UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

DANIELLA BASTOS DE ARAÚJO

AVALIAÇÃO ANTICONVULSIVANTE E PRÓ-CONVULSIVANTE DE ÓLEOS ESSENCIAIS DE *LIPPIA ORIGANOIDES* E *ROSMARINUS OFFICINALIS* EM RATOS: UM ESTUDO ELETROFISIOLÓGICO

BELÉM-PA

2025

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

D278a De Araújo, Daniella Bastos.
Avaliação Anticonvulsivante e Pró-Convulsivante de Óleos Essencias de Lippia Origanoides e Rosmarinus Officinalis em ratos: Um Estudo Eletrofisiológico / Daniella Bastos De Araújo, . — 2025.
29 f. : il. color.

Orientador(a): Prof. Dr. Moisés Hamoy
Tese (Doutorado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2025.

1. Óleo essencial, Lippia origanoides, rosmarinus officinalis, eletrofisiologia, eletrocardiograma. I. Título.

CDD 571

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

AVALIAÇÃO ANTICONVULSIVANTE E PRÓ-CONVULSIVANTE DE ÓLEOS ESSENCIAIS DE *LIPPIA ORIGANOIDES* E *ROSMARINUS OFFICINALIS* EM RATOS: UM ESTUDO ELETROFISIOLÓGICO

Autor(a): DANIELLA BASTOS DE ARAÚJO

Orientador(a): Prof. Dr. MOISÉS HAMOY

Documento de Tese apresentada ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Doutor em Farmacologia e Bioquímica.

BELÉM-PA

2025

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

D278a De Araújo, Daniella Bastos.
Avaliação Anticonvulsivante e Pró-Convulsivante de Óleos Essencias de Lippia Origanoides e Rosmarinus Officinalis em ratos: Um Estudo Eletrofisiológico / Daniella Bastos De Araújo, . — 2025.
29 f. : il. color.

Orientador(a): Prof. Dr. Moisés Hamoy
Tese (Doutorado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2025.

1. Óleo essencial, Lippia origanoides, rosmarinus officinalis, eletrofisiologia, eletrocardiograma. I. Título.

CDD 571

RESUMO

A epilepsia é um distúrbio neuronal caracterizado pela excitabilidade anormal do cérebro, levando a convulsões. Apenas cerca de 66% dos pacientes epilépticos respondem adequadamente ao tratamento com os anticonvulsivantes convencionais existentes, tornando necessária a investigação de novos medicamentos antiepilépticos. A crescente pesquisa sobre produtos naturais e suas propriedades farmacológicas tem se tornado cada vez mais promissora, particularmente no estudo dos óleos essenciais, já amplamente utilizados na cultura popular para o tratamento de diversas doenças. Os presentes estudos avaliaram os efeitos anti e pró-convulsivante dos óleos essenciais de *Lippia origanoides* (LOEO) e *Rosmarinus officinalis* (EORO) em ratos wistar. Avaliamos o óleo essencial de Lippia origanoides (LOEO) (100 mg/kg i. p.) em comparação ao diazepam (DZP) (5 mg/kg i. p.) e à administração combinada dessas duas substâncias para controlar convulsões induzidas por pentilenotetrazol (PTZ) (60 mg/kg i. p.). Essa avaliação foi realizada utilizando 108 ratos Wistar machos, que foram divididos em dois experimentos. Experimento 1 – Avaliação comportamental e experimento 2 – Avaliação eletrocorticográfica. Já com o óleo essencial de alecrim avaliamos altas doses em 54 ratos Wistar, com peso entre 180 e 200 g. O estudo consistiu em três experimentos: 1) monitoramento comportamental dos animais após administração de 500 mg/kg i.p.; 2) registros eletrocorticográficos após administração do fármaco; 3) reação a fármacos anticonvulsivantes, onde foram aplicados fenitoína, fenobarbital e diazepam (10 mg/kg i.p.). Com o LOEO os animais apresentaram uma diminuição mais intensa da frequência respiratória quando combinados com LOEO + DZP. Os registros de EEG mostraram uma redução na amplitude de disparo nos grupos tratados com LOEO. O tratamento combinado com diazepam resultou em aumento dos efeitos anticonvulsivantes, já com o EORO os resultados demonstraram aumento do tempo de latência para o aparecimento de crises clônicas isoladas sem perda do reflexo postural. Os animais apresentaram uma diminuição mais intensa da frequência respiratória quando combinados com LOEO + DZP. Os registros de EEG mostraram uma redução na amplitude de disparo nos grupos tratados com LOEO. O tratamento combinado com diazepam resultou em aumento dos efeitos anticonvulsivantes. O tratamento com LOEO foi eficaz no controle das convulsões, e sua combinação com diazepam pode representar uma opção futura para o tratamento de convulsões de difícil controle, já o tratamento com EORO demonstra uma atividade excitatória relacionada a redução da atividade GABAérgica.

Palavras-chave: Óleo essencial, *Lippia origanoides*, rosmarinus officinalis, eletrofisiologia, eletrocorticograma

ABSTRACT

Epilepsy is a neuronal disorder characterized by abnormal brain excitability, leading to seizures. Only about 66% of epileptic patients respond adequately to treatment with existing conventional anticonvulsants, making it necessary to investigate new antiepileptic drugs. The growing research on natural products and their pharmacological properties has become increasingly promising, particularly in the study of essential oils, already widely used in popular culture for the treatment of several diseases. The present studies evaluated the anti- and pro-convulsant effects of the essential oils of Lippia origanoides (LOEO) and Rosmarinus officinalis (EORO) in Wistar rats. We evaluated the essential oil of Lippia origanoides (LOEO) (100 mg/kg i. p.) in comparison with diazepam (DZP) (5 mg/kg i. p.) and the combined administration of these two substances to control seizures induced by pentylenetetrazole (PTZ) (60 mg/kg i. p.). This evaluation was carried out using 108 male Wistar rats, which were divided into two experiments. Experiment 1 – Behavioral evaluation and Experiment 2 – Electrocorticographic evaluation. With rosemary essential oil, we evaluated high doses in 54 Wistar rats, weighing between 180 and 200 g. The study consisted of three experiments: 1) behavioral monitoring of the animals after administration of 500 mg/kg i.p.; 2) electrocorticographic recordings after drug administration; 3) reaction to anticonvulsant drugs, where phenytoin, phenobarbital and diazepam (10 mg/kg i.p.) were administered. With LOEO, the animals presented a more intense decrease in respiratory rate when combined with LOEO + DZP. EEG recordings showed a reduction in firing amplitude in the groups treated with LOEO. Combined treatment with diazepam resulted in increased anticonvulsant effects, while with EORO, the results demonstrated an increase in the latency time for the onset of isolated clonic seizures without loss of the postural reflex. The animals showed a more intense decrease in respiratory rate when combined with LOEO + DZP. EEG recordings showed a reduction in firing amplitude in the groups treated with LOEO. Combined treatment with diazepam resulted in increased anticonvulsant effects. Treatment with LOEO was effective in controlling seizures, and its combination with diazepam may represent a future option for the treatment of difficult-to-control seizures, while treatment with EORO demonstrated an excitatory activity related to the reduction of GABAergic activity.

Keywords: Essential oil, *Lippia origanoides*, rosmarinus officinalis, electrophysiology, electrocorticogram

LISTA DE ILUSTRAÇÕES

FIGURAS DO ARTIGO 1

FIGURAS DO ARTIGO 2

LISTA DE TABELAS

TABELAS DO ARTIGO 1

TABELAS DO ARTIGO 2

LISTA DE SIGLAS E SÍMBOLOS

DAE´s	Drogas antiepliéticas
SNC	Sistema Nervoso Central
GABA	Ácido gama-aminobutírico
EO´s	Óleos essenciais
PTZ	Pentilenotetrazol

SUMÁRIO

1. VISÃO INTEGRADORA DO PROBLEMA

A epilepsia é um distúrbio neurológico carcterizado por crises convulsivas recorrentes causadas por uma atividade neural exacerbada resultando em descargas elétricas excessivas, sincrônicas e desordenadas. É uma das doenças cerebrais mais comuns em todo o mundo, afetando mais de 70 milhões de pessoas, com cerca de 80% vivendo em países de baixa renda, como o Brasil (Yacubian, 2008; Gallucci e Marchetti, 2005; Costa; Brandão; Segundo, 2020).

Nas últimas décadas, houveram avanços significativos em relação ao tratamento farmacológico das epilepsias. Estes avanços resultaram da maior elucidação dos mecanismos básicos da doença, levando ao desenvolvimento de um grupo de drogas antiepiléticas chamadas de primeira geração, e posteriormente de um novo grupo de drogas, conhecidas como de nova geração. Entretanto, quando a questão é o impacto clínico das drogas antiepiléticas de um modo geral, ainda são poucos os indivíduos cujas crises, que não são controladas com as drogas antiepiléticas convencionais, terão um controle satisfatório com o uso das novas drogas (Garzon, 2002; Loscher, 2011) (Fernandes, 2013; Rektor et al., 2013; Costa; Brandão, 2020)..

Aproximadamente 30-40% dos portadores de epilepsia não conseguem o controle adequado de suas crises no uso de terapias farmacológicas já disponíveis, sendo estas classificadas como epilepsias refratárias. Essa falta de controle em quadros epiléticos refratários pode culminar em déficits cognitivos, motores, psicológicos e sociais a seus portadores (Kwan et al., 2002; Hermann et al., 2006; Jacoby e Baker, 2008; Costa; Brandão; Segundo, 2020).

Neste contexto, é importante o estabelecimento de novas drogas antiepiléticas, porém, que são dependentes de novos modelos animais para o quadro de epilepsia refratária, que apesar de ser uma necessidade já conhecida, ainda não vem sendo muito explorada (Loscher, 2011).

Paralelamente, observa-se o crescimento de estudos criteriosos e sistemáticos relacionados a plantas medicinais e produtos naturais, visando avaliar suas ações farmacológicas e toxicológicas. Dentre as plantas medicinais, destaca-se um grupo denominado plantas aromáticas, que em sua composição contêm os chamados óleos essenciais. O potencial biológico dessa classe de plantas é conhecido há mais de seis mil anos pelos egípcios e vem se expandindo desde então.

Os óleos essenciais (EOs) são compostos bioquímicos naturais e voláteis extraídos da parte aromática de diversas plantas, como flores, folhas, troncos, galhos, raízes, casca de frutos e outros órgãos vegetais. São utilizados na indústria da perfumaria, cosméticos, culinária, medicina natural e aromaterapia. Diversas propriedades são atribuídas aos óleos essenciais como antibactreriano, antiinflamatório, antiviral e relaxante. São utilizados para promover relaxamento, reduzir estresse e melhorar o humor.

Plantas com atividade psicoativa exercem efeitos importantes no sistema nervoso central (SNC) e têm sido utilizadas para fins terapêuticos. Na medicina tradicional, bem como na terapêutica, plantas que contêm derivados terpênicos apresentam efeitos sedativos e anticonvulsivantes. Muitos óleos voláteis apresentam uma variedade de atividades farmacológicas, como ansiolíticas, anticonvulsivantes e antinociceptivas. Compostos como linalol, limoneno e citronelol apresentam ação anticonvulsivante, enquanto mentol e mirceno, atividade analgésica, e muitos derivados monoterpênicos demonstraram atividades no SNC (Pergentino de Souza et al., 2007; Sousa et al., 2007; Perazzo et al., 2007, 2008; Leite et al., 2008).

