRNA expression in middle brain • ↑Transport to the brain	• Therapeutic agent for METH-induced drug addiction and neuronal damage • Antiapoptotic • Antioxidant • Neuroprotective • Anti-inflammatory • The anxiolytic-like action • Relaxation	[122–126]
β -Pinene	Monoterpene	• ↓Hippocampal nitrite level and striatal content of dopamine and norepinephrine	• Anticonvulsant	[127]
Caryophyllene oxide	Sesquiterpene oxide	• ↓Ethanol-induced place conditioning • Modifying the reinforcing and appetitive conditioned effects of ethanol • Inhibited edema	• Sedative • Anti-inflammatory	[128,129]
α -Terpinene	Monoterpene	• Induced myorelaxant effects on top of contractions induced by KCl, ACh and 5-HT • Inhibited the contractions induced by barium in presence of High $[K^+]$ (80 mM) • Inhibition of L-type VGCC • Inhibiting the inward Ca^{2+} • Relaxation of the respiratory tract	• Antispasmodic	[130]
Copaene	Sesquiterpene	-	-	-
β -Gurjunene	Sesquiterpene	-	-	-
Isospathulenol	Sesquiterpene Alcohol	-	-	-
Sabinene	Monoterpene	-	-	-
8-Heptadecene	Alkene	-	-	-
Heptadecane	Alkene	-	-	-
γ -Murolene	Sesquiterpene	-	-	-

Linalool	Alcohol Monoterpene	• ↓The [Ca²⁺]_i increases induced by KCl and voltage-gated Ca²⁺ currents • ↓TRPA1 mediated nociceptive behaviors • ↓TNF-α, IL-6, IL-1β, IL-4, IL-5, IL-13, NO, PGE₂, MCP-1 • Phosphorylation of IκBα protein, p38, c-Jun terminal kinase, and extracellular signal-regulated kinase in LPS-stimulated RAW 264.7 cells was blocked • ↓Lung histopathologic changes in mouse models • Inhibited the levels of eosinophil numbers, Th2 cytokines and immunoglobulin E (IgE) caused by ovalbumin (OVA) exposure • ↓iNOS and protein kinase B (AKT) activation in the lung tissues • ↓MAPK and NF-κB • Inhibitory effect on OVA-induced airway hyperresponsiveness (AHR) • Secretion of MCP-1 was attenuated with linalool treatment in lipopolysaccharide (LPS)-stimulated H292 airway epithelial cells • ↑Induced nuclear translocation of Nrf2 and expression of HO-1 • Inhibited CS-induced lung MPO activity • Inhibited 5-HT₃ receptor, smooth muscle contractions in rat trachea, and guinea pig ileum	• Analgesic • Anti-inflammatory • Antioxidant • Anxiolytic • Sedative	[131–136]
trans-β-ocimene	Monoterpene	-	-	-
Camphene	Sesquiterpene	• ↓iNOS, NO • Inhibited PTGS-2, LOX-5, COX-1 and COX-2 • ↓PPAR-γ and C/ERPα • ↑IRS-1 and AKT • ↑SOD, GSH • ↓Lipid peroxidase	• Anti-inflammatory • Anti-diabetic • Antioxidant	[85]
Neral	Aldehyde Monoterpene	-	-	-
Geraniol	Alcohol Monoterpene	• Inhibits the proliferation of PC-3, A431, and A549 cells • Inhibited the DHFR • Suppressed COX-2 • Modulated LOX-5 • Relieved pentylentetrazol (PTZ)-induced seizures • ↓Altered the electroencephalogram (EEG) • Facilitated general anesthesia • ↑Spinal c-Fos • Suppressed the calcium activity in the paraventricular thalamic nucleus (PVT) • Prolonging spontaneous inhibitory postsynaptic currents and inducing tonic inhibitory currents via GABA_A receptors • ↓DCP and AEW-induced chronic itch • ↑Spinal GRPR • Modulating the spinal GABA/GRPR signaling	• Antiproliferative • Sedatives and anaesthetics • Antipruritic	[137–139]

(Continued)

Table 4 (Continued).

