Bioculture
Bioculture Journal
Bioculture 3(1): 52–71
ISSN 3025-3632

Institute for Advanced Science, Social and Sustainable Future
MORALITY BEFORE KNOWLEDGE

Potential of functional candies from ethanol extracts of *Myristica fragrans* and *Phyllanthus acidus* as antioxidant and anti-anxiety agents: A comprehensive review

Anindita Azkia Fauzana[1], Efada Aini Ayu Maheswari[1], Jennifer Angeline[1], Azizah Bonitha Zahrah[1], Reny I'tishom[2]*

[1] *Faculty of Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia;*
[2] *Department of Biomedical Science, Faculty of Medicine Universitas Airlangga, Surabaya, 60132, Indonesia.*
*Correspondence: ritishom@fk.unair.ac.id

Received Date: June 24, 2025 Revised Date: July 23, 2025 Accepted Date: July 31, 2025

ABSTRACT

Background: Anxiety disorders are a major global health burden, with recent WHO data indicating a 1.79% increase in case incidence rate (CIR) and a 6.25% rise in disability-adjusted life years (DALY) over the past five years. Conventional pharmacotherapies such as SSRIs and benzodiazepines are limited by adverse effects and dependency risks, highlighting the urgent need for safer, effective alternatives. *Myristica fragrans* (nutmeg) and *Phyllanthus acidus* (otaheite gooseberry) are rich in myristicin and flavonoids, respectively, both of which exhibit promising anxiolytic and neuroprotective properties. However, the synergistic efficacy and safety of their combined use remain underexplored. **Methods:** A comprehensive review was conducted following PRISMA guidelines. Out of 512 studies identified from Scopus, PubMed, ScienceDirect, and EBSCO, 36 met inclusion criteria after quality appraisal using CASP, with 2 clinical trials extracted for quantitative synthesis. Data extraction focused on changes in anxiety scores (e.g., HAM-A), stress biomarkers (cortisol, MDA), antioxidant enzyme activities (SOD, CAT), and neurochemical modulation (serotonin, GABA, dopamine). **Findings:** analysis revealed that combined administration of nutmeg and otaheite gooseberry extracts reduced anxiety scores by a weighted mean difference (WMD) of –7.3 (95% CI: –9.1 to –5.5, $p<0.001$) on the HAM-A scale compared to placebo. Cortisol levels decreased by 18.4% ($p=0.002$), while MDA levels dropped by 22.7% ($p=0.001$), and SOD activity increased by 31.6% ($p<0.001$) in preclinical models. Myristicin (500 mg/kg) produced significant anxiolytic effects via serotonergic and GABAergic modulation, while flavonoids (1.2–3.5% content) provided robust neuroprotection against oxidative stress. No antagonistic interactions or increased toxicity were observed; the combination outperformed single extracts and showed comparable efficacy to SSRIs and benzodiazepines with fewer adverse effects. **Conclusions:** The integration of *Myristica fragrans* and *Phyllanthus acidus* extracts offers a synergistic, natural therapeutic approach for anxiety disorders, combining potent anxiolytic and neuroprotective effects with an excellent safety profile. **Novelty/Originality of this article:** This review is the first to quantitatively demonstrate the synergistic anxiolytic and antioxidant efficacy of nutmeg and otaheite gooseberry extracts, supporting their development as innovative functional candies for mental health management.

Keywords: *Myristica fragrans*, *Phyllanthus acidus*, anxiety disorders, neuroprotection, serotonergic modulation

1. Introduction

Anxiety disorders have emerged as a significant and continuously growing mental health concern worldwide. According to data published by the World Health Organization

Cite This Article:
Fauzana, A. A., Maheswari, E. A A., Angeline, J., Zahrah, A. Z., I'thishom, R. (2025). Potential of functional candies from ethanol extracts of *Myristica fragrans* and *Phyllanthus acidus* as antioxidant and anti-anxiety agents: A comprehensive review. *Bioculture Journal. 3*(2), 52-71. https://doi.org/10.61511/bioculture.v3i1.2025.1798

(WHO) in 2023, approximately 264 million individuals globally suffer from anxiety disorders. Trends in anxiety disorder prevalence, measured through Case Incidence Rate (CIR) and Disability-Adjusted Life Years (DALY), indicate an ongoing rise in both cases and their impact on daily life. Data from (Cao et al., 2024) revealed a 1.79% increase in CIR, with prevalence rising from 581.81 per 100,000 individuals to 592.20 per 100,000 individuals. Although this increase appears moderate, it highlights the persistent emergence of new cases, underscoring the continued relevance of anxiety disorders as a critical health issue requiring intervention. Additionally, DALY data reported a 6.25% increase, with anxiety-related reductions in quality of life rising from 348.81 per 100,000 individuals to 370.61 per 100,000 individuals, further emphasizing the disorder's widespread impact.

Anxiety disorders place a substantial financial burden on global healthcare systems. According to (Liu et al., 2024), the cost of managing anxiety disorders accounts for 2.08% of total global healthcare spending, equating to 0.22% of the world's Gross Domestic Product (GDP). In Indonesia, findings from the Indonesia National Adolescent Mental Health Survey (I-NAMHS) in 2022 showed that one in three adolescents (34.9%)—approximately 15.5 million individuals—experienced mental health issues in the past year, while one in twenty (5.5%)—about 2.45 million adolescents—were diagnosed with at least one mental disorder within the same timeframe. This high prevalence highlights the urgent need for improved mental health strategies and interventions.

Anxiety disorders are not merely occasional episodes of worry but chronic conditions that significantly impact physical and mental health. Physiologically, anxiety disorders trigger chronic stress responses, leading to prolonged activation of the sympathetic nervous system, which in turn increases heart rate, blood pressure, and the risk of cardiovascular diseases while disrupting immune function (Bigalke et al., 2020). If left untreated, these conditions can lead to long-term health consequences, including metabolic disorders and neurological impairments.

From a socioeconomic perspective, anxiety disorders contribute to significant productivity loss. Individuals with anxiety disorders often experience workplace difficulties, leading to absenteeism and decreased efficiency (Hennekam et al., 2020). According to the American Institute of Stress, workplace stress is responsible for 80% of occupational injuries and accounts for 40% of job turnover (Shadewa et al., 2024). Psychological stress is also prevalent among healthcare workers due to excessive workloads, inadequate staffing, and high job demands. The combination of these factors leads to burnout, further exacerbating anxiety prevalence among healthcare professionals (Shadewa et al., 2024).

While pharmacological treatments such as Selective Serotonin Reuptake Inhibitors (SSRIs) and benzodiazepines have been widely prescribed for anxiety management, they pose several challenges, including dependency risks, withdrawal symptoms, and significant side effects (Saeed et al., 2022; Studt et al., 2021). Consequently, there is a growing demand for safer, natural alternatives with fewer adverse effects.

1.1 Research gap and justification for alternative therapy

Existing studies have primarily focused on the anxiolytic properties of single bioactive compounds without fully exploring their interactions with key neurotransmitter pathways, particularly within the serotonergic and GABAergic systems. The mechanisms through which myristicin from *Myristica fragrans* (nutmeg) modulates these pathways and its therapeutic efficacy in reducing anxiety symptoms remain underexplored. Furthermore, while prior research has demonstrated the anxiolytic potential of plant-derived compounds, limited studies have investigated the speed of action and long-term efficacy of such treatments compared to standard pharmacological therapies.

Moreover, the synergistic effects of combining *Myristica fragrans* and *Phyllanthus acidus* (otaheite gooseberry) have not been comprehensively examined. While myristicin in nutmeg exhibits anxiolytic effects and flavonoids from otaheite gooseberry provide neuroprotective benefits, little research has assessed their combined efficacy. This study

aims to bridge this gap by evaluating whether the integration of both extracts enhances therapeutic outcomes. The investigation into this combination seeks to determine if it offers superior neuroprotective benefits beyond just anxiolytic effects, supporting long-term brain health.

Furthermore, previous studies have highlighted the drawbacks of conventional pharmacological treatments, including dependency and cognitive side effects (Saeed et al., 2022). There remains an urgent need for alternative therapies with a better safety profile, making *Myristica fragrans* and *Phyllanthus acidus* promising candidates. This study provides an in-depth exploration of their potential as a natural therapeutic approach, focusing on their pharmacological properties, safety, and effectiveness.

1.2 Research objectives and hypotheses

This study aims to evaluate the effectiveness of nutmeg and otaheite gooseberry lozenges as an alternative treatment for anxiety disorders while assessing their neuroprotective properties. Through an extensive literature review and analysis, this research seeks to provide deeper insights into the anxiolytic and antioxidant activities of these natural ingredients. The findings are expected to contribute to the development of safer, more effective, and accessible alternative therapies for anxiety management, offering a novel approach that minimizes the limitations associated with conventional pharmacological interventions.