O gênero Lippia (Verbenaceae) é bem conhecido por seu caráter aromático e compreende cerca de 200 espécies de ervas, arbustos e pequenas árvores distribuídas do sul da América do Norte ao norte da América do Sul, com ocorrência proeminente na região Amazônica e Nordeste do Brasil, Guianas, Venezuela e Colômbia (Terblanché e Kornelius, 1996). Na medicina tradicional tem sido relatado seu uso no tratamento de doenças gastrintestinais, infecções de garganta, pele e couro cabeludo, além de apresentar analgésicos, sedativos, atividade expectorante e doenças respiratórias (O'Leary et al., 2012, Oliveira et al., 2007, Oliveira et al., 2014, Santos et al., 2004, Albuquerque et al., 2007, Pascual et al., 2001, Barreto et al., 2014, Oliveira et al., 2014, Coelho et al., 2015, Oliveira et al., 2007, Damasceno et al., 2011,Lobo et al., 2010; Amorati et al., 2013).

Rosmarinus officinalis (alecrim), pertencente à família botânica Lamiaceae, é uma planta aromática cultivada em diferentes regiões do mundo, tendo o Mediterrâneo como centro de origem (Murata et al., 2013; Oliveira et al., 2019). O alecrim é usado para acelerar a digestão, desobstruir as vias nasais, estimular o crescimento capilar e aliviar dores reumáticas, bem como mialgias, neuralgias e fadiga física e mental.

Sendo assim, este trabalho objetiva-se em avaliar a possível atividade anticonvulsivante ou pró-convulsivante de óleos essenciais de *Lippia origanoides e Rosmarinus officinalis* em ratos wistar, através do modelo de pentilenotetrazol (PTZ) e avaliar os tempos de latências para o aparecimento dos comportamentos avaliando posteriormente os padrões de convulsões após aplicação de óleos essenciais.

2. ARTIGO 1: O óleo essencial de *Lippia origanoides* possui efeito anticonvulsivante em convulsões induzidas por pentilenotetrazol em ratos: um estudo comportamental, eletroencefalográfico e eletromiogáfico

Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 28 November 2023
DOI 10.3389/fphar.2023.1289336

Lippia origanoides essential oil possesses anticonvulsant effect in pentylenetetrazol-induced seizures in rats: a behavioral, electroencephalographic, and electromyographic study

Daniella Bastos de Araújo*, Anthony Lucas Gurgel do Amaral, Suzane Maia da Fonseca, Keyla Rodrigues de Souza, Allane Patrícia Santos da Paz, Vanessa Jóia de Mello, Gabriela Brito Barbosa, Maria Klara Otake Hamoy and Moisés Hamoy*

Laboratory of Pharmacology and Toxicology of Natural Products, Institute of Biological Science, Federal University of Pará, Belém, Brazil

Check for updates

OPEN ACCESS

EDITED BY
Mahmoud Rafieian-Kopaei, Shahrekord University of Medical Sciences, Iran

REVIEWED BY
Hossein Amini-khoei, Shahrekord University of Medical Sciences, Iran
Fatemeh Hoseinpour, Razi University, Iran

*CORRESPONDENCE
Daniella Bastos de Araújo,
danivivaf@gmail.com
Moisés Hamoy,
hamoyufpa@gmail.com

RECEIVED 05 September 2023
ACCEPTED 30 October 2023
PUBLISHED 28 November 2023

CITATION
Bastos de Araújo D, Gurgel do Amaral AL, Maia da Fonseca S, Rodrigues de Souza K, Santos da Paz AP, Jóia de Mello V, Barbosa GB, Otake Hamoy MK and Hamoy M (2023), Lippia origanoides essential oil possesses anticonvulsant effect in pentylenetetrazol-induced seizures in rats: a behavioral, electroencephalographic, and electromyographic study.
Front. Pharmacol. 14:1289336.
doi: 10.3389/fphar.2023.1289336

Epilepsy is a neuronal disorder characterized by abnormal excitability of the brain, leading to seizures. Only around 66% of the epileptic patients respond adequately to treatment with existing conventional anticonvulsants, making it necessary to investigate new antiepileptic drugs. The growing research into natural products and their pharmacological properties has become increasingly promising, particularly in the study of essential oils, which are already widely used in popular culture for treating various diseases. The present study evaluated the anticonvulsant effects of Lippia origanoides essential oil (LOEO) (100 mg/kg i. p.) compared to diazepam (DZP) (5 mg/kg i. p.), and the combined administration of these two substances to control convulsions induced by pentylenetetrazol (PTZ) (60 mg/kg i. p.). This evaluation was carried out using 108 male Wistar rats, which were divided into two experiments. Experiment 1–Behavioral assessment: The animals were divided into 4 groups ($n = 9$): (I) saline solution + PTZ, (II) DZP + PTZ, (III) LOEO + PTZ, (IV) LOEO + DZP + PTZ. The convulsive behavior was induced 30 min after the administration of the tested anticonvulsant drugs, and the observation period lasted 30 min. Experiment 2- Electrocorticographic evaluation: The animals were divided into 8 groups ($n = 9$): (I) saline solution; (II) LOEO; (III) DZP; (IV) LOEO + DZP; (V) saline + PTZ, (VI) DZP + PTZ (VII) LOEO + PTZ, (VIII) LOEO + DZP + PTZ. PTZ was administered 30 min after LOEO and DZP treatments and electrocorticographic activity was assessed for 15 min. For the control groups, electromyographic recordings were performed in the 10th intercostal space to assess respiratory rate. The results demonstrated that Lippia origanoides essential oil increased the latency time for the appearance of isolated clonic seizures without loss of the postural reflex. The animals had a more intense decrease in respiratory rate when combined with LOEO + DZP. EEG recordings showed a reduction in firing amplitude in the LOEO-treated groups. The combining treatment with diazepam resulted in increased anticonvulsant effects. Therefore, treatment with Lippia origanoides essential oil was effective in controlling seizures, and its combination with diazepam may represent a future option for the treatment of difficult-to-control seizures.

KEYWORDS

seizures, pentylenetetrazol, electrocorticographic recordings, essential oil, Lippia origanoides

1 Introduction

Epilepsy is a neurological disorder that affects around 45.9 million people globally and is characterized by a predisposition to suffer spontaneous seizures (Fisher et al., 2014). Its pathophysiology consists of the appearance of abnormal foci of cerebral electrical activity caused by the imbalance between excitatory and inhibitory neurotransmitters in the central nervous system, in such a way as to make it prone to functioning in an excessive oscillatory pattern. Conventional antiepileptic drugs act through these two pathways, either by enhancing inhibitory neurotransmitters or reducing excitatory signaling (Fisher et al., 2005; Sultana et al., 2021).

Currently available drug therapies are effective in only 66% of cases in developed countries (Duncan et al., 2006; Brodie et al., 2012) and are associated with various side effects (Perucca and Gilliam, 2012), highlighting the need for research to identify alternative treatments that target seizure mechanisms and have minimal side effects (Sultana et al., 2021).

One promising option is the use of essential oils (EOs) in the treatment of epilepsy. Essential oils are volatile substances extracted from plant parts, made up of a mixture of various components with therapeutic properties, widely used in popular culture to treat various ailments (de Almeida et al., 2011; Dobetsberger and Buchbauer, 2011). Recent studies have shown that several essential oils from aromatic plants have potential neuroprotective effects in age-related neurodegenerative diseases such as Alzheimer's and dementia and other neurological diseases such as anxiety, depression, epilepsy and seizures (Ayaz et al., 2017; Rashed et al., 2021; Sattayakhom et al., 2023).

Behavioral assessment and electrocorticography are of paramount importance in evaluating and comparing the changes caused by neuronal discharge that trigger seizures and epilepsy. In recent studies using natural products with potential anticonvulsant activities, data have shown that in the behavioral assessment, there were increases in seizure latencies and in the seizure threshold, confirmed by electrocorticographic records, along with a decrease in the peak and energy of the waves (Souza-Monteiro et al., 2015; Hamoy et al., 2018; Nascimento et al., 2022; Muto et al., 2022).

Lippia origanoides is a shrub with a strong aroma found mainly in the Amazon territory (Pascual et al., 2001; Oliveira et al., 2014) with medicinal properties well-known in popular culture (Siqueira-Lima et al., 2019). In Central America and Colombia, it is used to treat respiratory diseases, gastrointestinal discomfort such as gastralgia, nausea and antiseptic. In the countryside of Pará, in Brazil, Lippia origanoides, known as "salva-do-marajó," is commonly administered to combat intestinal colic, indigestion, diarrhea, burns, vaginal discharge, menstrual cramps, and fever (Oliveira et al., 2014). It is also notable for its use in food preparation, and in Venezuela, it is employed as an appetite stimulant (Morton, 1981).

Regarding the anticonvulsant properties of LOEO (Lippia origanoides essential oil), no studies have been found directly linking this plant to such effects. However, there are articles that suggest anticonvulsant effects of Lippia alba due to the high presence of flavonoids in its composition (Zétola et al., 2002; Neto et al., 2009; Siqueira-Lima et al., 2019), as well as L. Citriodora (Rashidian et al., 2016). In this context, the objective of the present work was to evaluate the treatment with essential oil of *L. origanoides* in the control of seizures triggered by pentylenetetrazol and compare its effects with those of diazepam, through behavioral analysis, electrocorticography and assessment of respiratory movements (electromyogram).

2 Materials and methods

2.1 Animals

For the execution of this research, 108 adult Wistar rats were obtained from the Central Bioterium of the Federal University of Pará. All animals were housed under controlled conditions, with a temperature of approximately 23°C–25°C and a light-dark cycle of 12/12 h, receiving filtered water and rat food on free demand. The work was performed at the Laboratory of Pharmacology and Toxicology of Natural Products (Laboratório de Farmacologia e Toxicologia de Produtos Naturais)—ICB—UFPA. The project was approved by the Animal Ethics Committee (CEUA—UFPA) number 1395260821.

2.2 Drugs used

Lippia origanoides essential oil (LOEO) was purchased from Olinda pharmaceutical company (essential oils) and administered intraperitoneally at a dose of 100 mg/kg, while Diazepam (DZP) 10 mg/2 mL (União Química, Embu-Guaçu, SP, Brazil), was administered at a dose of 5 mg/kg intraperitoneally (i.p). Ketamine hydrochloride (50 mg/kg i. p.) was purchased from Köing Laboratory (Santana de Parnaíba, SP, Brazil), xylazine hydrochloride (5 mg/kg i. p) was purchased from Vallée Laboratory (Montes Claros, MG, Brazil), while the local anesthetic lidocaine was obtained from Hipolabor Laboratory (Sabará, MG, Brazil). Pentylenetetrazol (PTZ) was obtained from Sigma Chemical Co. (St. Louis, MO, United States) and administered intraperitoneally at a dose of 60 mg/kg (Santos et al., 2021; Muto et al., 2022).