Component	Group Compound	Mechanism of Action	Effect	Reference
Citral	Aldehyde Monoterpene	• ↓NF-κB, IL-6, IL-1β, TNF-α, ROS, Nrf2, MAPK • ↓The number of border crossings and time spent in borders • The number of grooming and rearing in OFT without altering the exploratory behaviour of mice • ↑Number of entries in open arms and time spent in open arms • ↓Time spent in closed arms • Modulated GABAA-benzodiazepam, 5HT1A, and 5HT2A • Inhibited 5-HT3 receptor, smooth muscle contractions in rat trachea, and guinea pig ileum • TRPV1 Agonist • voltage-gated Na⁺ channel blocker • non-N-methyl-D-aspartate (NMDA) glutamate receptor antagonist	• Anti-inflammatory • Anxiolytic • Sedative • Gastro-protective effect against NSAID-induced ulcers	[134,140–144]
Eucalyptol	Terpenoid Oxide	• Inhibited 5-HT3 receptor, smooth muscle contractions in rat trachea, and guinea pig ileum • ↓IFN-γ, IL-1β, TNF-α and IL-6 • ↓Leukocyte infiltration, myeloperoxidase activity • Mediated TRPM8 • ↑Transport to the brain • ↑SOD, CAT • Inhibited TRPA1 • Modulated GABA_A-benzodiazepam • Activation Nrf2 and Sirt1 • Regulation of NF-κB	• Anxiolytic • Sedative • Anti-inflammatory • Antioxidant	[124,134,145–147]

Abbreviations: TR, Trikatu; TREO, Trikatu essential oil; BP, Black pepper; BPEO, Black pepper essential oil; JLP, Javanese long pepper; JLPEO, Javanese long pepper essential oil; RG, red ginger; RGEO, red ginger essential oil; SOD, superoxide dismutase; Mn-SOD, mangan superoxide dismutase; CAT, catalase; GST, glutathione S-transferase; GR, glutathione reductase GSPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; MDA, malondialdehyde; HO-1, heme oxygenase-1; Nrf2, nuclear factor erythroid-2-related factor 2; IL-1, interleukin-1; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; NF-κB, nuclear factor kappa B; MCP-1, monocyte chemoattractant protein 1; MAPK, mitogen activated protein kinase; MDA, malondialdehyde; AChE, acetylcholinesterase; CRP, c-reactive protein; TLR, toll-like receptor; NO, nitrogen oxide; Sirt1, sirtuin 1; GABA_A, γ-aminobutyric acid; CB2, Cannabinoid type 2; IFN-γ, Interferon-γ; 5-HT, 5-hydroxytryptamine; 5-HT₃, 5-hydroxytryptamine-3; 5-HT_{1A}, 5-hydroxytryptamine-1A; 5-HT_{2A}, 5-hydroxytryptamine-2A; NMDA, non-n-methyl-d-aspartate; TRPV1, transient receptor potential cation channel subfamily V member 1; ROS, reactive oxygen species; GRPR, gastrin-releasing peptide receptor; COX, cyclooxygenase; COX-2, cyclooxygenase-2; COX-1, cyclooxygenase-1; LOX, lipoxygenase; LOX-5, 5-lipoxygenase; PTGS-2, prostaglandin-endoperoxide synthase-2; BDNF, brain-derived neurotrophic factor; TrkB, tyrosine receptor kinase B; CREB, cAMP-response element-binding protein; GC-MS, gas chromatography – mass spectrometry; PGE-2, prostaglandin E2; PDN, painful diabetic neuropathy; IC₅₀, inhibitory concentration 50%; DPPH, 2,2-diphenyl-1-picrylhydrazyl; COPD, chronic obstructive pulmonary disease; ROS, reactive oxygen species; NE, neutrophils; JNK, p-c-Jun N-terminal kinase; p-ERK1/2, p-extracellular signal-regulated kinase 1/2; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; Bcl-2, B-cell lymphoma 2; SSRIs, Selective Serotonin Reuptake Inhibitors; GluN1, glutamate ionotropic receptor NMDA type subunit 1; GluN2B, glutamate ionotropic receptor NMDA type subunit 2B; GluA2, glutamate ionotropic receptor AMPA type subunit 2; EEG, electroencephalogram; PPARs, peroxisome proliferator – activated receptors; GCS, gamma – glutamylcysteine synthetase; ERK, extracellular signal-regulated kinase; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; Ca²⁺, calcium cation.