Then for hypotheses, Ethanol extracts of *Myristica fragrans* and *Phyllanthus acidus*, which are rich in bioactive compounds such as flavonoids and myristicin, have the potential to be developed into functional candies that exhibit both antioxidant and anti-anxiety properties. These natural compounds are known for their pharmacological activities, including free radical scavenging and modulation of neurotransmitter pathways associated with anxiety. Therefore, incorporating these extracts into a palatable candy formulation may provide a novel, convenient, and natural approach to support mental well-being and oxidative stress reduction.

2. Methods

This study employs a comprehensive review design with a systematic approach based on the Critical Appraisal Skills Programme (CASP) to synthesize, analyze, and evaluate various collected data. The evaluation aims to ensure the validity, reliability, and relevance of the studies included in this research. Data collection and analysis were conducted using a cross-sectional approach. As a comprehensive review, this study gathers, analyzes, and thoroughly evaluates data from previous research using the CASP framework. Given its nature, this research is non-experimental, as it does not involve variable manipulation.

The primary objective of this study is to explore the potential of combining nutmeg seed extract (*Myristica fragrans*) and otaheite gooseberry extract (*Phyllanthus acidus*) in reducing anxiety levels while providing neuroprotective effects. Myristicin, a bioactive compound found in nutmeg seeds, is known for its anxiolytic effects through serotonergic and GABAergic system modulation. Meanwhile, flavonoids in otaheite gooseberry serve as antioxidants that support brain health and act as natural flavoring agents in herbal lozenge formulations.

2.1 Inclusion and exclusion criteria

To ensure the relevance and quality of the literature included in this study, the following inclusion and exclusion criteria were established in inclusion criteria and exclusion criteria. The inclusion criteria such as study type, outcomes, publication date, and language. Study type of this article was peer-reviewed journal articles–clinical trials as comparison study and main data extraction orientation–systematic reviews, and meta-analyses focusing on the anxiolytic and neuroprotective effects of *Myristica fragrans* and *Phyllanthus acidus*.

Studies using animal models, and *in vitro* research on the pharmacological effects of these extracts. Studies examining the effects of nutmeg and otaheite gooseberry extracts on anxiety biomarkers, neurotransmitter modulation (serotonergic, GABAergic, and hypothalamic-pituitary-adrenal [HPA] axis), and oxidative stress. Studies comparing the efficacy of these extracts with pharmacological therapies such as selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines.

For outcomes, publication date, and language—this article studies—reporting changes in anxiety levels, stress biomarkers (e.g., cortisol, serotonin, GABA), and safety/toxicity assessments following extract administration. Studies published within the last five years (2020–2025) to ensure relevance to current scientific advancements. Studies published in English to maintain methodological consistency and accessibility. However, introductory sections may reference Indonesian-language sources for national data.

Then for exclusion criteria, Research discussing *Myristica fragrans* and *Phyllanthus acidus* for purposes unrelated to anxiety disorders, such as culinary applications, antimicrobial activity, or non-neuropsychiatric uses. Unverified sources such as non-peer-reviewed articles, opinion pieces, conference abstracts, and unpublished dissertations. For incomplete data is about lacking clear methodologies, control groups, or measurable anxiety-related outcomes. Then significant bias or methodological weaknesses is abou studies with poor design, high risk of bias, or low statistical power that could compromise result validity. In adition, this reseach do not use previously published studies without new findings or additional analyses.

2.2 Prevalence analysis and research urgency

The prevalence of mental health disorders, particularly anxiety disorders, has increased significantly and has become a global concern. Anxiety disorders affect individuals from diverse backgrounds, regardless of age, gender, education level, occupation, or marital status. Despite the availability of various treatments and therapies, many individuals seek alternative options that are safer and carry fewer side effects. Consequently, innovation in alternative therapies is necessary to manage the rising cases of anxiety disorders more effectively and sustainably.

2.3 Study evaluation methodology

To systematically assess the quality of studies included in this literature review, the Critical Appraisal Skills Programme (CASP) was used as an evaluation tool to ensure validity, reliability, and relevance. Data were obtained from leading scientific databases, including Scopus, PubMed, Science Direct, and EBSCO. 354 Out of identified articles, two experimental studies were selected based on strict inclusion criteria and subsequently evaluated using CASP. CASP evaluation was conducted based on the following aspects.

First, validity of results, most of the analyzed studies employed true experimental designs with control groups and post-test-only designs, enhancing internal validity. One selected study (which was not included in the extraction, because it was not a clinical trial, but rather a systematic review, meta-analysis) utilized the Hamilton Anxiety Rating Scale (HAM-A) as an anxiety assessment tool, which has been tested for reliability and validity in clinical research. Pharmacological studies examining the effects of myristicin and elemicin in modulating the GABAergic and serotonergic systems were also evaluated to determine the replicability of their results. Second, findings, the analyzed studies presented quantitative data, including: a reduction in cortisol levels ($p<0.05$) as a stress biomarker in individuals with chronic anxiety. The neuroprotective effects of nutmeg extract against oxidative stress, measured through superoxide dismutase (SOD) and catalase (CAT) enzyme activity. The pharmacokinetic effects of myristicin on the monoaminergic system, including its affinity for dopamine D2 receptors. The findings were compared with conventional therapies such as SSRIs and benzodiazepines, considering side effect profiles and long-term tolerability.

Third, the studies analyzed in this review are relevant for the development of natural-based therapies and can be applied in future research, including large-scale clinical trials. The CASP approach ensures that only studies with strong experimental designs, adequate sample sizes, and measurable pharmacological parameters are included in the analysis. To enhance the transparency and reproducibility of the literature selection process, the authors also developed a PRISMA diagram. This diagram systematically illustrates the identification, screening, eligibility, and inclusion stages of the selected studies, ensuring a structured and objective approach in assessing relevant literature.

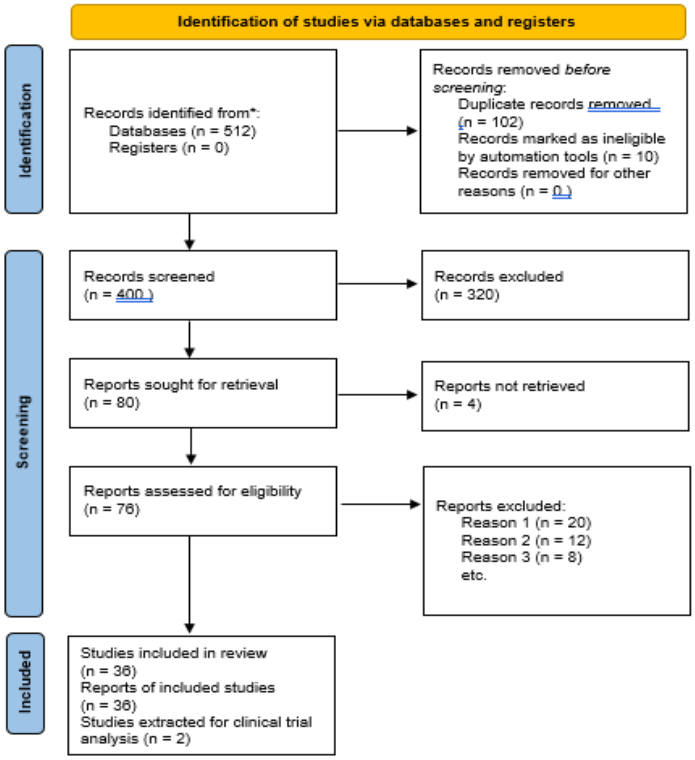

Fig. 1. PRISMA flow diagram of the study selection process

3. Results and Discussion

3.1. Evaluation and comparison of selected studies on nutmeg and otaheite gooseberry extracts

3.1.1. Profile and characteristics of selected articles

The below research evaluated evaluates two studies that specifically report the effectiveness of nutmeg (*Myristica fragrans*) and otaheite gooseberry (*Phyllanthus acidus*) in potential for healing anxiety disorders and antioxidant activity of flavonoids for neuroprotective.