2.3 Test to obtain the dose of LOEO used

To obtain the dose used of Lipia Origanoides extract (LOEO), a fixed time of 30 min was considered to achieve muscle relaxation

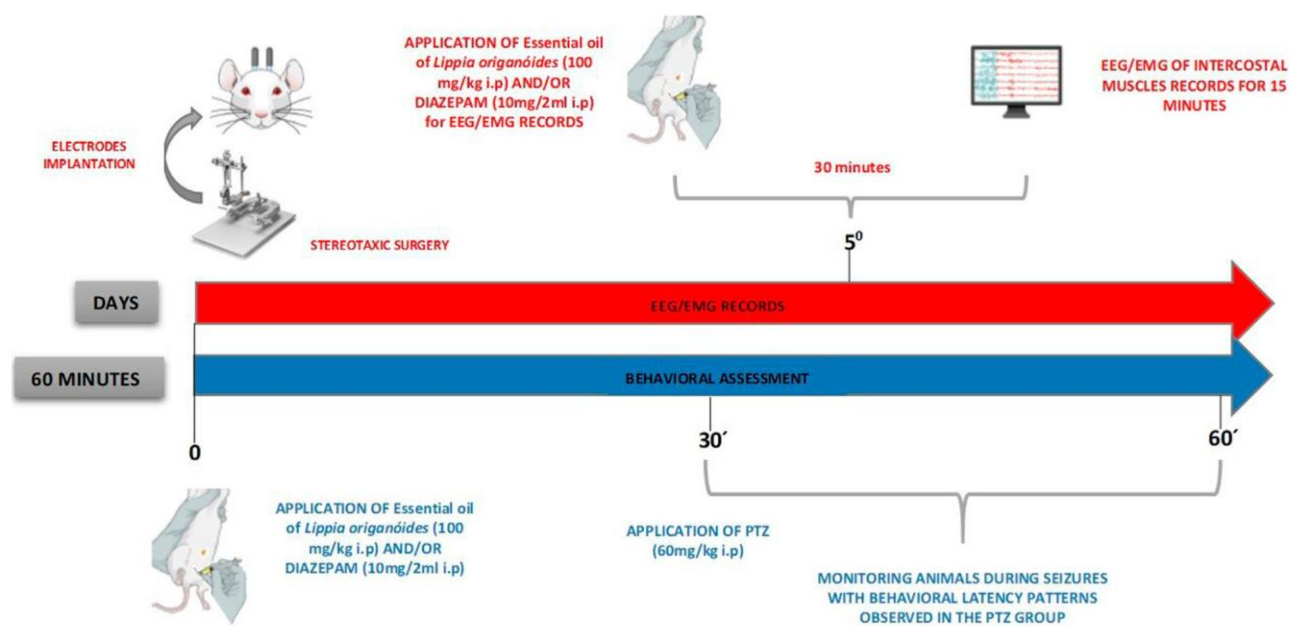

FIGURE 1
Experimental design. PTZ, pentylenetetrazol; EEG, electroencephalograph; EMG, electromyogram.

and animal sedation behavior at tested doses of 50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg i. p. the best response obtained was 100 mg/kg i. p. as higher doses caused myorelaxation with manifestation of respiratory depression. Therefore, a dose of 100 mg/kg i. p. was used 30 min before the onset of the seizure using PTZ (60 mg/kg).

2.4 Experimental design

The animals were kept at the research center for at least 7 days before the experiment for adaptation and acclimatization. The electrodes were implanted in the cortex 5 days before the application of the treatments. For the behavioral assessment the animals were divided into 4 groups (n = 9): (I) saline + PTZ, (II) DZP + PTZ, (III) LOEO + PTZ, (IV) LOEO + DZP + PTZ. The convulsant drug PTZ was administered 30 min after administration of the drugs tested as anticonvulsants and the observation time for behavior analysis was 30 min (Figure 1).

For electrocorticographic evaluation, animals were divided into 8 groups (n = 9): (I) saline; (II) LOEO; (III) DZP; (IV) LOEO + DZP; (V) saline + PTZ, (VI) DZP + PTZ (VII) LOEO + PTZ, (VIII) LOEO + DZP + PTZ. PTZ was administered 30 min after treatments and electrocorticographic activity was assessed for 15 min (Figure 1).

2.5 Evaluation of respiratory activity during sedation

For the analysis of respiratory frequency and muscle contraction power (Santos et al., 2021), electrodes were conjugated 2 mm apart and were prepared with a length of 2 mm and a diameter of 0.2 mm. These electrodes were inserted into the 10th intercostal space to record muscle activity. The recordings were conducted for a duration of 5 min for the Control, LOEO, DZP, and LOEO/DZP groups.

2.6 Description of seizure related behavior

The animals' behavior was monitored during the seizures and compared with latency patterns of behaviors observed in the PTZ-induced group (de Almeida. et al., 2020). Latency was measured concerning the initiation of the following behaviors: (I) whisker piloerection, (II) orofacial movements, (III) generalized tremor, (IV) anterior limb spasms, (V) isolated clonic seizures without loss of postural reflex, (VI) generalized clonic seizures with transient loss of postural reflex, and (VII) tonic-clonic seizures with complete loss of postural reflex.

2.7 Electrocorticographic recordings and data analysis

The animals were anesthetized and placed in a stereotaxic apparatus for the implantation of electrodes (with an exposed tip diameter of 1.0 mm) onto the dura mater above the prefrontal cortex at the coordinates of bregma–0.96 mm and ±1.0 mm lateral. The electrodes were secured using dental acrylic cement. Data were recorded using the electrodes with the assistance of a digital data acquisition system composed of a high-impedance amplifier (Grass Technologies, P511, United States), set with a filtering range of 0.3 Hz to 0.3 KHz. The data were monitored using an oscilloscope (Protek, Model 6510) and continuously digitized at a rate of 1 KHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX).

During the recording sessions, the animals were confined within acrylic boxes (20 cm × 45 cm × 15 cm), and ECoG activity was recorded for 15 min immediately after the application of PTZ or physiological solution. The data collected through the digital data acquisition system were analyzed offline. The analyses were performed in frequencies up to 40 Hz and then divided into five bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12 Hz–28 Hz), and gamma (28 Hz–40 Hz) [30].

The characterization of the aspects of neuronal hyperexcitability in seizures caused by PTZ, as well as the reversal of the condition by the control drugs, were performed using the Signal ® 3.0 and Pyton 5.0 programs, which allowed the analysis of the frequency domain of brain waves, in addition to the visual inspection of wave patterns.

2.8 Chromatographic analysis of Lippia origanoides essential oil

Gas Chromatography-Mass Spectrometry (GC-MS) analysis, with Agilent Model MSD 5977B apparatus, was carried out by the company Olinda (essential oils) to certify the chromatographic analysis of *Lippia origanoides*. The analysis was performed on a batch with manufacturing date of February 2022, labeled as lot: 180002.

Organoleptic Properties: The essential oil appeared as a liquid with a golden-yellow color, free of impurities. It exhibited a pungent, fresh, and herbal scent, and had a density of 0.935 at 20°C. It originated from Brazil and was obtained through steam distillation.

The components were identified based on the Chemical Abstracts Service (CAS) registry number, which assigns a unique number to each chemical compound described in literature. The major components identified were Thymol (57.46%) and Carvacrol (1.42%).

2.9 Statistical analysis

The results were subjected to descriptive statistics, including mean and standard deviation. One-way Analysis of Variance (ANOVA) was employed, followed by Tukey's *post hoc* test. A significance level of $*p < 0.05$, $**p < 0.001$, and $***p < 0.0001$ was adopted. The analyses were performed using GraphPad Prism, version 9 (GraphPad Software Inc., San Diego, CA, United States).

3 Results

3.1 Respiratory evaluation after administration of isolated and associated drugs

There was a reduction in respiratory rate when compared to the control group (60.22 ± 2.906/minute), LOEO group (52.44 ± 2.78/minute), DZP group (52.89 ± 3.480/minute) and LOEO/DZP group (45.33 ± 3.162/minute). The LOEO and DZP groups did not show a significant difference ($p = 0.990$), however, there was a decrease in respiratory frequency for the LOEO/DZP combination (Figures 2A–E).

To evaluate the muscle contraction power of the 10th intercostal muscle during treatment, it was observed that the control group had a mean power (3.215 ± 0.196 $mV^2/Hz \times 10–1$) and presented greater power compared to the other groups: LOEO group (2.254 ± 0.3382 $mV^2/Hz \times 10–1$), DZP group (2.523 ± 0.2479 $mV^2/Hz \times 10–1$) and LOEO/DZP group (1.976 ± 0.1767 $mV^2/Hz \times 10–1$). The LOEO and DZP groups did not show a significant difference ($p = 0.1181$). The LOEO and LOEO/DZP groups were also similar ($p = 0.1023$). The muscle contraction power of the DZP group was greater than that of the LOEO/DZP group (Figure 2F).

3.2 Behavioral assessment

The behavioral assessment of the animals was conducted to determine the progression of seizures (Table 1). Animals treated with PTZ rapidly progressed to tonic-clonic seizures with loss of postural reflex.

The group treated with DZP + PTZ exhibited the longest latency to the onset of convulsive seizures compared to the LOEO + PTZ group. However, when compared to the LOEO/DZP + PTZ combination, it showed significantly shorter latency. In the LOEO + PTZ group, animals did not progress to generalized clonic seizures. In the LOEO/DZP + PTZ group, animals only displayed whisker piloerection and generalized tremor, indicating greater stabilization of convulsive symptoms compared to DZP + PTZ. These results suggest that LOEO, when combined with DZP, can provide effective control of convulsive seizures by potentiating its effects.

3.3 Electrocorticographic evaluation

The animals in group I (physiological saline) exhibited amplitudes below 0.1 mV in the trace, and it can be observed in the corresponding spectrogram that the highest energy concentrations are below 10 Hz (Figure 3A). Group II (LOEO) showed little variation compared to the control group, although the spectrogram displayed greater intensity in oscillations up to 40 Hz (Figure 3B). Group III (DZP) displayed oscillations with amplitudes below 0.5 mV in the trace, with energy concentration below 10 Hz (Figure 3C). These groups did not maintain statistical differences and showed trace characteristics similar to the control group. In contrast, group IV (LOEO/DZP) (Figure 3D) displayed oscillations with amplitudes below 0.5 mV in the trace, and energy concentration below 15 Hz. On the other hand, group V (PTZ) exhibited significant alterations in the EEG trace, with peak amplitudes exceeding 0.5 mV, and activities characterized by constant levels of high-frequency and high-amplitude wave peaks (Figure 3E).

For group VI (LOEO + PTZ), the electrocorticographic trace did not show variations above 0.5 mV in amplitude, indicating seizure control. The spectrogram demonstrated an increase in power below 20 Hz (Figure 3F). For group VII (DZP + PTZ), the electrocorticographic trace did not show variations above 0.1 mV