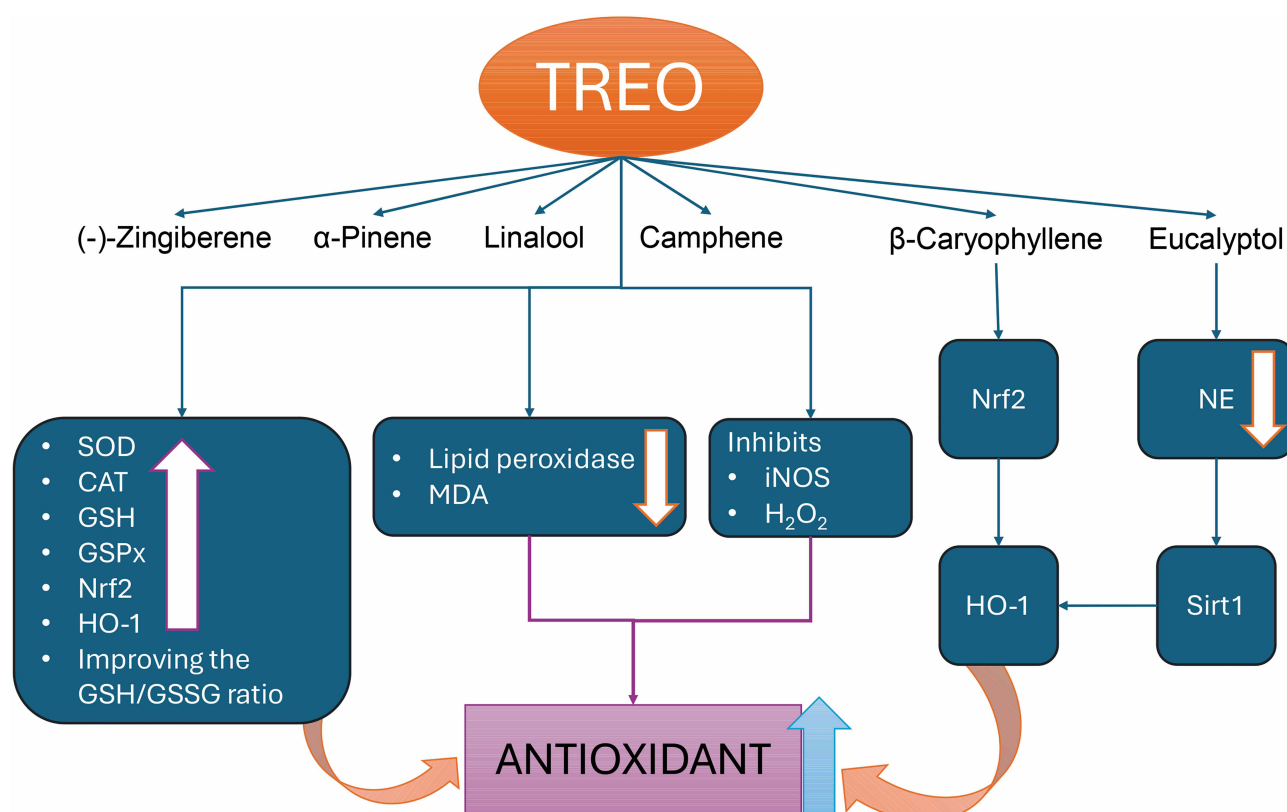


Figure 1 Mechanisms of Trikatu essential oil compound as antioxidant.