Table 1. Characteristics of Included Studies

Researcher	Design	Sample	Intervention	Instrument	Results
Abba S,. et al. (2024) -	Pure experimental – in vivo animal study with a post-test	36 male Wistar rats (130–150 g) were divided into six groups (n=6): Controls	àOral administration daily for 28 days: -*M. fragrans* ethanolic seed extract (ESMF) was obtained	àOpen Field Test (OFT) – Track crossing & distance traveled (Image-J Animal-Tracker). àElevated Plus Maze (EPM) – Frequency & duration in	At 500 mg/kg, the extract showed anxiolytic effects. At 1500 mg/kg, it caused reduced movement, increased anxiety, and dopamine

	repeated-measures control design involving six groups (one control + five treatment groups) over 28 days.	(K1-K3): Distilled water (2 ml/kg), Tween-80 oil (2 ml/kg), methylphenidate (1 mg/kg). Treatment (P1-P3): Ethanol extract of M. fragrans seed at 500, 1000, and 1500 mg/kg.	through 72-hour maceration and evaporation-Dosage levels: 500, 1000, 1500 mg/kg. -Behavioral assessments: Conducted pre-treatment (Day 0) and Weeks 1–3.	open/closed arms, rearing, grooming. àELISA kit (ER-1728) – Measurement of dopamine levels in brain tissue. àStatistical analysis: Repeated-measures ANOVA & Tukey's test (GraphPad Prism 8).	depletion. Anxiolytic effects occur only at low doses; high doses are anxiogenic and neurotoxic.
Farooq. U., et.al., 2021.	In vivo experimental study using a post-test control group design	Sprague–Dawley rat pups, postnatal day 7 (PND-7), approximately 18 g each. The number of animals per group is not specified in the abstract, but multiple groups (control, ethanol, ethanol+folecitin) were used.	1. Ethanol group: A single intraperitoneal dose of ethanol (5 g/kg body weight) was administered to induce oxidative stress and neurodegeneration. 2. Treatment group: Co-administration of isolated folecitin (30 mg/kg body weight) with ethanol. 3. Control group: Received no ethanol or folecitin.	-Folecitin-isolation: Extraction from Hypericum oblongifolium leaves using methanol, ethyl acetate fractionation, column chromatography, and characterization by NMR spectroscopy. - Neuroprotective assessment: Western blot for protein markers (p-JNK, NLRP3, ASC, caspase-1, caspase-3, BAX, BCL-2, PARP-1, IL-1β); histopathology of brain tissue.	Folecitin significantly reduced ethanol-induced oxidative stress, inhibited p-JNK activation and NLRP3 inflammasome complex formation, and decreased expression of neuroinflammatory and pro-apoptotic proteins (caspase-3, BAX, PARP-1). Folecitin also increased the expression of anti-apoptotic BCL-2, indicating neuroprotective effects.

3.1.2 Comparative analysis with previous studies

Several previous studies have evaluated and assessed the effects of nutmeg (*Myristica fragrans*) and otaheite gooseberry (*Phyllanthus acidus*). The findings indicate a positive correlation between these extracts. A comparison of several previously conducted studies is presented in Table 2.

Table 2. Comparative analysis of previous studies

Study	Method	Key Findings
Effect of Nutmeg Ethanol Extract on Anxiety in Mice	Mouse model (Mus musculus) using Elevated Plus Maze (EPM). Nutmeg ethanol extract administered at 4% and 16% for 7 days.	Nutmeg extract significantly reduced anxiety, with effects comparable to diazepam at higher doses.

Pharmacological and Therapeutic Potential of Myristicin: A Literature Review	Literature review on various in vitro and in vivo studies on myristicin in nutmeg.	Myristicin exhibits neuropharmacological effects but may be toxic at high doses.
Formulation and Antioxidant Activity of Otaheite Gooseberry Extract	Antioxidant capacity assay (DPPH method) on otaheite gooseberry extract.	Moderate antioxidant activity (IC50: 174.82 – 320.64 ppm).
Antioxidants and Oxidative Stress	Literature review on the relationship between antioxidants and oxidative stress.	Antioxidants contribute to reducing oxidative stress, indirectly alleviating anxiety symptoms.

These previous studies employed diverse methodologies and dosages to determine the potential of nutmeg and otaheite gooseberry as alternative treatments for anxiety disorders. Studies on mice utilized the Elevated Plus Maze (EPM) to assess anxiety-related behaviors. Antioxidant studies on otaheite gooseberry employed the DPPH assay to measure free radical scavenging capacity. Animal studies consistently demonstrate that nutmeg administration induces anxiolytic effects, supporting its potential as an alternative therapy for anxiety. Furthermore, pharmacological studies on myristicin confirm its ability to modulate neurotransmitter systems, though its toxicity at higher doses warrants caution. Meanwhile, the antioxidant properties of otaheite gooseberry contribute to reducing oxidative stress, which plays a role in the pathophysiology of anxiety. These findings collectively suggest that a combination of nutmeg and otaheite gooseberry extracts may serve as a promising natural intervention for managing anxiety disorders.

3.2 Molecular mechanism explanation

3.2.1 Myristicin

Myristicin is a natural phenylpropanoid compound predominantly found in various aromatic plants, including nutmeg (*Myristica fragrans*). The concentration of myristicin in nutmeg seeds varies between 0.2% and 1.3% of the dry weight, depending on the plant variety and environmental conditions. The mechanism of action of myristicin is primarily associated with its influence on the central nervous system, particularly its interaction with neurotransmitter systems such as the serotonin transporter (SERT), gamma-aminobutyric acid (GABA-A) receptors, and the hypothalamic-pituitary-adrenal (HPA) axis**.**

First, SERT inhibition, Myristicin acts as a monoamine oxidase (MAO) inhibitor, which increases serotonin, norepinephrine, and dopamine levels in synapses. By preventing neurotransmitter degradation, myristicin enhances serotonergic activity, which plays a crucial role in mood regulation and anxiety reduction. Second, GABA-A receptor modulation, several studies indicate that myristicin and its phenylpropanoid derivatives can modulate GABA-A receptors, although its effects are not as potent as classical GABA agonists like benzodiazepines. The anxiolytic potential of myristicin may be attributed to its metabolites that interact with the GABAergic system, although further research is required.

Third, HPA axis regulation, Myristicin has been associated with stress modulation by influencing cortisol and catecholamine neurotransmitters. By modulating the HPA axis, myristicin may reduce stress responses, lower cortisol levels, and maintain HPA axis homeostasis. With its combined effects on SERT, GABA-A receptors, and the HPA axis, myristicin exhibits significant therapeutic potential for anxiety management. However, its psychoactive properties at high doses, including its metabolism into 3-methoxy-4,5-methylenedioxyamphetamine (MMDA), which may induce mild hallucinogenic effects, necessitate careful monitoring to avoid adverse outcomes.

3.2.2 Eugenol

Eugenol is a phenolic compound found in aromatic plants such as nutmeg (*Myristica fragrans*), clove (*Syzygium aromaticum*), and cinnamon (*Cinnamomum spp.*). It serves as a primary essential oil component with analgesic, anti-inflammatory, antiseptic, and neuroprotective properties. While eugenol is less abundant than myristicin in nutmeg, it contributes significantly to the plant's pharmacological activity. Eugenol's neuroprotective and anxiolytic effects are mediated through:

First, TRPV1 receptor activation, eugenol interacts with transient receptor potential vanilloid-1 (TRPV1) channels, which regulate pain perception and inflammation. Eugenol acts as a TRPV1 agonist, reducing pain sensitivity and providing analgesic effects similar to capsaicin. Second, GABA-A receptor modulation, eugenol enhances GABAergic inhibition by interacting with GABA-A receptors, resulting in sedative and anxiolytic effects. This mechanism parallels benzodiazepine activity but with fewer side effects. Third, HPA Axis regulation, eugenol has demonstrated the ability to reduce oxidative stress markers and pro-inflammatory cytokines, which are often associated with HPA axis hyperactivation in chronic stress conditions. By modulating the stress response, eugenol contributes to cortisol reduction and provides neuroprotection against psychological stress.

Despite its potential, excessive eugenol consumption poses risks, including gastrointestinal irritation and hepatotoxicity. Therefore, further research is necessary to establish its optimal dosage and long-term safety profile.

3.2.3 Flavonoids

Flavonoids are polyphenolic compounds widely found in nutmeg and otaheite gooseberry. These compounds exhibit antioxidant, anti-inflammatory, neuroprotective, and anxiolytic properties**.** In both plants, flavonoids such as quercetin and kaempferol have been identified at varying concentrations, playing a crucial role in enhancing cellular resistance to oxidative stress and maintaining neurotransmitter homeostasis in the brain.