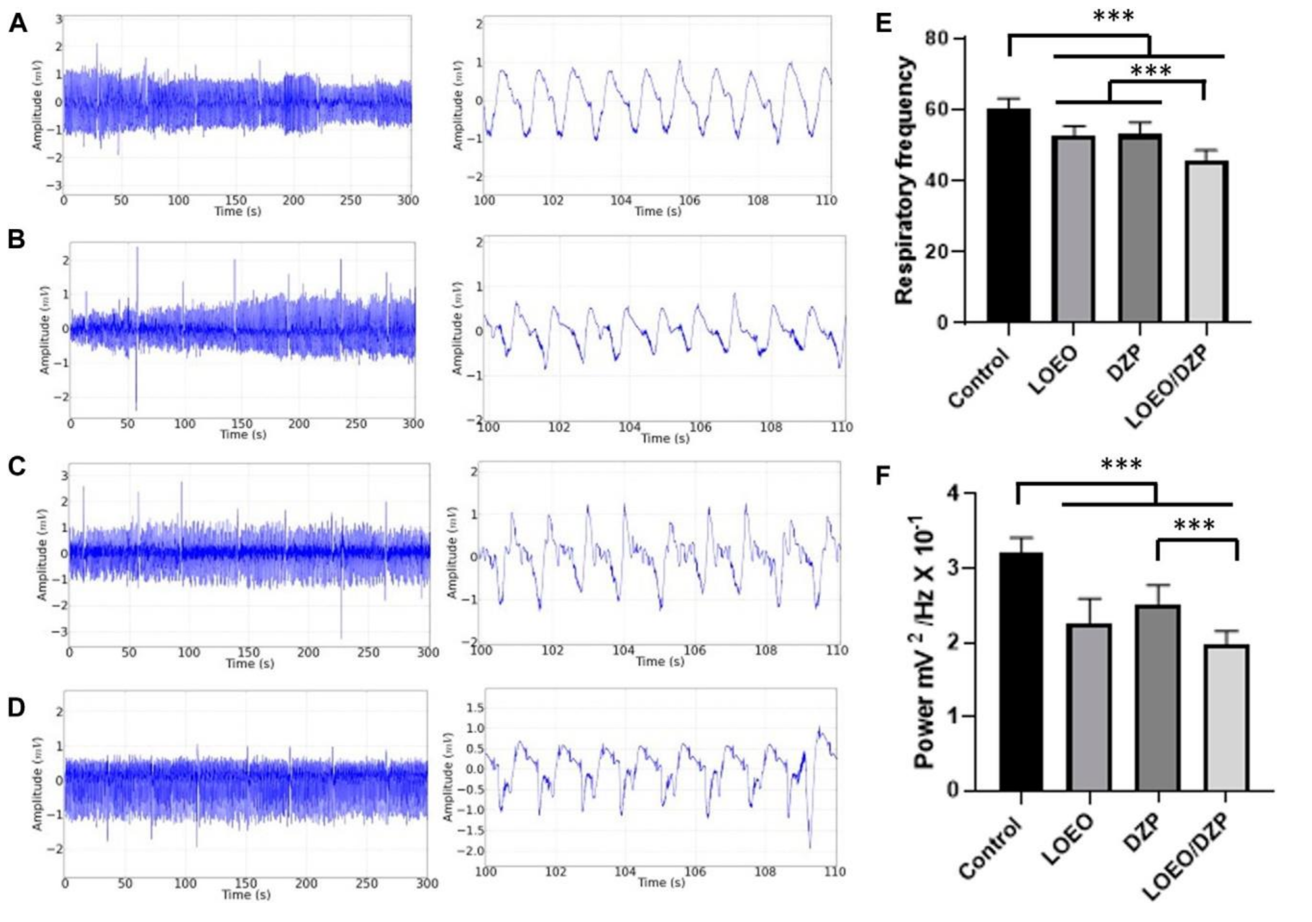

FIGURE 2
Demonstrations of electromyographic recordings performed in the 10th intercostal muscle of the rat. Control group (A); Lippia origanoides group (LOEO) (B); Diazepam group (DZP) (C); LOEO/DZP group (D); Graph showing the mean respiratory frequency for the groups during treatment (E); Graph depicting the mean power of intercostal muscle contractions in animals subjected to treatment (F). Recordings had a duration of 5 min. After ANOVA and Tukey's test, (***) $p < 0.0001$.

TABLE 1 Behavioral Characterization for Latencies of Excitability Behaviors Induced by PTZ (control group), Diazepam followed by PTZ application, and LOEO followed by PTZ application. (*) indicates statistical difference for the PTZ group, (#) represents statistical difference for the DZP + PTZ group, and (+) represents statistical difference for the LOEO group. After ANOVA followed by Tukey's test, a significance level of *$p < 0.05$, **$p < 0.001$, and ***$p < 0.0001$ was adopted.

Comportamento/ Latência (S)	Whisker piloerection	Orofaciais movements	Generalized tremor	Anterior limb espasms	Isolated clonic seizure without loss of postural reflex	Generalizes clonic seizure with transiente loss of postural reflex	Tonic-clonic seizure with loss of postural reflex
PTZ	59.0 ± 5.809	74.11 ± 8.738	84.67 ± 6.164	96.33 ± 4.637	113.1 ± 8.860	161.1 ± 22.74	207.43 ± 16.32
DZP + PTZ	131.6 ± 19.17***	180.9 ± 10.88***	242.6 ± 25.49***	304.2 ± 40.17***	—	—	—
LOEO + PTZ	117.7 ± 7.14***	153.0 ± 12.96***/###	193.4 ± 12.07***/###	280.3 ± 10.65***	338.7 ± 34.7***	—	—
LOEO/DZP + PTZ	172.0 ± 16.47***/ ###/+++	—	296.3 ± 21.17***/###/+++	—	—	—	—
F-value and *p*-value	$F(3,34) = 108.9$ $p < 0.0001$	$F(2,24) = 228.3$ $p < 0.0001$	$F(2,24) = 228.2$ $p < 0.0001$	$F(2,24) = 199.8$ $p < 0.0001$	—	—	—

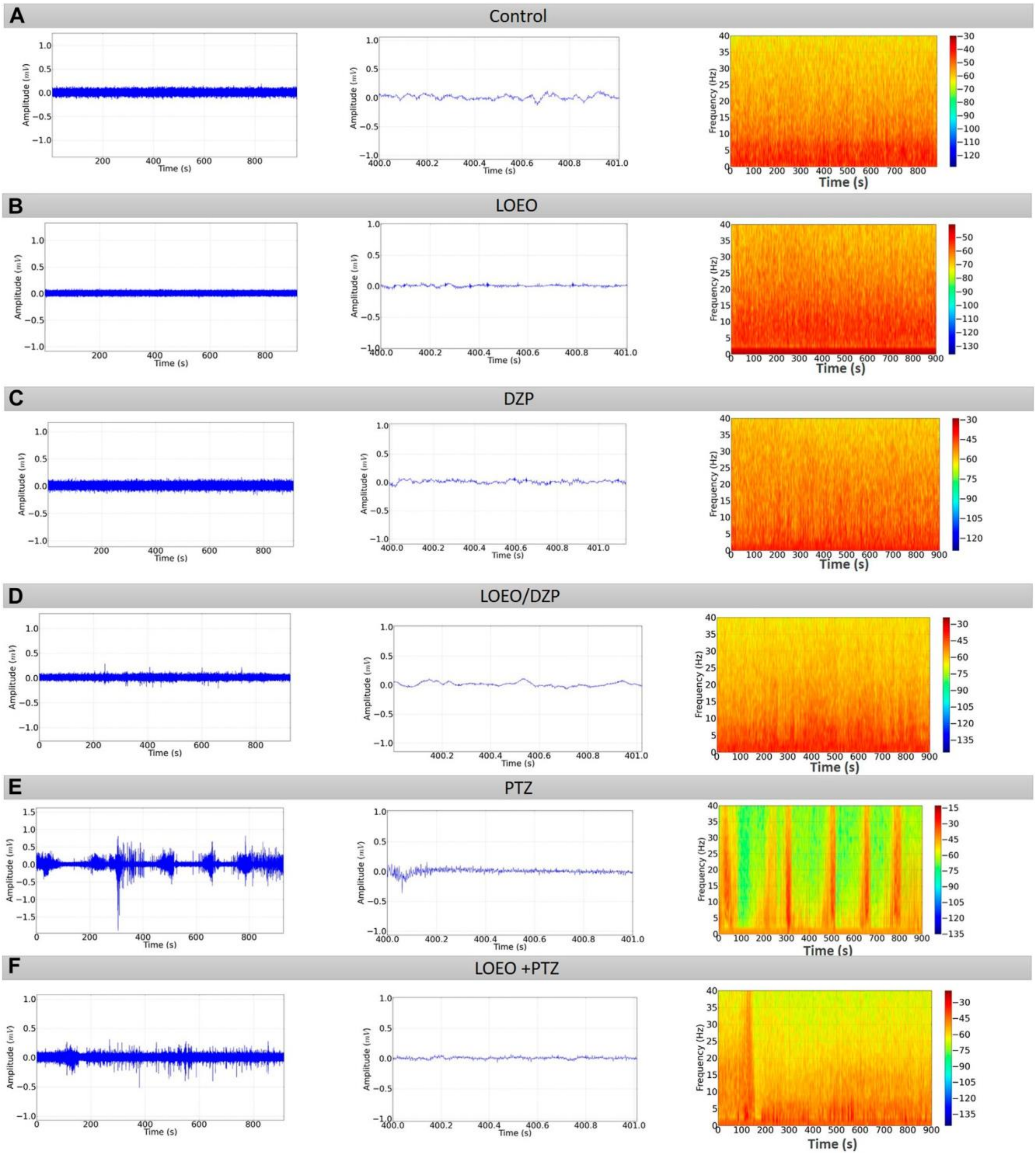

FIGURE 3

(Continued)

in amplitude, indicating seizure control. However, the spectrogram displayed an increase in power below 20 Hz (Figure 3G).

In group VIII, (LOEO/DZP + PTZ), the electrocorticographic trace did not show variations above 0.1 mV in amplitude, possibly indicating potentiation of the effect of DZP when combined with LOEO. The spectrogram displayed an increase in power below 15 Hz (Figure 3H).

Total power varied significantly between groups: group I $0.6268 \pm 0.1064\,mV^2/Hz \times 10^{-3}$ and group II $0.1946 \pm 0.06929\,mV^2/Hz \times 10^{-3}$ ($p = 0.0012$), group VI $0.9680 \pm 0.1696\,mV^2/Hz \times 10^{-3}$ ($p = 0.0211$). However, groups III $0.5868 \pm 0.06176\,mV^2/Hz \times 10^{-3}$ ($p > 0.9999$), group IV $0.6718 \pm 0.1204\,mV^2/Hz \times 10^{-3}$ ($p = 0.9998$), group VII $0.7690 \pm 0.1624\,mV^2/$

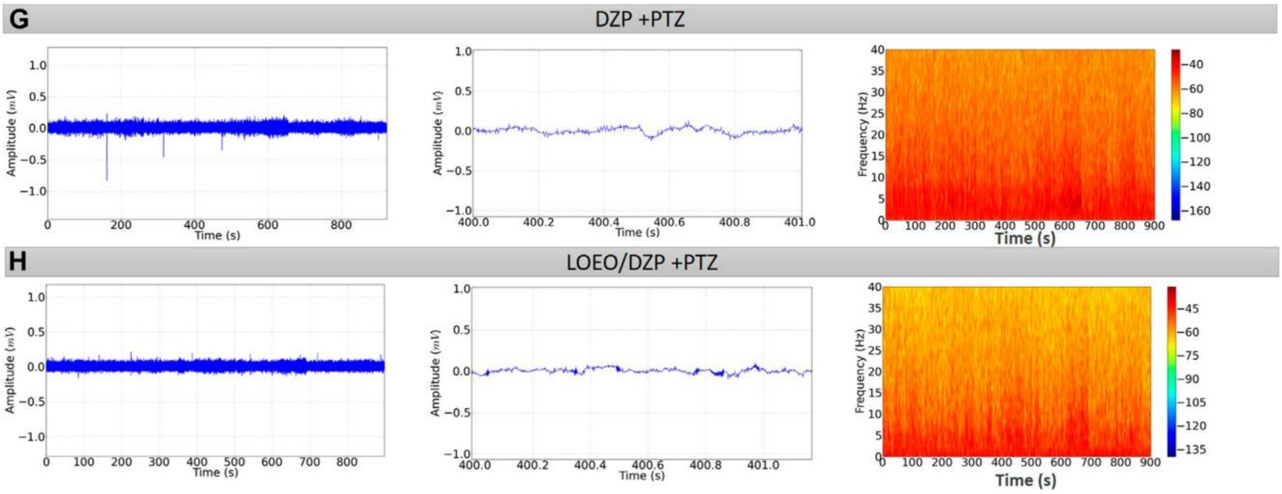

FIGURE 3
(Continued). Electrocorticography (ECoG) recording, representative 1s fragment (400–401 s) of the ECoG recording (center) and frequency spectrogram (right). Control group (A); Lippia origanoides group (LOEO) (B); Diazepam (DZP) (C); LOEO + DZP group (LOEO + DZP) (D); Pentylenetetrazol (PTZ) (E), LOEO + PTZ (F); DZP + PTZ (G) and LOEO/DZP + PTZ (H).

Hz × 10^{-3} ($p = 0.8363$), and group VIII 0.4031 ± 0.09328 mV^2/Hz × 10^{-3} ($p = 0.3313$) no showed a difference to the saline control group. The administration of PTZ 2.215 ± 0.5042 mV^2/Hz × 10^{-3} (group V) resulted in a significant increase in power and presented a statistical difference in relation to all groups [$F(7,64) = 77.25$; $p < 0.001$] (Figure 4A).