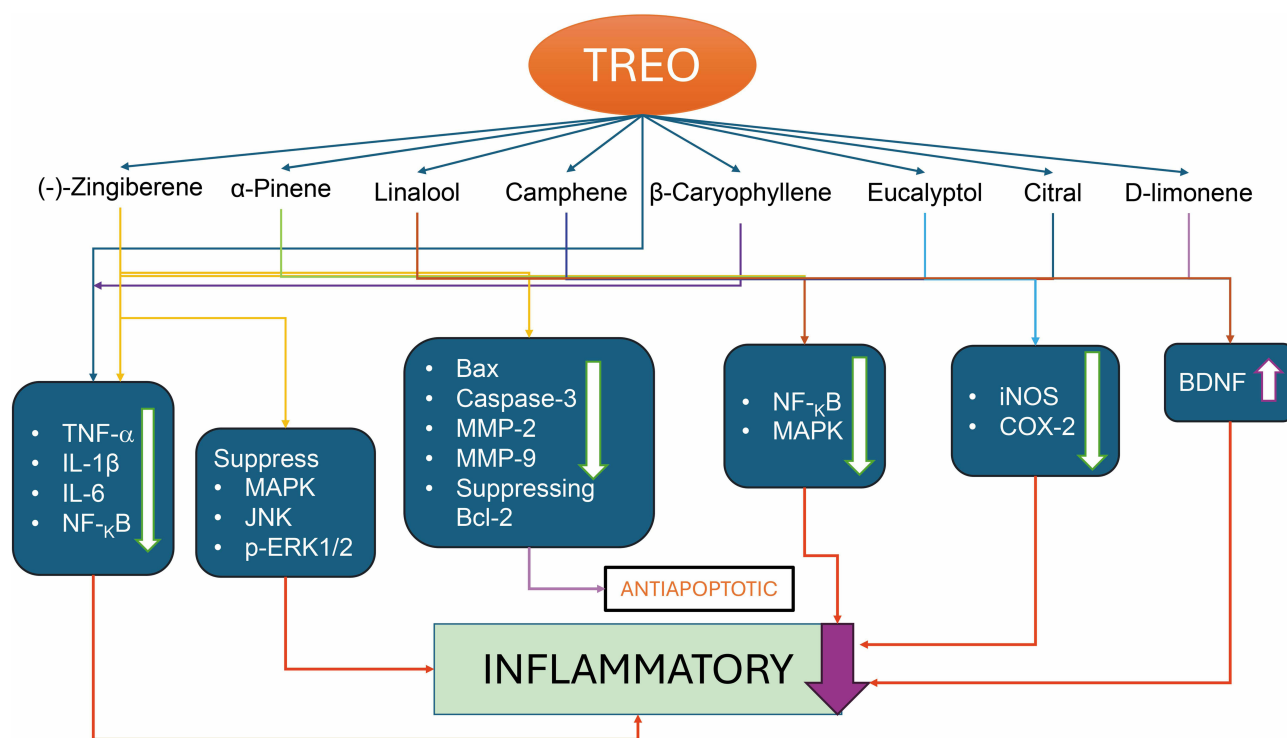


Figure 2 Mechanisms of Trikatu essential oil compound as anti-inflammatory.

reducing MAPK and NF- κ B activation, and can also reduce Iba-1, Bax, and increase Bcl-2 expression which indicates an antiapoptotic effect.^{122,132,133,140}

D-Limonene, linalool, and citral also show strong anti-inflammatory effects. D-limonene reduces neuroinflammation by decreasing IL-1 β and caspase-1 levels, and increasing BDNF expression, which is important in neuroprotection.¹¹⁶ Linalool and citral synergistically suppress inflammatory signaling pathways through inhibition of NF- κ B and MAPK, as well as activation of the Nrf2 pathway which plays a role in increasing the antioxidant response.^{132,133,140,141} Citral also has additional effects on the nervous system through interactions with GABA_A and 5-HT_{1A}, which not only provide anti-inflammatory effects but also reduce stress-induced anxiety.¹⁴² Other compounds such as camphene and eucalyptol also enhance the anti-inflammatory effects by suppressing the expression of iNOS, COX-2, and pro-inflammatory cytokines, and supporting the activity of Nrf2 and Sirt1 (two major pathways in controlling inflammation and oxidative stress).^{85,146}

Overall, the activity of the compounds in TREO works complementarily by suppressing major inflammatory signaling pathways such as NF- κ B, MAPK, COX/LOX, while increasing the activity of the antioxidant defense system through Nrf2/HO-1. This two-pronged approach, namely anti-inflammatory and antioxidant, helps break the mutually reinforcing cycle of oxidative stress and inflammation. With the potential to reach both the peripheral and central systems (neuroinflammation), TREO offers a holistic and prospective therapeutic approach for the development of evidence-based aromatherapy.

Anxiolytic

Anxiolytic, known as a minor tranquilizer and prescribed to treat and prevent anxiety symptoms or to manage anxiety disorders.¹⁵¹ Most compounds in TREO exhibit anxiolytic properties through several molecular targets (Table 4). Compounds in TREO can modulate the glutamate and GABA neurotransmitter systems which are important mechanisms and are responsible for sedative, anxiolytic, and anticonvulsant properties.⁷⁹ Compounds such as β -caryophyllene, α -pinene, linalool, geraniol, citral, and eucalyptol work synergistically by modulating neurotransmission, suppressing neuroinflammation, and restoring redox balance in the brain.^{62,124,125,137,138} One of the central anxiolytic mechanisms involves β -caryophyllene, a sesquiterpene that acts as a selective CB2 receptor agonist, reducing neuroinflammation and anxiety-related behaviors through modulation of the MAPK pathway, activation of Nrf2, and suppression of proinflammatory cytokines (TNF- α and IL-1 β).^{26,115} Importantly in this mechanism, β -caryophyllene also increases the release of β -endorphin, which contributes to emotion regulation.¹¹⁵ However, on the other hand, compounds such as α -pinene,¹²² α -terpinene,¹³⁰ linalool,¹³⁴ geraniol,^{137,138} citral,¹³⁴ and eucalyptol,¹³⁴ could modulate GABA_A, 5-HT, 5-HT_{1A}, 5-HT_{2A}, and inhibit 5-HT₃ mimicking the anxiolytic effects of benzodiazepine and Selective Serotonin Reuptake Inhibitors (SSRIs).