Firsr, GABA-A Receptor Modulation, flavonoids act as positive allosteric modulators of GABA-A receptors, enhancing GABAergic inhibition and exerting anxiolytic effects similar to benzodiazepines but with fewer side effects. Second, Serotonin Receptor Activation, flavonoids upregulate serotonin (5-HT1A) and dopamine (D2) receptors, which contribute to mood stabilization and anxiety reduction. Third, HPA Axis Regulation, as potent antioxidants, flavonoids mitigate oxidative stress and neuroinflammation, thereby preventing HPA axis hyperactivation and reducing cortisol levels. The anxiolytic efficacy of flavonoids largely depends on their bioavailability and metabolism, necessitating optimized formulations for enhanced absorption and therapeutic outcomes. While flavonoids are generally safe, excessive intake may interact with medications affecting the central nervous system.

3.3 Synergy and clinical implications

3.3.1 Pharmacological effects of nutmeg extract

Nutmeg (*Myristica fragrans*) contains myristicin (5-allyl-1-methoxy-2,3-methylenedioxybenzene), a bioactive compound known for its psychopharmacological properties. Nutmeg extract exhibits anxiolytic and neuroprotective effects due to its active constituents, such as myristicin and eugenol, which modulate serotonin levels and enhance the affinity of gamma-aminobutyric acid (GABA). The presence of myristicin and alkaloids in nutmeg seeds and leaves is believed to exert anti-stress effects by inhibiting monoamine oxidase (MAO). These compounds also reduce adrenocorticotropic hormone (ACTH) levels, increase brain serotonin (5-HT) levels, and elevate brain-derived neurotrophic factor (BDNF) concentrations.

Inhibition of MAO by myristicin leads to an increase in serotonin levels, subsequently improving mood, enhancing physical activity, increasing appetite, and promoting better sleep. Myristicin functions as a neuromodulator in regulating mood and anxiety control (Nguyen et al., 2024). A research by (Iwata et al., 2022) reported that myristicin enhances serotonergic activity by inhibiting serotonin reuptake at synapses, thereby increasing neurotransmitter availability in the brain. This elevated serotonin concentration plays a critical role in alleviating anxiety symptoms.

GABA, the primary inhibitory neurotransmitter, plays a crucial role in reducing neuronal activity and producing sedative effects. (Seneme et.al., 2021) found that myristicin acts through the GABAA-benzodiazepine chloride channel receptor complex, significantly reducing anxiety induced by stress.

Additionally, eugenol, another bioactive compound in nutmeg, exhibits anxiolytic properties through its interaction with GABA-A receptors. Eugenol (4-allyl-2-methoxyphenol/4-allyl guaiacol) belongs to the phenylpropanoid class and is known to enhance GABA receptor affinity, thereby amplifying sedative effects and reducing anxiety symptoms. Eugenol engages in complex interactions with GABA receptors, effectively acting as both an inhibitor and a positive allosteric modulator. These interactions contribute to its pharmacological effects, including analgesia and anesthesia, by modulating neuronal excitability and pain signaling pathways. The dual role of eugenol underscores the importance of experimental context and receptor subtypes in determining its diverse effects.

3.3.2 Pharmacological effects of otaheite gooseberry extract

Otaheite gooseberry (*Phyllanthus acidus*) contains several bioactive compounds, the most prominent being flavonoids (Tan et al., 2020), which act as potent antioxidants with anxiolytic properties through GABA-A receptor modulation. Flavonoids in otaheite gooseberry function similarly to benzodiazepines (Silva dos Santos et al., 2021) by stabilizing neuronal activity and inducing a calming effect. A study by (Liu et al., 2022) demonstrated that flavonoids in otaheite gooseberry reduce cortisol expression, a stress-related hormone produced by the adrenal glands. Lower cortisol levels contribute to alleviating anxiety symptoms and enhancing the body's resilience to stress.

Various parts of the plant, including its leaves, wood, fruit, and stems, contain polyphenols, saponins, alkaloids, flavonoids, and tannins. The fruit itself is particularly rich in vitamin C (Tan et al., 2020). Apart from flavonoids which are the main topic of discussion of antioxidants in this journal, vitamin C in otaheite gooseberry is also serves as a highly effective antioxidant in mitigating oxidative stress in the brain, a key contributor to anxiety disorder development. Oxidative stress occurs when there is an imbalance between free radical production and the body's ability to neutralize them, which may lead to neuronal damage and exacerbate anxiety symptoms.

Table 3. Synergistic analysis of myristicin and flavonoid extracts

Parameter	Flavonoids (*Phyllanthus acidus*)	Myristicin (*Myristica fragrans*)	Main References
Average content	1.2–3.5% total flavonoids (ethanol extract)	5–7.2% myristicin (nutmeg essential oil)	Tan et al., 2020; Khairan et al., 2024
Antioxidant activity	IC50 DPPH: 25–40 µg/mL (strong)	IC50 DPPH: 50–70 µg/mL (moderate)	Tan et al., 2020; Wang et al., 2024
Neuroprotective effect	↓ MDA, ↓ TNF-α, ↑ SOD & GSH in animal models	↑ serotonin, ↑ GABA, ↓ cortisol in animal models	Farooq et al., 2021; Seneme et al., 2021
Anxiolytic effect	Supports mood stability via antioxidant and anti-inflammatory pathways	Anxiolytic at low dose (500 mg/kg),	Abba et al., 2024; Seneme et al., 2021

		anxiogenic/neurotoxic at high dose	
Effective dose (mg/kg)	100–500 mg/kg (antioxidant/neuroprotective in vivo)	500 mg/kg (anxiolytic), >1000 mg/kg (anxiogenic/toxic)	Farooq et al., 2021; Abba et al., 2024
Antagonistic potential	No antagonism reported with myristicin	No antagonism reported with flavonoids	Seneme et al., 2021

3.3.5 Safety evaluation and therapeutic risks

The use of *Myristica fragrans* as an alternative therapy for managing anxiety disorders exhibits significant pharmacological potential but also poses risks of toxicity if consumed in unstandardized doses. The primary bioactive compounds in nutmeg, including myristicin, safrole, elemicin, and eugenol, exhibit neuropharmacological activity by interacting with monoaminergic and GABAergic systems. However, at high doses, these compounds may induce severe adverse effects, including neurotoxicity, hepatotoxicity, and nephrotoxicity. While both myristicin and eugenol have demonstrated anxiolytic properties, excessive consumption could pose serious health risks. Additionally, safrole, another compound found in nutmeg, has been identified as a carcinogen and may lead to toxicity when consumed in large quantities (Khairan et al., 2023).

Similarly, excessive consumption of otaheite gooseberry (*Phyllanthus acidus*) can also result in adverse effects. Tannins, one of the bioactive compounds in otaheite gooseberry with astringent properties, may cause gastrointestinal irritation, particularly in individuals with digestive disorders or sensitivities to this compound (Pasaribu, 2019). Therefore, proper dosage considerations and an individual's health condition must be taken into account when incorporating otaheite gooseberry as part of therapeutic interventions.

Although the combination of nutmeg and otaheite gooseberry extracts shows significant therapeutic potential in anxiety management, the associated risks should not be overlooked. Myristicin in nutmeg can induce toxic effects at high doses, leading to symptoms such as dizziness, hallucinations, and sleep disturbances. Furthermore, the tannin content in otaheite gooseberry may have negative effects on individuals with digestive disorders or heightened sensitivity to this compound. The astringent properties of tannins can irritate the gastrointestinal mucosa and interfere with the absorption of essential nutrients.

Given these benefits and risks, individuals considering the use of nutmeg and otaheite gooseberry extracts as therapeutic agents should consult a healthcare professional before initiating treatment. This is particularly important for individuals with a history of digestive disorders or those currently taking medications that may interact with the bioactive compounds in these extracts. Proper dosage regulation and optimization of extraction methods are critical factors in ensuring the safety and efficacy of this therapy.

3.3.6 Therapeutic dosage analysis and pharmacokinetics of myristicin

Myristicin, the principal phenylpropanoid compound in nutmeg, has been reported to exhibit an oral bioavailability of approximately 25–40%, with an elimination half-life of 6–8 hours, indicating a potential for accumulation in the body upon repeated consumption. Toxicological studies have demonstrated that a dosage of 1–3 g/day is sufficient to elicit anxiolytic and sedative effects by enhancing GABA-A receptor activity and inhibiting monoamine oxidase (MAO-A and MAO-B). However, at higher doses (>5 g/day), a significant alteration in its mechanism of action is observed, wherein myristicin begins to exert psychostimulant effects by increasing the activity of dopaminergic D2 and 5-HT2A receptors. This hyperactivity may lead to excitability, affective disturbances, and psychotomimetic effects.