Significant variation was found between groups II and VI (LOEO and LOEO + PTZ), groups II and VII (LOEO/DZP + PTZ), and between group VI and VIII (LOEO + PTZ and LOEO/DZP + PTZ).

For delta oscillations, the control group presented an average power of 0.1609 ± 0.02492 mV^2/Hz × 10^{-3}, showing significant variation between the following groups: group II with 0.02884 ± 0.004213 mV^2/Hz × 10^{-3} ($p = 0.0276$) and group VII with 0.2948 ± 0.03959 mV^2/Hz × 10^{-3} ($p = 0.0241$). However, it was similar to groups III with 0.1624 ± 0.01824 mV^2/Hz × 10^{-3} ($p > 0.9999$), group IV with 0.1487 ± 0.03489 mV^2/Hz × 10^{-3} ($p > 0.999$) and group VIII with 0.1313 ± 0.03135 mV^2/Hz × 10^{-3} ($p = 0.9949$). However, significant differences were observed between groups V with 0.7095 ± 0.2105 mV^2/Hz × 10^{-3} and VI with 0.4827 ± 0.08197 mV^2/Hz × 10^{-3}, for the other groups (Figure 4B).

For theta oscillations, group I presented an average power of 0.1626 ± 0.01493 mV^2/Hz × 10^{-3} and showed no statistical difference with group III 0.1272 ± 0.01868 mV^2/Hz × 10^{-3} ($p = 0.3057$), group IV 0.1275 ± 0.02840 mV^2/Hz × 10^{-3} ($p = 0.3139$) and group VI 0.2043 ± 0.03789 mV^2/Hz × 10^{-3} ($p = 0,1322$). Significant statistical differences were observed between groups II 0.1626 ± 0.01493 mV^2/Hz × 10^{-3}, V 0.5975 ± 0.06429 mV^2/Hz × 10^{-3} and VII 0.2595 ± 0.03483 mV^2/Hz × 10^{-3} and group VIII 0.1142 ± 0.01620 mV^2/Hz × 10^{-3} ($p = 0.0470$) (Figure 4C).

For alpha oscillations, group I presented an average linear power of 0.07834 ± 0.01241 mV^2/Hz × 10^{-3} and showed no statistical difference with group III (0.06866 ± 0.01372 mV^2/Hz × 10^{-3}, $p = 0.9226$), group VI (0.05895 ± 0.008050 mV^2/Hz × 10^{-3}, $p = 0.2384$) and group VII (0.08349 ± 0.01473 mV^2/Hz × 10^{-3}, $p = 0.9980$). Significant statistical differences were observed between groups II (0.02046 ± 0.002646 mV^2/Hz × 10^{-3}), group IV (0.03024 ± 0.008092 mV^2/Hz × 10^{-3}) and group V (0.3096 ± 0.03782 mV^2/Hz × 10^{-3}) and group VIII (0.05244 ± 0.003926 mV^2/Hz × 10^{-3}). The PTZ group had a higher mean linear power in alpha oscillations (Figure 4D).

For beta oscillations, the control group exhibited an average linear power of 0.08061 ± 0.01036 mV^2/Hz × 10^{-3}, with no statistical difference observed in comparison to group III (0.08187 ± 0.01351 mV^2/Hz × 10^{-3}, $p > 0.9999$), group IV (0.02291 ± 0.007150 mV^2/Hz × 10^{-3}, $p = 0.6753$), group VI (0.08120 ± 0.01311 mV^2/Hz × 10^{-3}, $p > 0.9999$), group VII (0.1060 ± 0.01166 mV^2/Hz × 10^{-3}, $p = 0.9948$), and group VIII (0.06128 ± 0.01399 mV^2/Hz × 10^{-3}, $p = 0.9991$). Significant statistical difference was observed only in group V (0.5655 ± 0.1993 mV^2/Hz × 10^{-3}) (Figure 4E).

For gamma oscillations, the control group exhibited an average linear power of 0.02402 ± 0.004807 mV^2/Hz × 10^{-3}, with no statistical difference observed for group II (0.007608 ± 0.003549 mV^2/Hz × 10^{-3}, $p = 0.9667$), group III (0.03010 ± 0.004594 mV^2/Hz × 10^{-3}, $p > 0.9999$), group IV (0.009768 ± 0.002647 mV^2/Hz × 10^{-3}, $p = 0.9849$), group VI (0.01174 ± 0.001755 mV^2/Hz × 10^{-3}, $p = 0.9938$), group VII (0.01953 ± 0.003204 mV^2/Hz × 10^{-3}, $p > 0.9999$), and group VIII (0.01391 ± 0.002762 mV^2/Hz × 10^{-3}, $p = 0.9982$). Significant statistical difference was observed only in group V (0.2121 ± 0.09447 mV^2/Hz × 10^{-3}) (Figure 4F).

4 Discussion

In this study, we have demonstrated, for the first time, that the essential oil of Lippia origanoides was able to increase the convulsive threshold induced by PTZ in rats. This was achieved through the analysis of behavior, electroencephalographic (EEG) and electromyographic patterns after the administration of LOEO alone, as well as the evaluation of the LOEO/DZP combination and its response compared to diazepam.

10.3389/fphar.2023.1289336

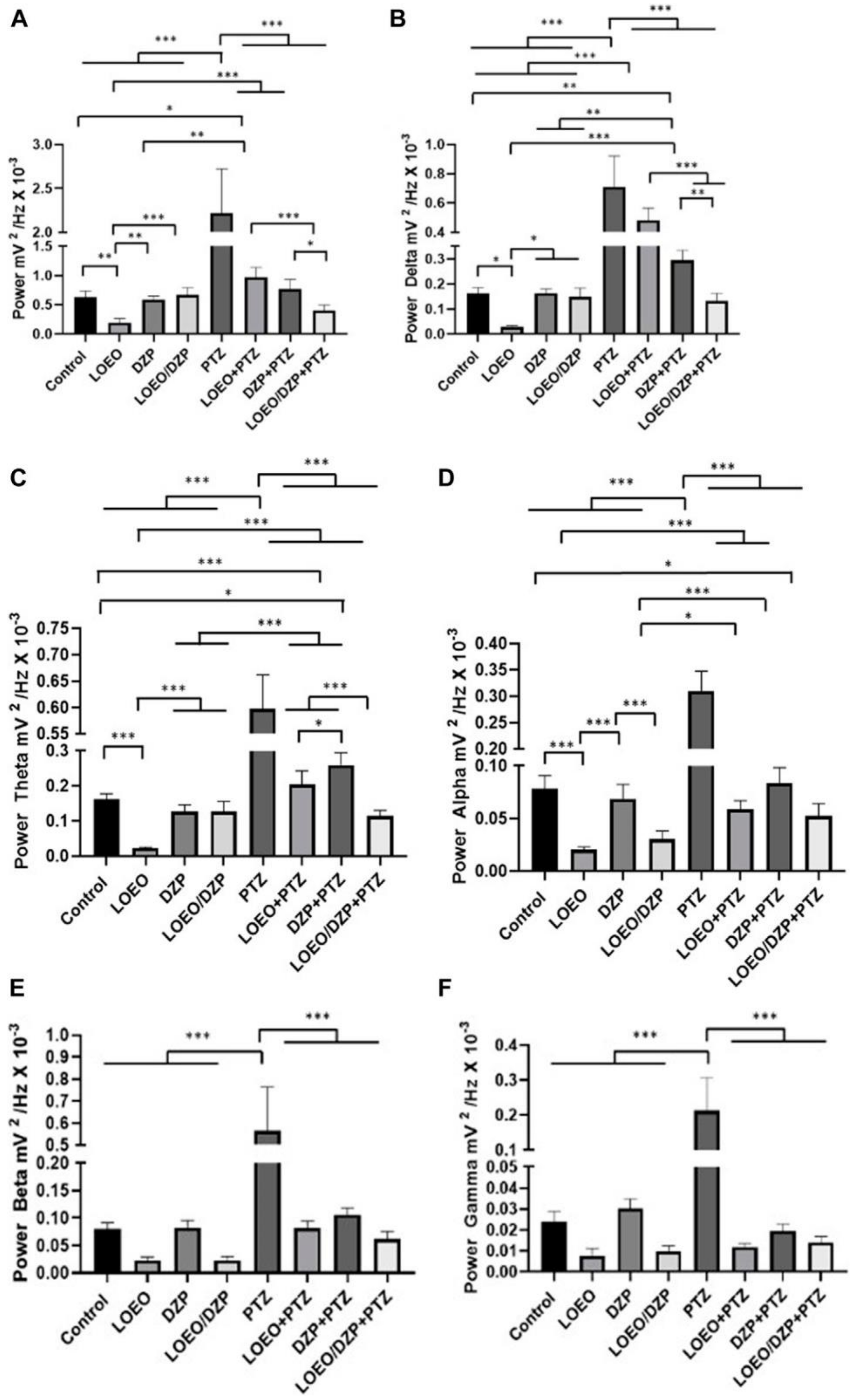

FIGURE 4
Total linear power analysis of brain waves up to 40 Hz (A) and quantitative linear frequency distribution of brain waves: (B) delta waves; (C) theta waves; (D) alpha waves; (E) beta waves and (F) gamma waves, recorded by electrocorticography. Data show drugs associated and not associated with pentylenetetrazole (*p < 0.05, **p < 0.001 and ***p < 0.0001).

The present study demonstrated significant differences in the respiratory frequency depression of the group treated with LOEO in combination with DZP compared to the other groups in relation to respiratory behavior patterns, as assessed through electromyography of the 10th intercostal muscle.

These respiratory depressant effects caused by the LOEO/DZP combination suggest various therapeutic targets. Some anesthetic medications are known respiratory depressants, such as propofol, sevoflurane, and midazolam, acting as GABA receptor agonists and NMDA receptor antagonists (Pattinson, 2008).

The depressant respiratory response shown in our results by the LOEO/DZP combination suggests the need for further investigations to elucidate the underlying mechanisms triggering the decrease in respiratory frequency after its combined administration.

In recent a study (Hamoy et al., 2018), behavioral and electrocorticographic assessments were extremely useful to evaluate and compare the changes arising from disordered neuronal excitability that generate seizures and consequent epileptic conditions. In this research, the electrocorticogram of rat cortex was examined and it was demonstrated that the administration of PTZ in rats presented continuous discharges and high amplitude waves, being this effect reversed by conventional anticonvulsants (phenobarbital, phenytoin and diazepam). Our results corroborate with the whole protocol of this study, using diazepam as conventional antiepileptic.

Thymol (2-isopropyl-5-methylphenol), the most abundant constituent (57.46%) in LOEO and several other essential oils, is an isomer of carvacrol (2-methyl-5-1 methylethylphenol), a monoterpene also present in LOEO (1.42%). Thymol can manifest as a white crystalline powder or colorless crystals and are constituents of the essential oils of several plants (Nunes et al., 2005; Guillen et al., 2007).

The effects of thymol that have been studied and described in the literature include its antimicrobial and antiseptic actions (Matos et al., 2000; Kachur and Suntres, 2020). Both carvacrol and thymol exhibit high antioxidant activity, serving as natural food preservatives that inhibit peroxidation of phospholipid liposomes and demonstrating antifungal activities (Milos et al., 2000; Teissedre and Waterhouse, 2000; Mastelic et al., 2008). Other natural monoterpenoids have a wide range of pharmacological properties, such as local anesthetic, anticancer, antihistaminic, anti-inflammatory, antiviral, and neuroprotective activities (Volcho et al., 2018).