Chronic stress and anxiety are often associated with microglial activity and oxidative damage. As we discussed earlier (Figure 2), some monoterpenes and sesquiterpenes in TREO exhibit dual antioxidant and anti-inflammatory effects, especially β -caryophyllene, (-)-zingiberene, α -pinene, linalool, camphene, eucalyptol which increase SOD, CAT, GSH, GSPx, Nrf2, HO-1, improve GSH/GSSG ratio, and simultaneously decrease TNF- α , IL-6, NF- κ B, IL-1 β , lipid peroxidase and MDA levels.^{83,85,114,122,131,145,146} The compound D-limonene can downregulate IL-1 β and caspase-1, which contribute to neuroimmune stabilization and alleviation of depressive-like symptoms.¹¹⁶ Linalool and citral, which inhibit the MAPK and NF- κ B pathways while increasing the expression of Nrf2/HO-1, thereby reducing anxiety caused by inflammation.^{132,133,140,141} These antioxidant and anti-inflammatory properties synergize in improving the overall anxiolytic results.

Several compounds also play a role in improving neuroplasticity and synaptic function, including D-Limonene and α -Pinene which increase BDNF expression and CREB phosphorylation, supporting the recovery of neural circuits disrupted by chronic stress.^{116,124} In addition, α -curcumen shows potential in modulating the glutamatergic system through strong binding to GluN1, GluN2B, and GluA2 receptors which are relevant in regulating mood and anxiety.¹⁷

The synergistic effects of these compounds arise through multitarget interactions, complementary pharmacokinetics, and strengthening physiological effects such as normalization of heart rate, modulation of EEG waves, and reduction of anxious behavior in test animals.⁶² Therefore, TREO has the potential as a complementary therapy candidate for anxiety disorders, with a comprehensive mechanistic approach based on the combined effects between active compounds.

Inhalation of essential oils is a very relevant approach because of its fast and direct mechanism of action towards the central nervous system through the olfactory pathway.⁶² Volatile vapors of terpenoid compounds in TR can reach the brain in a short time, affecting neuronal activity in the limbic system, especially the amygdala and hippocampus, which are involved in processing emotions and anxiety.¹⁵² Several brain imaging studies have shown that certain aromas can activate areas of the brain associated with feelings of calm and relaxation.^{79,87,112} On the other hand, the systemic pathway through the respiratory epithelium also contributes to long-term pharmacological effects. The use of essential oils through inhalation also shows a better safety profile than the oral route, because it avoids first-pass metabolism in the liver and reduces the risk of gastrointestinal side effects. Therefore, TREO inhalation not only offers the therapeutic benefits of its bioactive compounds but also presents a practical and effective route of administration to support emotional balance and alleviate anxiety disorders in a complementary manner.

Neuroprotective

Neuroprotective agents are compounds or drugs that can protect neuronal injury after acute illness or neuro-degradation in the brain after chronic neurodegenerative diseases.¹⁵³ One of the main compounds of TREO, β -caryophyllene, is a cannabinoid receptor agonist (CB2),⁶⁶ which has an important role in modulating PPARs,⁶⁵ and MAPK¹⁵⁴ aimed at protecting neurons through regulation of anti-inflammatory and antioxidant gene expression (Figure 3). Synergistically, α -Pinene also contributes through activation of Nrf2 and increased antioxidant enzymes such as Mn-SOD and GCS, as well as suppressing inflammation through inhibition of the NF- κ B pathway.¹²⁶ Not only that, α -pinene also shows a protective effect against neurodegeneration due to methamphetamine stimulation through regulation of BDNF/TrkB/CREB expression (Figure 3) in the hippocampus.¹²⁶