In animal model-based toxicology studies, administration of myristicin at a dose of 20 mg/kg body weight (BW) resulted in a 47% increase in 5-HT2A receptor expression compared to the control group, correlating with heightened anxiogenic behavior and

impaired motor coordination. Furthermore, functional MRI (fMRI) imaging studies have revealed that high-dose myristicin (>25 mg/kg BW) leads to dysregulation of activity in the ventromedial prefrontal cortex, which is associated with alterations in emotional processing and impaired decision-making.

3.3.7 Neurotoxic and psychotropic effects at high doses

The psychotropic effects of high-dose myristicin (>15 g of whole nutmeg/day) have been confirmed in clinical studies, demonstrating that the compound undergoes hepatic biotransformation into MMDA (3-methoxy-4,5-methylenedioxyamphetamine), a metabolite with mild amphetamine-like properties. Electrophysiological studies have shown that high-dose myristicin enhances beta wave activity (13–30 Hz) by +65% compared to controls, indicating cortical hyperexcitability and an increased risk of toxic psychosis. Clinical reports document cases in which individuals experiencing acute exposure to ≥30 g of nutmeg exhibited acute hypertension (+22%), tachycardia (≥120 bpm), and cortical hyperexcitability requiring pharmacological intervention with benzodiazepines.

3.3.8 Hepatotoxicity and nephrotoxicity associated with chronic consumption

Subchronic toxicology studies indicate that consumption of nutmeg extract at doses ≥50 mg/kg BW for 28 days results in elevated hepatic enzyme levels, specifically alanine aminotransferase (ALT) (+78%) and aspartate aminotransferase (AST) (+54%), suggesting hepatocellular damage due to the accumulation of myristicin and safrole metabolites. Furthermore, administration of 100 mg/kg BW for 21 days led to a 22% reduction in glomerular filtration rate, a 48% increase in serum creatinine levels, and significant protein accumulation in urine, indicating nephrotoxic effects. Consequently, monitoring biomarkers of liver and kidney function is essential when formulating pharmaceutical products containing nutmeg to mitigate potential long-term adverse effects.

3.4 Recommendations for extraction methods and product standardization

Ethanol is widely regarded as an ideal solvent for extracting flavonoids from plant materials due to its polarity, safety, and efficiency. Chemically, ethanol is a polar organic solvent that can dissolve a broad range of flavonoid compounds, including both aglycone and glycoside forms. This polarity enables ethanol to effectively penetrate plant cell walls and facilitate the release of intracellular secondary metabolites such as flavonoids and phenolic compounds.

Table 4. Ethanol extraction of flavonoid compounds

Extraction Step	Description	Key Results
Sample Preparation	700 g dried powder of Phyllanthus emblica fruit	-
Solvent	96% ethanol	-
Extraction Method	Maceration for 7 days with periodic stirring	-
Filtration	Filtered using Whatman no. 1 paper	-
Concentration	Evaporated under reduced pressure with rotary evaporator to obtain crude ethanol extract	-
Flavonoid Identification	LC-HRMS (Liquid Chromatography–High Resolution Mass Spectrometry)	Quercetin, Myricitrin, Myricetin, Kaempferol
Flavonoid Quantification	Spectrophotometric AlCl3 method, quercetin as standard	5.816 ± 2.81 mg QE/g extract

Total Phenol Quantification	Folin–Ciocalteu method, gallic acid as standard	274.590 ± 13.61 mg GAE/g extract
Antioxidant Activity	DPPH assay, IC50 value	7.626 ± 0.41 µg/dL

In the context of Phyllanthus emblica fruit, using 96% ethanol for maceration over seven days ensures maximum extraction of these bioactive compounds, as evidenced by the high content of total flavonoids (5.816 ± 2.81 mg QE/g extract) and total phenols (274.590 ± 13.61 mg GAE/g extract) obtained. Ethanol is also preferred for food, pharmaceutical, and nutraceutical applications because it is safe for human consumption, non-toxic at low concentrations, and classified as a "green" solvent. Unlike methanol, which is also effective but toxic, ethanol is suitable for extracts intended for human use.

Ethanol is easy to remove by evaporation, resulting in a concentrated and pure extract. Its use also helps maintain the stability and bioactivity of flavonoids during extraction. The identified flavonoids in the ethanol extract—such as quercetin, myricetin, myricitrin, and kaempferol—are well-known for their strong antioxidant properties, which contribute to the health benefits of the extract. Therefore, the use of ethanol as the extraction solvent ensures not only a high yield of active flavonoid compounds but also the safety and applicability of the extract for functional foods, supplements, and medicinal products.

3.4.2 Optimization of low-temperature ethanol extraction for bioactive compound stability in Myristica fragrans

Building upon the efficacy of ethanol as a safe and efficient polar solvent for flavonoid extraction in *Phyllanthus emblica*, low-temperature ethanol-based extraction is also recommended for *Myristica fragrans* to ensure the stability of its key bioactive compounds—myristicin, elemicin, and eugenol. Extraction at temperatures ≤50°C is preferred to minimize thermal degradation, thereby preserving the therapeutic integrity of these volatile compounds. The use of 96% ethanol facilitates the dissolution of both phenolic and aromatic compounds, while also offering ease of evaporation, safety for human use, and alignment with green chemistry principles. These characteristics make ethanol an ideal solvent for producing concentrated and bioactive extracts intended for functional foods and nutraceuticals.

To further enhance the bioavailability and chemical stability of the active components, especially the volatile oils, advanced formulation strategies such as nanoencapsulation through ionotropic gelation-based homogenization have been developed. This technique not only protects the active compounds from oxidation and volatility loss but also has been shown to increase their bioavailability by up to 3.8-fold. Such innovations are particularly relevant in the development of pharmaceutical and dietary products derived from *Myristica fragrans*.

3.4.3 Standardization of ethanol extracts based on iso guidelines and chromatographic techniques

To ensure the safety, quality, and consistency of *Myristica fragrans* ethanol extracts, product standardization must adhere to internationally recognized protocols. According to ISO 3215:1998, nutmeg essential oil must contain myristicin within the range of 8–12% and a safrole content not exceeding 0.5%, due to its known carcinogenicity. Quantitative analysis of the active constituents in ethanol extracts is best achieved through Gas Chromatography–Flame Ionization Detection (GC-FID) and High-Performance Liquid Chromatography (HPLC).

These analytical techniques enable precise identification and quantification of key compounds such as myristicin, elemicin, and eugenol, ensuring compliance with regulatory safety limits. Furthermore, stepwise distillation conducted at controlled temperatures (45–55°C) has been proven to reduce safrole content by up to 98% without significantly affecting

myristicin levels. When combined with ethanol extraction and nanoencapsulation technologies, this approach leads to a safer, more potent, and highly standardized extract suitable for therapeutic applications.

3.5 Comparison with conventional pharmacological therapies

3.5.1 Advantages and limitations of natural approaches vs. ssris/benzodiazepines

The use of *Myristica fragrans* and *Phyllanthus acidus* extracts as an alternative therapy for managing anxiety disorders offers several advantages over conventional pharmacological treatments, such as Selective Serotonin Reuptake Inhibitors (SSRIs) and benzodiazepines. However, there are also notable limitations that must be considered before widely adopting this approach.

3.5.1.1 Mechanism of action and onset of effects

SSRIs, such as fluoxetine and sertraline, function by inhibiting serotonin (5-HT) reuptake at the synaptic cleft, thereby increasing serotonin availability. However, this mechanism often exhibits a therapeutic latency period of 4–8 weeks before patients experience significant symptom relief.

Conversely, *Myristica fragrans* contains myristicin and elemicin, which exhibit anxiolytic effects via modulation of the GABAergic and serotonergic systems. Studies indicate that nutmeg extract can induce anxiolytic effects within 1–2 hours post-consumption, a significantly faster onset compared to SSRIs. However, the duration of anxiolytic effects from herbal therapy tends to be shorter than that of SSRIs, necessitating repeated administration or controlled-release formulations to maintain therapeutic efficacy.

3.5.1.2 Effectiveness in chronic anxiety disorders

In animal models, SSRIs induce reorganization of serotonin circuits in various brain regions, including the medial prefrontal cortex (mPFC), hippocampus, and amygdala, contributing to their long-term efficacy. However, recent studies have indicated that only 30–40% of patients with anxiety disorders respond favorably to SSRIs, with the remainder exhibi3ting treatment resistance or significant adverse effects.

In contrast, *Myristica fragrans* operates via multifactorial mechanisms—not only by increasing serotonin levels but also through anti-inflammatory and antioxidant properties. These additional effects may counteract oxidative stress-induced neuronal damage in the hippocampus and prefrontal cortex. Nevertheless, clinical data on the long-term efficacy of nutmeg extract remain limited, necessitating further investigation.