In previous studies on the central action of carvacrol, its effects on experimental models of anxiety and depression in mice were demonstrated, suggesting involvement of the GABAergic system through the GABA-A receptor, similarly to benzodiazepines that have high affinity for these receptors. In evaluating the antidepressant effects of carvacrol, the mechanism of action was shown to be associated with the dopaminergic system, possibly through stimulation of D1 and D2 receptors (Melo et al., 2010; Melo et al., 2011). Other studies have also shown central nervous system actions of monoterpenes, exhibiting anxiolytic and antidepressant effects (Umezu and Morita, 2003; Silva et al., 2007).

In a study the authors suggested that the mechanism of action of the isomers carvacrol and thymol is related to the modulation of GABAergic ionotropic receptors with Cl^- channels, as the monoterpenes bound to GABA receptors increased the uptake of $^{36}Cl^-$ (Tong and Coats, 2010).

Analogues of carvone, such as carvacrol, were able to inhibit neuronal excitability in the sciatic nerve of rats, probably by blocking voltage-dependent sodium channels. The authors also observed that the structure of the compounds interfered with their ability to block channels. This capability of these compounds to alter their chemical structures can be effective in delivering drugs that act directly on their targets, without affecting other organisms (Gonçalves et al., 2010).

To evaluate the mechanisms by which carvacrol promoted the inhibition of neuronal excitability, the authors demonstrated through several tests that carvacrol is able to block neuronal excitability in a reversible and concentration-dependent manner through direct inhibition of voltage-dependent sodium channels, which suggests its effect as a local anesthetic (Joca et al., 2012).

Studies using oils and plant extracts have demonstrated the potentiation of GABAergic pathways in the control of convulsive crises triggered by pentylenetetrazole (de Oliveira et al., 2022; Muto et al., 2022; Nascimento et al., 2022). This effect is allosterically potentiated by benzodiazepines such as Diazepam, which favors the hyperpolarization of the neuronal membrane (Aburawi et al., 2021). LOEO demonstrated the ability to mitigate the intensity of PTZ-induced seizures, increasing the latency for the onset of behavior, as evidenced by ECoG. It was observed that the anticonvulsant activity of LOEO increased seizure control when associated with Diazepam, which demonstrated a potentiating effect on seizure control.

In summary, the outcomes of this study underscore the potential utility of LOEO in managing convulsive seizures, while its synergistic combination with DZP opens a promising pathway for the development of new agents targeting refractory epilepsy. Moreover, these findings contribute significantly to the deeper comprehension of the mechanisms underlying epilepsy.

5 Conclusion

The current study revealed that treatment with LOEO led to distinct alterations in electrocorticographic tracings, showcasing attributes of a potent anticonvulsant agent. Moreover, the combination of LOEO with diazepam yielded a more favorable response compared to any individual drug administration, resulting in increased convulsive threshold and respiratory depression. This finding holds significant implications, as the synergistic effect of Lippia origanoides essential oil with diazepam may represent a valuable therapeutic approach for the treatment of epilepsy, enhancing therapeutic efficacy while minimizing adverse effects.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by the Animal Ethics Committee (CEUA—UFPA) number 1395260821. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

Conceived and designed the experiments: D.B.d.A., M.H. Performed the experiments: D.B.d.A., A.L.G.d.A., G.B.B., M.K.O.H and M.H. Wrote the paper: all authors. Financial support and administrative support: M.H and V.J.d.M. All authors have read and agreed to the published version of the manuscript.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by Fundação Amazônia de Amparo a Estudos e Pesquisas do Estado do Pará (FAPESPA) and The APC was funded by Pró-Reitoria de Pesquisa e Pós-Graduação—PROPESP/UFPA.

Acknowledgments

The authors would like to acknowledge Fundação Amazônia de Amparo a Estudos e Pesquisas do Estado do Pará (FAPESPA) for their financial support provided of this research and Programa de Pós graduação em Farmacologia e Bioquímica (FARMABIO).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Aburawi, S., Shushni, M., and Alkateb, M. (2021). Effect of Moringa Oleifera extract on the behavior of male albino rats. *AlQalam J. Med. Appl. Sci.* 4 (2), 1–12. doi:10.5281/zenodo.4667396

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Subhan, F., and Ahmed, J. (2017). Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants. *Front. Aging Neurosci.* 9, 168. doi:10.3389/fnagi.2017.00168

Brodie, M. J., Barry, S. J., Bamagous, G. A., Norrie, J. D., and Kwan, P. (2012). Patterns of treatment response in newly diagnosed epilepsy. *Neurology* 78, 1548–1554. doi:10.1212/WNL.0b013e3182563b19

de Almeida, L. C. N., de Andrade Marques, B., Silva, R. L., Hamoy, A. O., de Mello, V. J., Borges, R. D. S., et al. (2020). New nanocarried phenobarbital formulation: maintains better control of pentylenetetrazole-Induced seizures. *Biotechnol. Rep. (Amst)* 9, e00539, e00539. doi:10.1016/j.btre.2020.e00539

de Almeida, R. N., Agra Mde, F., Maior, F. N., and de Sousa, D. P. (2011). Essential oils and their constituents: anticonvulsant activity. *Molecules* 16, 2726–2742. doi:10.3390/ molecules16032726

de Oliveira, F. R., da Silva, N. M., Hamoy, M., Crespo-López, M. E., Ferreira, I. M., da Silva, E. O., et al. (2022). The GABAergic system and endocannabinoids in epilepsy and seizures: what can we expect from plant oils? *Molecules* 27 (11), 3595. doi:10.3390/molecules27113595

Dobetsberger, C., and Buchbauer, G. (2011). Actions of essential oils on the central nervous system: an updated review. *Flavour Fragr. J.* 26, 300–316. doi:10.1002/ffj.2045

Duncan, J. S., Sander, J. W., Sisodiya, S. M., and Walker, M. C. (2006). Adult epilepsy.

Lancet 367, 1087–1100. doi:10.1016/S0140-6736(06)68477-8

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., et al. (2014). ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 55, 475–482. doi:10.1111/epi.12550

Fisher, R. S., Boas, W. V. E., Blume, W., Elger, C., Genton, P., Lee, P., et al. (2005). Epileptic seizures and epilepsy: definitions proposed by the international league against epilepsy (ILAE) and the international bureau for epilepsy (IBE). *Epilepsia* 46, 470–472. doi:10.1111/j.0013-9580.2005.66104.x

Gonçalves, J. C. R., Alves, A. M. H., Araújo, A. E. V., Cruz, J. S., and Araújo, D. A. M. (2010). Distinct effects of carvone analogues on the isolated nerve of rats. *Eur.*

J. Pharmacol. 645, 108–112. doi:10.1016/j.ejphar.2010.07.027

Guillen, F. P. J., Zapata, D., Martinez-Romero, S., Castillo, M. S., and Valero, D. (2007). Improvement of the overall quality of table grapes stored under modified atmosphere packaging in combination with natural antimicrobial compounds. *J. Food Sci.* 72, 185–190. doi:10.1111/j.1750-3841.2007.00305.x

Hamoy, M., Batista, L. S., de Mello, V. J., Gomes-Leal, W., Farias, R. A. F., Batista, P. S., et al. (2018). Cunaniol-elicited seizures: behavior characterization and electroencephalographic analyses. *Toxicol. Appl. Pharmacol.* 360, 193–200. doi:10.1016/j.taap.2018.10.008

Joca, H. C., Cruz-Mendes, Y., Oliveira-Abreu, K., Maia-Joca, R. P. M., Barbosa, R., Lemos, T. L., et al. (2012). Carvacrol decreases neuronal excitability by inhibition of voltage-gated sodium channels. *J. Nat. Prod.* 75, 1511–1517. doi:10.1021/np300050g

Kachur, K., and Suntres, Z. (2020). The antibacterial properties of phenolic isomers, carvacrol and thymol. *Crit. Rev. Food Sci. Nutr.* 60, 3042–3053. doi:10.1080/10408398.2019.1675585

Mastelic, J., Jerković, I., Blazević, I., Poljak-Blazi, M., Borović, S., Ivancić-Baće, I., et al. (2008). Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. *J. Agric. Food Chem.* 6, 3989–3996. doi:10.1021/jf073272v

Matos, F. J. A., Machado, M. I. L., Craveiro, A. A., Alencar, J. W., and Silva, M. G. V. (2000). Medicinal plants of Northeast Brazil containing thymol and carvacrol-*Lippia sidoides* Cham.and *L.gracilis* H.B.K (Verbenaceae). *J. Essent. Oil Res.* 11, 666–668. doi:10.1080/10412905.1999.9711990

Melo, F. H. C., Moura, B. A., Sousa, P. D., Vasconcelos, S. M. M., Macedo, D. S., Fonteles, M. M. F., et al. (2011). Antidepressant-like effect of Carvacrol (5-isopropyl-2-methylphenol) in mice: involvement of dopaminergic system. *Fundam. Clin. Pharmacol.* 25, 362–367. doi:10.1111/j.1472-8206.2010.00850.x

Melo, F. H. C., Venâncio, E. T., Sousa, D. P., Fonteles, M. M. F., Vasconcelos, S. M. M., Viana, G. S. B., et al. (2010). Anxiolytic-like effect of Carvacrol (5-isopropyl-2 methylphenol) in mice: involvement with GABAergic transmission. *Fundam. Clin. Pharmacol.* 24, 437–443. doi:10.1111/j.1472-8206.2009.00788.x

Milos, M., Mastelic, J., and Jerkovic, I. (2000). Chemical composition and antioxidante effect of glycosidically bound volatile compouds from orégano (*Origanum vulgare L. ssp hirtum*). *Food Chem.* 71, 79–83. doi:10.1016/S0308-8146(00)00144-8

Morton, J. F. (1981). *Atlas of medicinal plants of Middle America, Bahamas to yucatan*. Springfield (IL): C. Thomas.

Muto, N. A., Hamoy, M., da Silva Ferreira, C. B., Hamoy, A. O., Lucas, D. C. R., de Mello, V. J., et al. (2022). Extract of *Euterpe oleracea* martius stone presents anticonvulsive activity *via* the GABAA receptor. *Front. Cell Neurosci.* 11 (16), 872743. doi:10.3389/fncel.2022.872743

Nascimento, C. P., Ferreira, L. O., Silva, A. L. M., Silva, A. B. N., Rodrigues, J. C. M., Teixeira, L. L., et al. (2022). Combination of *curcuma longa* and diazepam attenuates seizures and subsequent hippocampal neurodegenaration. *Front. Cell. Neurosci.* 16, 884813. doi:10.3389/fncel.2022.884813

Neto, A. C., Netto, J. C., Pereira, P. S., Pereira, A. M. S., Taleb-Contini, S. H., França, S. C., et al. (2009). The role of polar phytocomplexes on anticonvulsant effects of leaf extracts of *Lippia alba* (Mill.) N.E. Brown chemotypes. *J. Pharm. Pharmacol.* 61, 933–939. doi:10.1211/jpp/61.07.0013

Nunes, R. S., Lira, A. M., Ximenes, E., Silva, J. A., and Santana, D. P. (2005). Characterization of the *Lippia sidoides* in vegetable raw material for pharmaceutical products. *Scentia Plena* 1, 182–184.