In addition, linalool (Figure 2) has a strong protective effect against oxidative stress and nerve tissue damage through inhibition of p38 MAPK, ERK, and JNK activation, as well as increased HO-1 expression and Nrf2 translocation. Its

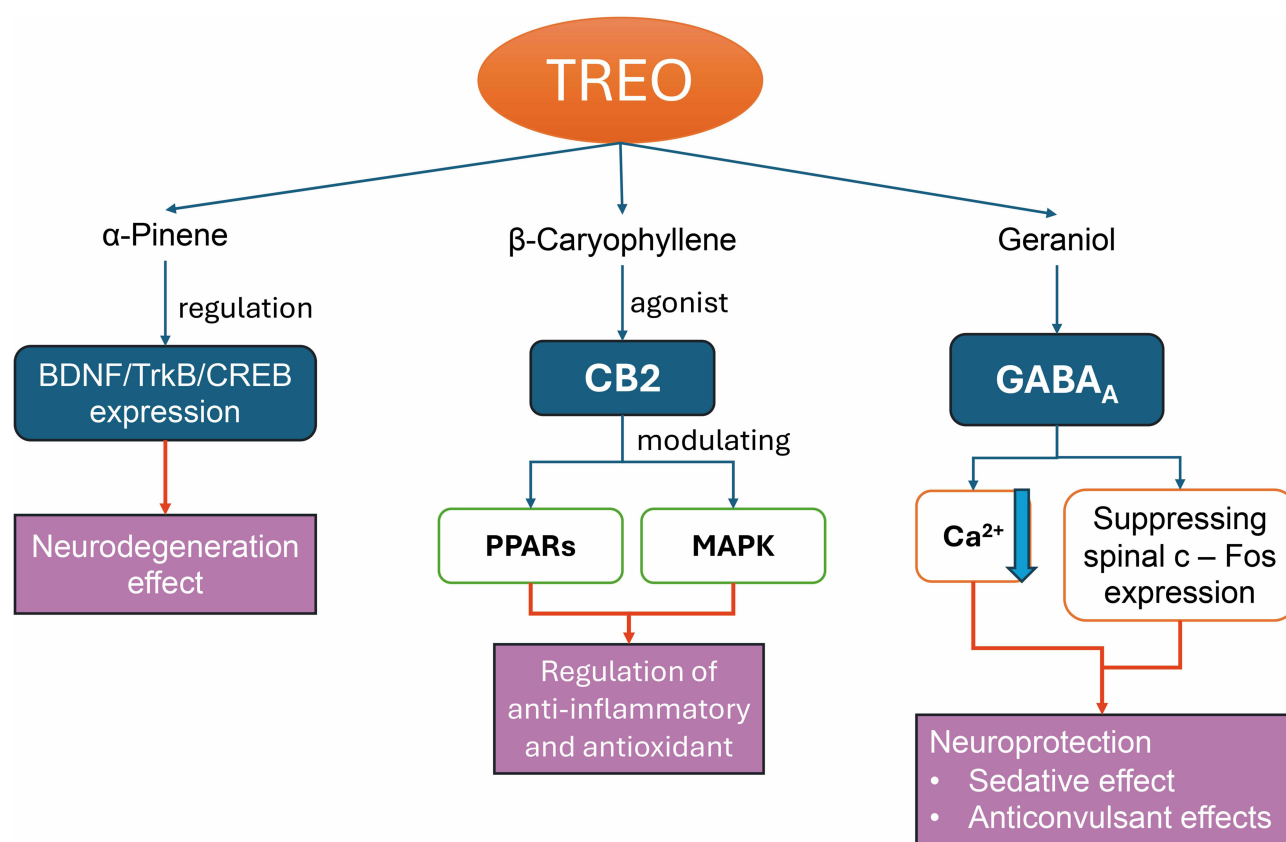


Figure 3 Mechanisms of Trikatu essential oil compound as Neuroprotective.

ability to inhibit the release of pro-inflammatory cytokines (such as TNF- α , IL-1 β , and IL-6) and suppress iNOS and MPO strengthens its contribution to maintaining the integrity of the nervous system against various inflammatory and toxic challenges.^{131–135} Other components such as geraniol also play an important role in neuroprotection, by showing sedative and anticonvulsant effects associated with strengthening inhibitory currents through GABA_A, decreasing Ca²⁺ activity of neurons in the thalamus, and suppressing spinal c-Fos expression as a marker of nociceptive nerve activation (Figure 3).^{137–139}

The possibility of a synergistic effect between compounds such as β -caryophyllene, α -pinene, linalool, and geraniol is very large, considering the overlapping action pathways such as Nrf2/HO-1, NF- κ B inhibition, and strengthening the GABAergic system. These collective protective effects have the potential to prevent or slow the development of neurodegeneration induced by chronic oxidative stress, systemic inflammation, and neurotransmitter dysfunction. Therefore, TREO has the potential to be developed as a holistic approach to protecting the nervous system, both in the context of neuropsychiatric and neurodegenerative stress.