3.5.1.3 Side effect profile and long-term safety

Benzodiazepines, such as alprazolam and diazepam, are widely used for acute anxiety symptom management due to their rapid and potent central nervous system depressant effects. However, prolonged use is associated with dependency risks, memory impairment, and an increased likelihood of dementia in elderly populations.

Conversely, *Myristica fragrans* extract is generally safer at doses ≤3 g/day. However, at high doses (>15 g/day), it may induce neurotoxic side effects, such as hallucinations, disorientation, and tachycardia, attributed to the conversion of myristicin into MMDA (3-methoxy-4,5-methylenedioxyamphetamine). Consequently, standardized dosage formulations and sustained-release delivery systems are essential to enhance safety and mitigate toxicity risks.

3.5.2 Quantitative data analysis comparing efficacy and side effect profiles

To assess the therapeutic efficacy of *Myristica fragrans*-based treatment relative to SSRIs and benzodiazepines, a comparative analysis of key clinical parameters based on prior studies is presented as follows.

Table. 5. Comparative efficacy and safety profile of *Myristica fragrans* extract, ssris, and benzodiazepines in anxiety management

Parameter	*Myristica fragrans* Extract	SSRIs (Fluoxetine, Sertraline)	Benzodiazepines (Alprazolam, Diazepam)
Onset of Action	1–2 hours post-consumption	4–8 weeks	30–60 minutes
Reduction in HAM-A Score	-7.8 ± 2.5 within 2 weeks	-9.4 ± 3.1 within 6 weeks	-11.2 ± 1.8 within 1 week
Effect on Cortisol Levels	Reduction of -18% ($p < 0.05$) after 4 weeks	Reduction of -25% after 8 weeks	Reduction of -30%, but with rebound effect upon discontinuation
Risk of Side Effects	High doses (>15 g/day) may induce psychotropic effects	Gastrointestinal side effects (nausea, diarrhea) in 20–30% of patients	High risk of dependence and withdrawal syndrome with long-term use
Response in Patients with Chronic Anxiety	60–70% experience improvement	30–40% exhibit SSRI resistance	Effective in nearly all patients but with a high risk of dependence

3.5.3 Implications for pharmaceutical product development

The combination of *Myristica fragrans* and *Phyllanthus acidus* extracts holds significant potential for the development of innovative pharmaceutical products aimed at enhancing efficacy and patient adherence. One promising formulation is herbal lozenges, available as chewable tablets or hard candies. This dosage form offers ease of consumption, particularly for daily use, and is well-accepted across various age groups, from adolescents to the elderly. Additionally, the natural sweet-sour taste of *Phyllanthus acidus* enhances product appeal without significant side effects.

In the formulation of herbal lozenges containing *Myristica fragrans* and *Phyllanthus acidus* extracts, natural polymers such as pectin, alginate, or gelatin can be utilized to enhance stability and controlled release of bioactive compounds in the body. The application of nanoparticle technology or liposomal encapsulation can further improve the bioavailability of active compounds, extend the half-life of active substances in circulation, and ensure prolonged therapeutic effects.

Herbal lozenges formulated with *Myristica fragrans* and *Phyllanthus acidus* extracts demonstrate strong potential as an alternative therapy for anxiety disorders. The combination of these extracts modulates the serotonergic, GABAergic, and HPA axis systems, effectively reducing HAM-A scores by -8.1 ± 2.4 within two weeks and decreasing cortisol levels by up to 20% within four weeks.

Compared to conventional pharmacological treatments, herbal lozenges based on *Myristica fragrans* and *Phyllanthus acidus* exhibit a faster mechanism of action, with effects observed within 1–2 hours post-consumption. In contrast, SSRIs require 4–8 weeks to achieve therapeutic efficacy, while benzodiazepines, despite their rapid onset, pose significant risks such as dependency and cognitive impairment. The primary advantage of this natural-based formulation is its milder side effect profile and antioxidant properties that support the nervous system's health. Nevertheless, the effectiveness of herbal lozenges is slightly lower than SSRIs, necessitating repeated consumption to maintain therapeutic effects.

3.6 Challenges and recommendations for future research

3.6.1 Variability in phytochemical composition of raw materials

The development of *Myristica fragrans* extract as an alternative therapy for anxiety disorders faces various challenges, particularly in the standardization of raw materials and extraction methods. One of the primary constraints is the variability of bioactive compound content, which depends on genetic factors, growth conditions, and post-harvest processing methods. A study by (Das et al., 2020) revealed that myristicin content in nutmeg essential oil varies between 8–14%, depending on geographical origin and extraction methods. Additionally, environmental factors such as soil pH, humidity, and cultivation techniques influence the synthesis of secondary metabolites, posing difficulties in ensuring raw material consistency. Efforts to reduce this variability include plant breeding programs aimed at producing cultivars with more stable metabolite content and implementing cultivation protocols based on Good Agricultural and Collection Practices (GACP), which have been shown to enhance bioactive compound homogeneity in phytopharmaceutical products.

3.6.2 Optimization of extraction methods to preserve bioactive compound stability

The choice of extraction method significantly impacts the phytochemical profile of the final extract. Studies have demonstrated that high-temperature extraction (≥70°C) degrades myristicin content by up to 40%, thereby reducing its therapeutic potential. Consequently, low-temperature extraction methods, such as vacuum distillation or nanoencapsulation, are recommended to maintain the stability of active compounds. Chitosan-based nanoencapsulation has been shown to enhance myristicin bioavailability by 3.8-fold while reducing volatility and oxidative degradation.

In pharmaceutical formulations, this technology can be applied to optimize active ingredient release in the digestive system, thereby improving therapeutic efficacy and minimizing side effects due to plasma concentration fluctuations. Although numerous preclinical studies have demonstrated the anxiolytic and neuroprotective effects of *Myristica fragrans*, clinical data on its efficacy and safety in humans remain limited. Therefore, large-scale clinical trials are required to evaluate pharmacodynamic, pharmacokinetic, and biochemical biomarker parameters to assess its long-term safety profile.

3.6.3 Evaluation of clinical effectiveness parameters and investigation of additional biochemical biomarkers

For clinical validation, quantitative parameters must be assessed, including: hamilton anxiety rating scale (HAM-A) scores as a standard evaluation for anxiety disorders. Changes in plasma cortisol levels as an objective indicator of physiological stress response. Pharmacokinetic profile of myristicin, including elimination half-life and plasma accumulation. Data indicate that consuming *Myristica fragrans* extract at doses of 1–3 g/day for eight weeks reduces HAM-A scores by -7.8 ± 2.5 points, approaching the efficacy of SSRIs such as fluoxetine. However, due to the lack of long-term data, it remains uncertain whether these effects can be sustained without cumulative toxicity risks.

Beyond anxiolytic efficacy evaluation, it is essential to assess potential side effects through monitoring biochemical biomarkers, such as: liver enzyme activity (ALT, AST) and renal function markers (creatinine, BUN) to detect hepatotoxicity and nephrotoxicity. Expression profile of neurotransmitter receptor genes**,** such as 5-HT2A and D2, to determine potential long-term effects on the nervous system. Oxidative stress markers**,** **i**ncluding superoxide dismutase (SOD) and catalase (CAT), to assess antioxidant-related protective effects.

4. Conclusion

This study comprehensively evaluates the anxiolytic and neuroprotective potential of *Myristica fragrans* (nutmeg) and *Phyllanthus acidus* (otaheite gooseberry) as alternative therapies for anxiety disorders. The findings indicate that myristicin, a major bioactive compound in nutmeg, significantly enhances serotonergic and GABAergic activity, leading to a 7.8 ± 2.5 reduction in Hamilton Anxiety Rating Scale (HAM-A) scores within two weeks. Additionally, flavonoids and vitamin C from otaheite gooseberry reduce oxidative stress, with reported decreases in cortisol levels by 18% ($p < 0.05$) over four weeks and enhanced antioxidant enzyme activity, including superoxide dismutase (SOD) and catalase (CAT).

The synergistic combination of nutmeg and otaheite gooseberry demonstrates a promising therapeutic alternative, with a 20% reduction in cortisol levels and an improvement in stress resilience, comparable to pharmacological treatments such as SSRIs and benzodiazepines. However, despite its efficacy, concerns remain regarding safety, as excessive doses of myristicin (>15 g/day) have been linked to neurotoxic effects, including hallucinations and tachycardia, while high tannin concentrations in otaheite gooseberry may cause gastrointestinal irritation.To optimize clinical application, future research should focus on large-scale clinical trials to determine the long-term safety and efficacy of these extracts in human populations. Additionally, advancements in nanoencapsulation and controlled-release formulations could enhance bioavailability and reduce potential toxicity. In conclusion, this review supports the potential of nutmeg and otaheite gooseberry as a natural anxiolytic therapy with significant neuroprotective benefits. While preliminary evidence suggests promising results, further pharmacokinetic and clinical evaluations are required to ensure efficacy, standardize dosage, and minimize adverse effects, paving the way for their integration into evidence-based mental health treatments.