Oliveira, D. R., Leitão, G. G., Fernandes, P. D., and Leitão, S. G. (2014). Ethnopharmacological studies of Lippia origanoides. *Braz. J. Pharmacogn.* 24, 206–214. doi:10.1016/j.bjp.2014.03.001

Pascual, M. E., Slowing, K., Carretero, E., Sánchez Mata, D., and Villar, A. (2001). Lippia: traditional uses, chemistry and pharmacology: a review. *J. Ethnopharmacol.* 76, 201–214. doi:10.1016/s0378-8741(01)00234-3

Pattinson, K. T. S. (2008). Opioids and the control of respiration. *Br. J. Anaesth.* 100, 747–758. doi:10.1093/bja/aen094

Perucca, P., and Gilliam, F. G. (2012). Adverse effects of antiepileptic drugs. *Lancet Neurol.* 11, 792–802. doi:10.1016/S1474-4422(12)70153-9

Rashed, A. A., Rahman, A. Z. A., and Rathi, D. N. G. (2021). Essential oils as a potential neuroprotective remedy for age-related neurodegenerative diseases: a review. *Molecules* 19 (4), 1107. doi:10.3390/molecules26041107

Rashidian, A., Farhang, F., Vahedi, H., Dehpour, A. R., Mehr, S. E., Mehrzadi, S., et al. (2016). Anticonvulsant effects of *Lippia citriodora* (Verbenaceae) leaves ethanolic extract in mice: role of gabaergic system. *Int. J. Prev. Med.* 7, 97. doi:10.4103/2008-7802.187251

Santos, G. F. S., Ferreira, L. O., Gerrits Mattos, B., Fidelis, E. J., de Souza, A. S., Batista, P. S., et al. (2021). Electrocorticographic description of the effects of anticonvulsant drugs used to treat lidocaine-induced seizures. *Brain Behav.* 11 (2), e01940. doi:10.1002/ brb3.1940

Sattayakhom, A., Wichit, S., and Koomhim, P. (2023). The effects of essential oils on the nervous system: a scoping review. *Molecules* 28 (9), 3771. doi:10.3390/ molecules28093771

Silva, M. I. G., Neto, M. R. A., Neto, P. F. T., Moura, B. A., Amaral, J. F., Sousa, D. P., et al. (2007). Central nervous system activity of acute administration of isopulegol in mice. *Pharmacol. Biochem. Behav.* 88, 141–147. doi:10.1016/j.pbb. 2007.07.015

Siqueira-Lima, P. S., Passos, F. R. S., Lucchese, A. M., Menezes, I. R. A., Coutinho, H. D. M., Lima, A. A. N., et al. (2019). Central nervous system and analgesic profiles of Lippia genus. *Braz. J. Pharmacogn.* 29, 125–135. doi:10.1016/j.bjp.2018.11.006

Souza-Monteiro, J. R., Hamoy, M., Santana-Coelho, D., Arrifano, G., Paraense, R. S. O., Costa-Malaquias, A., et al. (2015). Anticonvulsant properties of Euterpe oleracea in mice. *Neurochem. Int.* 90, 20–27. doi:10.1016/j.neuint.2015.06.014

Sultana, B., Panzini, M.-A., Veilleux Carpentier, A., Comtois, J., Rioux, B., Gore, G., et al. (2021). Incidence and prevalence of drug-resistant epilepsy: a systematic review and meta-analysis. *Neurology* 96, 805–817. doi:10.1212/WNL. 0000000000011839

Teissedre, P. L., and Waterhouse, A. L. (2000). Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *J. Agric. Food Chem.* 48, 3801–3805. doi:10.1021/jf990921x

Tong, F., and Coats, J. R. (2010). Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl− uptake in American cockroach ventral nerve cord. *Biochem. Physiol.* 98, 317–324. doi:10.1016/j. pestbp.2010.07.003

Umezu, T., and Morita, M. (2003). Evidence for the involvement of dopamine in ambulation promoted by menthol in mice. *J. Pharmacol. Sci.* 91, 125–135. doi:10.1254/ jphs.91.125

Volcho, K. P., Laev, S. S., Ashraf, G. M., Aliev, G., and Salakhutdinov, N. F. (2018). Application of monoterpenoids and their derivatives for treatment of neurodegenerative disorders. *Curr. Med. Chem.* 25, 5327–5346. doi:10.2174/ 0929867324666170112101837

Zétola, M., de Lima, T. C. M., Sonaglio, D., González-Ortega, D., Limberger, R. P., Petrovick, P. R., et al. (2002). CNS activities of liquid and spray-dried extracts from *Lippia alba* – verbenaceae (Brazilian false melissa). *J. Ethnopharmacol.* 82, 207–215. doi:10.1016/s0378-8741(02)00187-3

3. ARTIGO 2: Óleo essencial de *Rosmarinus officinalis* desencadeia depressão seguida de excitabilidade do SNC em ratos Wistar

frontiers | Frontiers in Natural Products

TYPE Original Research
PUBLISHED 12 August 2024
DOI 10.3389/fntpr.2024.1394657

Rosmarinus officinalis essential oil triggers depression followed by CNS excitability in Wistar rats

Daniella Bastos de Araújo*, Ysis Nayhara Raiol de Almeida, Maria Klara Otake Hamoy, Luana Vasconcelos de Souza, Priscille Fidelis Pacheco Hartcopff, Rodrigo Gonçalves dos Santos, Lívia Letícia Santos da Silva, Lucas Lima da Rocha, Murilo Farias dos Santos, Yris da Silva Deiga, Rayllan da Cunha Ferreira, Raíssa Vieira de Souza, Gabriela Brito Barbosa and Moisés Hamoy*

Laboratory of Pharmacology and Toxicology of Natural Products, Institute of Biological Science, Federal University of Pará, Belém, Brazil

OPEN ACCESS

EDITED BY
Ping-Jyun Sung,
National Museum of Marine Biology and Aquarium, Taiwan

REVIEWED BY
Ankanahalli N. Nanjaraj Urs,
Washington University in St. Louis, United States
Atallah F. Ahmed,
King Saud University, Saudi Arabia

*CORRESPONDENCE
Daniella Bastos de Araújo,
danivivaf@gmail.com
Moisés Hamoy,
hamoyufpa@gmail.com

RECEIVED 01 March 2024
ACCEPTED 16 May 2024
PUBLISHED 12 August 2024

CITATION
Bastos de Araújo D, Raiol de Almeida YN, Otake Hamoy MK, Vasconcelos de Souza L, Pacheco Hartcopff PF, Gonçalves dos Santos R, Santos da Silva LL, Lima da Rocha L, Farias dos Santos M, da Silva Deiga Y, da Cunha Ferreira R, Vieira de Souza R, Barbosa GB and Hamoy M (2024), *Rosmarinus officinalis* essential oil triggers depression followed by CNS excitability in Wistar rats.
Front. Nat. Produc. 3:1394657.
doi: 10.3389/fntpr.2024.1394657

The essential oil of rosemary (*Rosmarinus officinalis*) (EORO) is widely used in folk medicine and has proven therapeutic effects. Our research evaluated high doses of rosemary essential oil in 54 Wistar rats between 180 and 200 g. The study consisted of three experiments: 1) behavioral monitoring of the animals after administration of 500 mg/kg i.p.; 2) electrocorticographic records after drug administration; 3) anticonvulsant drug reaction, where phenytoin, phenobarbital, and diazepam 10 mg/kg i.p were applied. The results showed that the application of EORO presented two phases. Phase 1 was characterized by the appearance of myorelaxation and a reduction in the power of the electrocorticogram in low-frequency cerebral oscillations. Phase 2 was characterized by increased excitability, with the appearance of convulsions and the increased power of electrocorticographic recordings in cerebral oscillations up to 40 Hz. In this phase, three tracing patterns were observed. Beta oscillations were the most prevalent and were better controlled by diazepam, which demonstrates that the excitatory activity of EORO is related to the reduction of GABAergic activity.

KEYWORDS
ethnopharmacology, electrocorticographic record, behavioral 7. characterization, rosemary essential oil, Rosmarinus officinalis

1 Introduction

Essential oils and their constituents have been presented as possible modulators of the central nervous system (CNS) (Figuêredo et al., 2019), and the presence of components such as terpenes in their constitutions is known to have anxiolytic, antidepressant, analgesic, and anticonvulsant activities, and to provoke excitability of the central nervous system (de Sousa et al., 2015; Chen et al., 2020). *Rosmarinus officinalis* (rosemary), belonging to the Lamiaceae botanical family, is an aromatic plant cultivated in different regions worldwide, with the Mediterranean as its center of origin (Murata et al., 2013; Oliveira et al., 2019). Rosemary is used to accelerate digestion, clear nasal passages, stimulate hair growth, and relieve rheumatic pain, as well as myalgias, neuralgias, and physical and mental fatigue.

Essential oil of *R. officinalis* (EORO) is also used as a memory and cognition stimulator, have antidiabetic, anti-inflammatory, and hepatoprotective properties, relieve dyslipidemia, and protect against glial cell tumors (Rašković et al., 2014; Rodrigues et al., 2020; Olah et al., 2017; Ozdemir and Goktuk, 2018; Borges et al., 2019; Allegra et al., 2020; Bao et al., 2020; Chen et al., 2020; Zappalà et al., 2021). According to Bellumori et al. (2021), Ahmed and Babakir-Mina (2020) and Paixão and de Carvalho (2021), rosemary has immunomodulatory and antimicrobial activity against bacteria (*Staphylococcus epidermidis, S. aureus, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa*, and *Escherichia coli*) and fungi (*Candida albicans and Aspergillus niger*). Several studies have demonstrated the interactive relationship of EORO with the CNS, having activity in the cholinergic and dopaminergic pathways (Park et al., 2010; Sasaki et al., 2012; Borrás-Linares et al., 2014; Kayashima et al., 2020). Studies of stimulant effects are related to the components 1,8-cineole (oxide) and α-pinene (monoterpene) which, through sympathetic activity, stimulate the autonomic nervous system and increase blood pressure and respiratory rate measurements (Howes and Houghton, 2003; Sasaki et al., 2012; Kayashima et al., 2020). In line with these studies, Schriever et al., 2017 and DeGuzman et al., 2020 recorded by electroencephalography a significant decrease in the power of alpha waves in the bilateral middle frontal regions; they associated this result with increased alertness they clinically observed.

It is known that despite advances in the understanding of epilepsy, the mechanisms responsible for the epileptic phenomenon and its cellular bases are still not fully understood, although existing studies indicate that EORO interacts with the CNS. The behavioral and electrocorticographic characterization during these interactions caused by EORO have never been described and can help us understand the pathophysiological mechanisms that underlie epilepsies.

2 Materials and methods

2.1 Animals

For this study, 54 heterogeneous male Wistar rats, aged 8–10 weeks and weighing 180–200 g were used. They originated from the Central Animal Facility of the Federal University of Pará ICB (UFPA) and accommodated in the vivarium of its Laboratory of Pharmacology and Toxicology of Natural Products (LFTPN/UFPA). The animals were acclimatized to laboratory conditions 5 days before the experimental manipulation in boxes measuring 50 cm × 60 cm × 20 cm (height × width × depth) with wood shavings, at a temperature adjusted to 25–28 °C, 12-h light/dark cycle, receiving rodent food and filtered water during the tests. The experimental procedures followed the guidelines of the Ethics Committee in Research with Experimental Animals of the Federal University of Pará – (CEPAE–UFPA) under CUS approval number 6301260821.

2.2 Acquisition and composition of essential oil

Essential oil of *R. officinalis* (EORO) was purchased from Harmonie Aromatherapy (Florianópolis, SC, Brazil, CNPJ: 11.938.821/0001-90). It was extracted by steam distillation and analyzed by high-performance gas chromatography on an AGILENT 7820A Gas Chromatograph under the following conditions. Column: RXi-5MS 30 m × 0.25 mm × 0.25 μm (Restek). Temp.: column: 50 °C (0 min), 3° C/min at 200 °C; injector: 200 °C split: 1/50; FID detector: 220 °C. Vol. injection: 1 µL (1% in ethyl acetate) (Figure 1). The phytoconstituents that make up the oil are eucalyptol (47.5%), camphor (19.3%), α-pinene (12.2%), β-pinene (7.8%), and β-caryophyllene (4.6%) (Table 1).