Limitations and Future Directions

One significant drawback of TREO (a blend of *Piper nigrum*, *Piper retrofractum*, and *Zingiber officinale* var. *rubrum*) is the absence of strong clinical validation, despite encouraging data from in vitro and in silico investigations about its pharmacological potential. Most current research investigates the constituent plants separately, without assessing the synergistic properties of their combined essential oils. Furthermore, the conversion of TREO into evidence-based therapeutic uses is hindered by the lack of in vivo and clinical trials. Therefore, in order to prove safety and effectiveness in human patients, future research should include pharmacokinetic profiles, toxicological evaluations, and carefully planned randomized clinical trials.^{155–157}

Another critical challenge is the high variability in the chemical composition of essential oils, which results from variations in extraction methods, plant parts used, geographical origin, harvesting time, and storage conditions. This chemical variability can significantly influence biological activity and hinder the standardization necessary for clinical reproducibility. Addressing this issue requires optimized and reproducible extraction protocols (eg, steam distillation, hydro-distillation, or supercritical fluid extraction), alongside routine chemical profiling using analytical techniques such as GC-MS. Additionally, metabolomics and chemometric tools can be used to identify key bioactive markers for standardization purposes.^{28,158} Establishing chemical consistency in TREO is crucial for its development as a reliable and scientifically supported aromatherapeutic agent.

Conclusion

Combining black pepper (BP), Javanese long pepper (JLP), and red ginger (RG) to create Trikatu essential oil (TREO) has enormous promise as an aromatherapy agent with synergistic pharmacological activity. High and consistent essential oil yields have been successfully obtained by the hydro-distillation method with a Clevenger apparatus, particularly when consistently sized dry (40 mesh) and standardized distillation time (6 hours).

TREO exhibits multiple bioactive components, including β -caryophyllene, (-)-zingiberene, α -pinene, linalool, and eucalyptol, which support its antioxidant, anti-inflammatory, anxiolytic, and neuroprotective properties. The Nrf2/HO-1 and Sirt1 pathways are activated to carry out antioxidant activity, which contributes to enhancing cell defense against oxidative stress. The compound-to-compound synergy strengthens this action. In addition to suppressing pro-inflammatory enzymes like COX-2 and LOX and a variety of inflammatory signaling pathways including NF- κ B and MAPK, chemicals in TREO also act as an anti-inflammatory drug by lowering the expression of pro-inflammatory cytokines like TNF- α , IL-1 β , and IL-6. This impact is further enhanced by compounds like (-)-zingiberene, citral, and linalool, which makes TREO useful in the treatment of chronic inflammation.

Furthermore, by blocking 5-HT₃ and modifying CB2, GABA_A, 5-HT, 5-HT_{1A}, and 5-HT_{2A} receptors, TREO exhibits strong anxiolytic potential. It is well known that substances like β -Caryophyllene and linalool increase the activity of the serotonergic and GABAergic systems, which helps to reduce neuroendocrine activity in response to stress and promote relaxation. Moreover, substances like D-limonene, eucalyptol, and linalool have been shown to have neuroprotective effects by lowering neuroinflammation, boosting the expression of neurotrophins like BDNF, and preventing neuronal

apoptosis by controlling the expression of Bcl-2 and lowering caspase 3 and Bax. The defense of these nerve cells also involves signaling pathways like p38 MAPK.

Overall, these results show that TREO's many bioactive chemicals work in concert to provide a wide range of therapeutic benefits. TREO's multi-target and multi-mechanism approach supports both physical and mental health holistically and creates opportunities for growth as an evidence-based inhalation aromatherapy agent for the prevention and supplemental treatment of anxiety disorders, chronic inflammatory conditions, and neurodegenerative risks.

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Disclosure

The authors report no conflicts of interest in this work.

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