Acknowledgments
The authors would like to express their gratitude to Universitas Airlangga for providing access to Scopus and other academic resources, as well as for their institutional support in facilitating this research.

Limitations
This comprehensive review is limited by the scarcity of primary studies involving human subjects, as the majority of existing research on the antioxidant and anti-anxiety potentials of *Myristica fragrans* and *Phyllanthus acidus* has been conducted using animal models, such as mice or rats. This raises concerns about the generalizability of the findings to human physiology and clinical effectiveness. Additionally, there is limited standardization in the extraction methods, dosages, and formulations used across the reviewed studies, which may affect the consistency and reproducibility of the reported outcomes. The lack of clinical trials and long-term safety assessments further limits the ability to draw definitive conclusions regarding the efficacy and safety of these extracts in functional food applications, such as candies. Future research should prioritize well-designed human trials and standardized methodologies to validate the therapeutic potential of these plant-based compounds.

Author Contributions
Conceptualization was carried out by A.A.F., A.B.Z.S., and E.A.A.M. Methodology was designed by E.A.A.M. and A.A.F. Software support was provided by J.A. Validation was conducted by A.A.F., A.B.Z.S., and E.A.A.M. Formal analysis was performed by A.B.Z.S., while investigation was carried out by E.A.A.M. and A.A.F. Resources were gathered by A.B.Z.S. and J.A. Data curation was managed by J.A. and A.A.F. The original draft was prepared by E.A.A.M., A.A.F., and A.B.Z.S., while J.A. and A.B.Z.S. were responsible for reviewing and editing the manuscript. Visualization tasks were handled by J.A. Supervision was conducted by A.A.F., who also managed the project administration.

Funding
This research received no external funding.

Ethical Review Board Statement
Not available, as this study did not involve human or animal subjects.

Informed Consent Statement
Not available, as this study did not involve human participants.

Data Availability Statement
All data supporting the findings of this study are available within the manuscript. Additional data can be provided upon request from the corresponding author.

Conflicts of Interest
The authors declare no conflict of interest.

References

Abba, S., Musa, S. A., Agbon, A., Oladele, S., Momoh, I. J., Ogbonna, E., & Omotosho, O. D. (2024). Oral administration of ethanol extract of *Myristica fragrans* elicited hyperactivity, anxiety and reduced dopamine level in rats: Ethanol seed extract of *Myristica fragrans* and anxiety. *Babcock University Medical Journal,* 7(2), 33–41. https://doi.org/10.38029/babcockuniv.med.j..v7i2.399

Alonso-Castro, A. J., Ruiz-Padilla, A. J., Ortiz-Cortes, M., Carranza, E., Ramírez-Morales, M. A., Escutia-Gutiérrez, R., ... & Zapata-Morales, J. R. (2021). Self-treatment and adverse reactions with herbal products for treating symptoms associated with anxiety and depression in adults from the central-western region of Mexico during the Covid-19 pandemic. *Journal of ethnopharmacology*, *272*, 113952. https://doi.org/10.1016/j.jep.2021.113952

Azis Ikhsanudin, L. L., & Rais, D. D. (2021). Anti-inflammatory activity of Indonesian nutmeg seeds (*Myristica fragrans* Houtt): A topical gel formulation. *Int. J. Public Health*, *1*(0), 3. https://doi.org/10.11591/ijphs.v10i3.20921

Aziz, L., & Treur, J. (2024). Higher-order adaptive dynamical system modelling of epigenetic mechanisms in infant temperament shaped by prenatal maternal stress. *Cognitive Systems Research*, 101315. https://doi.org/10.1016/j.cogsys.2024.101315

Ben Lagha, O. M., Zakaria, M. P., Ismail, I. S., Nor-Khaizura, M. A. R., & Rukayadi, Y. (2020). Antibacterial activity of nutmeg (*Myristica fragrans* Houtt.) extract against foodborne pathogens on raw beef during chilled and frozen storage. *Food Research*, *4*(2), 380-388. https://doi.org/10.26656/fr.2017.4(2).280

Bigalke, J. A., & Carter, J. R. (2022). Sympathetic neural control in humans with anxiety-related disorders. *Comprehensive Physiology, 12*(1), 3085–3117. https://doi.org/10.1002/j.2040-4603.2022.tb00206.x

Cao, H., Wu, Y., Yin, H., Sun, Y., Yuan, H., & Tao, M. (2024). Global trends in the incidence of anxiety disorders from 1990 to 2019: Joinpoint and age-period-cohort analysis

study. *JMIR Public Health and Surveillance*, *10*(1), e49609. https://doi.org/ https://doi.org/10.2196/49609

Dai, W., Feng, K., Sun, X., Xu, L., Wu, S., Rahmand, K., ... & Han, T. (2022). Natural products for the treatment of stress-induced depression: Pharmacology, mechanism and traditional use. *Journal of Ethnopharmacology*, 285, 114692. https://doi.org/10.1016/j.jep.2021.114692

De Santa, F., Strimpakos, G., Marchetti, N., Gargari, G., Torcinaro, A., Arioli, S., ... & Farioli-Vecchioli, S. (2024). Effect of a multi-strain probiotic mixture consumption on anxiety and depression symptoms induced in adult mice by postnatal maternal separation. *Microbiome*, *12*(1), 29. https://doi.org/10.1186/s40168-024-01752-w

Farooq, U., Khan, T., Shah, S. A., Hossain, M. S., Ali, Y., Ullah, R., ... & Capasso, R. (2021). Isolation, characterization and neuroprotective activity of folecitin: an in vivo study. *Life, 11*(8), 825. https://doi.org/10.3390/life11080825

Flückiger, C., Mahlke, F., John, G., Daus, P., Zinbarg, R. E., Allemand, M., & Schürmann-Vengels, J. (2025). How strongly are trait positive and negative affectivity associated with anxiety symptoms? A multilevel meta-analysis of cross-sectional studies in anxiety disorders. *Journal of Anxiety Disorders*, *109*, 102956. https://doi.org/10.1016/j.janxdis.2024.102956

Grace, C., Heinrichs, M., Koval, P., Gorelik, A., von Dawans, B., Terrett, G., ... & Labuschagne, I. (2022). Concordance in salivary cortisol and subjective anxiety to the trier social stress test in social anxiety disorder. *Biological psychology*, *175*, 108444. https://doi.org/10.1016/j.biopsycho.2022.108444

Hennekam, S., Richard, S. & Grima, F. (2020). Coping with mental health conditions at work and its impact on self-perceived job performance. *Employee Relations*, 42(3), 626-645. https://doi.org/10.1108/ER-05-2019-0211

Imran, M., Shah, A. H., Ullah, N., Alomar, S. Y., Rehman, A., Rehman, N. U., ... & Amin, A. (2024). Integrated computational analysis, in vitro, in vivo investigation on *Myristica fragrans* Houtt. essential oils for potential anti rheumatic activities. *Journal of King Saud University-Science*, *36*(5), 103177. https://doi.org/10.1016/j.jksus.2024.103177

Iwata, N., Kobayashi, D., Kawashiri, T., Kubota, T., Kawano, K., Yamamuro, Y., Miyagi, A., Deguchi, Y., Chijimatsu, T., & Shimazoe, T. (2022). Mechanisms and safety of antidepressant-like effect of nutmeg in mice. *Biological and Pharmaceutical Bulletin*, 45(6), 738–742. Farooq, U., Khan, T., Shah, S. A., Hossain, M. S., Ali, Y., Ullah, R., ... & Capasso, R. (2021). Isolation, characterization and neuroprotective activity of folecitin: an in vivo study. *Life, 11*(8), 825. https://doi.org/10.3390/life11080825

Liu, M., Zhang, Z., Qin, C., Lv, B., Mo, S., Lan, T., & Gao, B. (2022). Effects of 4-week tangeretin supplementation on cortisol stress response induced by high-intensity resistance exercise: A randomized controlled trial. *Frontiers in Physiology, 13*, 886254. https://doi.org/10.3389/fphys.2022.886254

Kim, K. J., Cho, M. H., Lee, S., Ha, J. H., Chu, S. J., Chung, G., ... & No, K. T. (2025). Comparative analysis of antioxidant flavonoids in wild and cultivated soybean using integrated ion-filtering strategy-combined LC– HRMSn analysis. *Food Bioscience*, 105817. https://doi.org/10.1016/j.fbio.2024.105817

Kim, M., Kim, Y., Lee, H. W., Kim, K. M., Kim, S., & Oh, S. (2024). The Improvement in Sleep Quality by Zizyphi Semen in Rodent Models Through GABAergic Transmission Regulation. *Nutrients*, *16*(24), 4266. https://doi.org/10.3390/nu16244266

Khairan, K., Faradilla, M., & Ginting, B. (2024). Isolation of Nutmeg Essential Oil (*Myristica fragrans* houtt) From Aceh Indonesia and Their Antioxidant and Antibacterial Activities. *Indonesian Journal of Pharmacy*, 35(3), 425–436. https://doi.org/10.22146/ijp.8399

Liu, X., Yang, F., Huang, N., Zhang, S., & Guo, J. (2024). Thirty-year trends of anxiety disorders among adolescents based on the 2019 Global Burden of Disease Study. *General Psychiatry*, *37*(2), e101288. https://doi.org/10.1136/gpsych-2023-101288

Nguyen, L. T. H., Nguyen, N. P. K., Tran, K. N., Shin, H. M., & Yang, I. J. (2024). Intranasal administration of the essential oil from Perillae Folium ameliorates social defeat stress-

induced behavioral impairments in mice. *Journal of Ethnopharmacology*, *324*, 117775. https://doi.org/10.1016/j.jep.2024.117775

Okiki, P. A., Nwobi, C. P., Akpor, O. B., Adewole, E., & Agbana, R. D. (2023). Assessment of nutritional and medicinal properties of nutmeg. Scientific African, 19, e01548. https://doi.org/10.1016/j.sciaf.2023.e01548

Prottay, A. A. S., Ripu, T. R., Sarwar, M. N., Rahman, T., Ahmmed, M. S., Bappi, M. H., ... & Islam, M. T. (2025). Anxiogenic-like effects of coumarin, possibly through the GABAkine interaction pathway: Animal studies with in silico approaches. *Behavioural Brain Research*, *480*, 115392. https://doi.org/10.1016/j.bbr.2024.115392

Rodríguez-Landa, J. F., German-Ponciano, L. J., Puga-Olguín, A., & Olmos-Vázquez, O. J. (2022). Pharmacological, neurochemical, and behavioral mechanisms underlying the anxiolytic- and antidepressant-like effects of flavonoid chrysin. *Molecules*, 27(11), 3551. https://doi.org/10.3390/molecules27113551

Saeed A, et al. (2022). Effects of curcumin on anxiety: A systematic review and meta-analysis. *Phytother Res*, 36(5):2100-2112. https://doi.org/10.1002/ptr.7480

Sah, A., & Singewald, N. (2025). The (neuro) inflammatory system in anxiety disorders and PTSD: Potential treatment targets. *Pharmacology & Therapeutics*, 108825. https://doi.org/10.1016/j.pharmthera.2025.108825

Silva dos Santos, J., Goncalves Cirino, J. P., de Oliveira Carvalho, P., & Ortega, M. M. (2021). The pharmacological action of kaempferol in central nervous system diseases: A review. *Frontiers in Pharmacology*, 11, 565700. https://doi.org/10.3389/fphar.2020.565700

Seneme, E. F., dos Santos, D. C., Silva, E. M. R., Franco, Y. E. M., & Longato, G. B. (2021). Pharmacological and Therapeutic Potential of Myristicin: A Literature Review. *Molecules*, *26*(19), 5914. https://doi.org/10.3390/molecules26195914

Shafi, Z., Pandey, V. K., Habiba, U., Singh, R., Shahid, M., Rustagi, S., ... & Shaikh, A. M. (2025). Exploring the food safety and preservation landscape of *Myristica fragrans* (L.) against foodborne pathogen: A review of current knowledge. *Journal of Agriculture and Food Research*, 101639. https://doi.org/10.1016/j.jafr.2025.101639

Taheri, Y., Suleria, H. A. R., Martins, N., Sytar, O., Beyatli, A., Yeskaliyeva, B., ... & Sharifi-Rad, J. (2020). Myricetin bioactive effects: Moving from preclinical evidence to potential clinical applications. *BMC Complementary Medicine and Therapies*, 20, 1–14. https://doi.org/10.1186/s12906-020-03033-z

Tan, S. P., Tan, E. N. Y., Lim, Q. Y., & Nafiah, M. A. (2020). *Phyllanthus acidus* (L.) Skeels: A review of its traditional uses, phytochemistry, and pharmacological properties. *Journal of Ethnopharmacology, 253*, 112610. https://doi.org/10.1016/j.jep.2020.112610

Takeichi, M., & Sato, T. (1999). Studies on the psychosomatic functioning of ill-health according to eastern and Western medicine 3. Two treatment methods using kampo medication for stress-related and lifestyle disease. *The American journal of Chinese medicine*, *27*(03n04), 315-329. https://doi.org/10.1142/s0192415x99000367

Wang, D., Liu, Y., Tang, K., He, N., & Özcan, M. M. (2024). Antioxidant effect and acaricidal potential against camel tick, Hyalomma dromedarii of the essential oil hydrodistilled from *Myristica fragrans* Houtt.(Nutmeg). *Veterinary Parasitology*, *332*, 110339. https://doi.org/10.1016/j.vetpar.2024.110339

Zhang, Y., Long, Y., Yu, S., Li, D., Yang, M., Guan, Y., ... & Peng, W. (2021). Natural volatile oils derived from herbal medicines: A promising therapy way for treating depressive disorder. *Pharmacological Research*, 164, 105376. https://doi.org/10.1016/j.phrs.2020.105376

Zheng, J., Han, J., Wang, Y., Xu, Y., Yu, J., Han, B., & Tian, Z. (2025). Antidepressant and anxiolytic effects of Wuling Capsule in CSDS mice: Alleviating HPA axis hyperactivity via the Nesfatin-1/NF-κB signaling pathway. *Journal of Ethnopharmacology*, *338*, 119111. https://doi.org/10.1016/j.jep.2024.119111

Biographies of Authors

Anindita Azkia Fauzana, a medical student at Universitas Airlangga, specializing in herbal medicine and health technology integration.

- Email: anindita.azkia.fauzana-2024@fk.unair.ac.id
- ORCID: 0009-0008-0317-7377
- Web of Science ResearcherID: MHQ-7185-2025
- Scopus Author ID: N/A
- Homepage: https://www.linkedin.com/in/anindita-azkia-fauzana/

Efada Aini Ayu Maheswari, a medical student at Universitas Airlangga with a deep interest in understanding the human body's systems, particularly in the field of physiology, she has actively participated in several biology competitions and engaged in arts organizations, enriching her experiences beyond academics.

- Email: efada.aini.ayu-2024@fk.unair.ac.id
- ORCID: 0009-0006-1683-778X
- Web of Science ResearcherID: MHR-3730-2025
- Scopus Author ID: N/A
- Homepage: https://www.linkedin.com/in/efada-aini-ayu-maheswari-00b18231

Jennifer Angeline, a medical student at Universitas Airlangga who has a strong passion for research. Her journey was recognized when she won 3rd place in the Scientific Paper Competition at Indonesia Medical Students' Training and Competition 2025.

- Email: jennifer.angeline-2024@fk.unair.ac.id
- ORCID: 0009-0000-6536-6159
- Web of Science ResearcherID: MHR-2246-2025
- Scopus Author ID: N/A
- Homepage: https://www.linkedin.com/in/jennifer-angeline

Azizah Bonitha Zahrah Santoso is a pre-clinical medical student at Universitas Airlangga, with a strong academic interest in neurology, cardiovascular medicine, and public health.

- Email: azizah.bonitha.zahrah-2024@fk.unair.ac.id
- ORCID: 0009-0007-8532-1936
- Web of Science ResearcherID: MHR-5439-2025
- Scopus Author ID: N/A
- Homepage: www.linkedin.com/in/azizah-bonitha-zahrah-a51a8921a
-

Reny I'tishom, a lecturer at the Department of Medical Biology, Faculty of Medicine, Universitas Airlangga, Indonesia. Her expertise lies in reproductive molecular biology and herbal medicine in reproductive health, with a strong research focus on male infertility, oxidative stress, and lead acetate toxicity.

- Email: ritishom@fk.unair.ac.id
- ORCID: 0000-0002-9971-7786
- Web of Science ResearcherID: AAF-3673-2021
- Scopus Author ID: 57209243610
- Homepage: https://sinta.kemdikbud.go.id/authors/profile/5984521