2.3 Drugs used

The drugs were acquired as follows: ketamine hydrochloride from König (Santana de Parnaíba, SP, Brazil); xylazine hydrochloride from Vallée (Montes Claros, MG, Brazil); phenobarbital anticonvulsant compounds from Aventis-Pharma (Ribeirão Preto, SP, Brazil), and phenytoin and diazepam from União Química (Embu-Guaçu, SP, Brazil).

2.4 Experimental design

2.4.1 Experiment I: Behavioral characterization

The convulsant doses used were 300 mg/kg and 600 mg/kg i.p; through linear regression, considering the behavior of clonic seizure with partial loss of posture reflex, the indicated dose was 500 mg/kg. The drug was previously diluted in reconstituted peanut oil at 10% (100 mg/mL). The control group just with the injection of peanut oil in a volume equivalent did not present any behavioral alterations. The animals were submitted to behavioral analysis to evaluate the latency period of the appearance of behaviors after the administration of 500 mg/kg i.p. EORO forf 60 min (n = 9). Preliminary tests made it possible to determine the effective dose (ED50) at 500 mg/kg to trigger seizures with loss of postural reflex.

2.4.2 Experiment II: Electrocorticographic characterization

Five days after electrode implantation, electrocorticograms (ECoGs) were performed for 50 min after the injection of EORO 500 mg/kg i.p. This experiment was designed as follows. The control group received i.p. of 0.9% saline solution in equivalent volume, then ECoG recording was performed (n = 9). The animals in the EORO group received an EORO injection of 500 mg/kg i.p., then ECoG recording was performed (n = 9).

2.4.3 Experiment III: Action of anticonvulsants

EORO-induced seizures were attenuated with three different anticonvulsants: phenytoin (PHT) 10 mg/kg i.p., phenobarbital (PBT) 10 mg/kg i.p., and diazepam (DZP) 10 mg/kg i.p. The

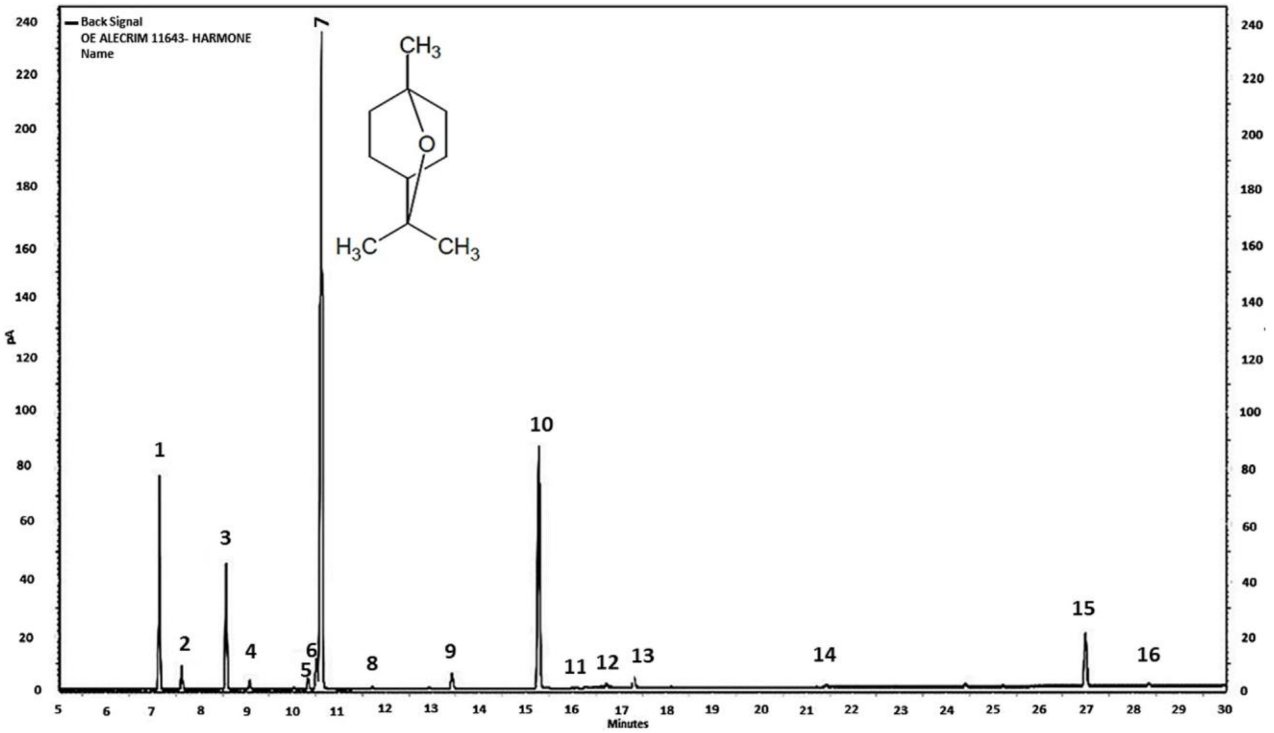

FIGURE 1
Chromatogram of *R. officinalis* essential oil sample Chromatography Laboratory, Department of Chemistry—Federal University of Minas Gerais, Belo Horizonte, 19 August 2019. 1 (alpha-pinene), 2 (camphene), 3 (beta-pinene), 4 (myrcene), 5 (para-cymene), 6 (limonene), 7 (eucalyptol), 8 (gamma), 9 (linalool), 10 (camphor), 11 (borneol), 12 (terpinen-4-ol), 13 (alpha terpineol), 14 (bornyl acetate), 15 (beta-caryophyllene), and 16 (humolene).

TABLE 1 Chemical composition of *Rosmarinus officinalis* essential oil.

Retention index	Identification	Percentage (%)	Peak
1003	Eucalyptol	47.5	7
1121	Camphor	19.3	10
913	Alpha-pinene	12.2	1
950	Beta-pinene	7.8	3
1425	Beta-caryophyllene	4.6	15
1000	Limonene	2.3	6
925	Camphene	1.4	2
1073	Linalool	1.2	9
1174	Alpha terpineol	1.0	13
995	Para-cymene	0.7	5
963	Myrcene	0.5	4
1159	Terpinen-4-ol	0.3	12
1281	Bornyl acetate	0.2	14
1461	Humolene	0.2	16
1031	Gamma terpinene	0.1	8
1148	Borneol	0.1	11
	Others	0.2	

groups were organized as follows. a) EORO (500 mg/kg i.p.) 10 min before the application of phenytoin at a dose of 10 mg/kg i.p. followed by the ECoG recording for 30 min. b) EORO (500 mg/kg i.p.) 10 min before application of phenobarbital at a dose of 10 mg/kg i.p. followed by the ECoG recording for 30 min. c) EORO (500 mg/kg i.p.) 10 min before the application of diazepam at a dose of 10 mg/kg i.p. followed by the ECoG recording for 30 min. To evaluate the seizure control by anticonvulsants, the animals were submitted to the same electrocorticographic recording protocol as step two and received intraperitoneal EORO and 10 min later received phenytoin, phenobarbital, and diazepam, followed by ECoG recording for 30 min.

2.5 Surgery for electrode implantation

The animals were anesthetized by intraperitoneal injection of an association of ketamine hydrochloride at a dose of 100 mg/kg and xylazine hydrochloride at a dose of 10 mg/kg. The degree of anesthetic depth was evaluated. After anesthesia, the animals were placed in a stereotaxic apparatus. Stainless steel electrodes, with an exposed tip 1.0 mm in diameter, were placed on the dura above the frontal cortex at the bregma coordinates −0.96 mm and ±1.0 mm laterally (Hamoy et al., 2018) in the motor cortex region. A screw was fixed in the skull, and the electrodes were fixed with dental acrylic cement (self-curing acrylic).

TABLE 2 Characterization of the phases and latency of the occurrence of behaviors (60-min observation) (n = 9).

Phase	Behavior	Latency (seconds)
Phase 1	1. Immobility	301.2 ± 42.78
	2. Myorelaxation	458.9 ± 62.20
Phase 2	1. Head and neck spasms	974.0 ± 49.64
	2. Clonic seizures of the forelimbs	1084 ± 68.71
	3. Generalized clonic seizure with transient loss of posture reflex	1213.0 ± 93.18
	4. Generalized clonic seizure with loss of posture reflex	1348.0 ± 49.92

2.6 Electrocorticography record

The recordings were obtained through a differential amplifier with high impedance AC input (Grass Technologies, Model P511) adjusted with 0.3 Hz and 0.3 KHz filtering, monitored with an oscilloscope (Protek, Model 6510), and continuously digitized at a 1 KHz rate by a computer equipped with a data acquisition card (National Instruments, Austin, TX). The analyses were performed using a tool built using the Python programming language (version 5.0). The "Numpy" and "Scipy" libraries were used for mathematical processing, and the "matplolib" library was used to obtain graphs and plots. The results were submitted to descriptive statistics as mean and standard deviation. One-way analysis of variance (ANOVA) was used, followed by the Tukey test. A significance index of $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ was adopted. Analyses were performed at a frequency of up to 40 Hz, and divided into bands according to Jalilifar et al. (2018) in beta (1–4 Hz), theta (4–8 Hz), alpha (8–12), beta (12–28), and gamma (28–40 Hz) for the interpretation of dynamics during the development of crises.

3 Results

3.1 Behavioral characterization

The behavioral observations obtained after the administration of essential oil of *R. officinalis* (EORO) at a dose of 500 mg/kg i.p, were characterized by two phases: CNS depression (phase 1) followed by CNS excitability (phase 2) (Table 2). In phase 1, it is possible to perceive two distinct stages: immobility and myorelaxation. Immobility starts after approximately 5 min, with a latency of 301.2 ± 42.78 s. Myorelaxation, in turn, starts at approximately 8 min, with a latency of 458.9 ± 62.20 s (Table 2). Phase 2 begins approximately 15 min after the application of EORO, characterized by excitability and the appearance of generalized clonic convulsion. The convulsion observed in animals demonstrated four characteristics: head and neck spasms, clonic convulsion of the thoracic limbs, generalized clonic convulsion with transient loss of postural reflex, and generalized clonic convulsion with loss of postural reflex (Table 2).

3.2 ECoG according to phases of depression and cerebral excitability with different tracing patterns caused by EORO

The animals in the control group walked normally, showed ECoG characteristics with low tracing amplitude (Figure 2A, left), and revealed a frequency spectrogram with energy intensity concentrated at frequencies below 10 Hz (Figure 2A, right). During phase 1, which occurred shortly after the application of EORO, muscle relaxation was observed in behavioral analysis. Furthermore, the ECoG recorded a decrease in the amplitude of the trace (Figure 2B, left), with a slight reduction of the energy level in the frequency oscillations up to 40 HZ (Figure 2B, right) compared to the control spectrogram (Figure 2A, right). During phase 2, much larger amplitude traces can be noticed (Figure 2C, left) with signal intensification according to the frequency spectrogram achieved (Figure 2C, right), contrasting with the patterns observed in control and during phase 1. The behavioral responses were in line with the two distinct phases observed in the ECoG analysis (Figure 3). In phase 2, three tracing patterns were observed: pattern A characterized by a change in the tracing at the beginning of excitability with an amplitude below 0.5 mV; pattern B characterized by a polypoint wave that repeats with an amplitude of 2 mV but at a lower frequency than pattern A; Pattern C that presents a high-frequency polypoint with a burst firing pattern of potentials with an amplitude of 4 mV (Figures 3A,B).

3.3 Analysis of brain oscillations during the phases and tracing patterns observed in phase 2

The linear power between control and phase 1 were similar (p = 0.999). However, the control group was smaller than the other groups in phase 2. Phase 2 of the registration was similar to pattern B (=0.9551). Linear power was increased according to patterns A, B, and C (Figure 4A). For linear power in delta oscillations, the control group was similar to phase 1 (p = 0.9701) and pattern A group (p = 0.9785). The phase 1 group was similar to pattern A (p = 0.6673). Phase 2 was larger than pattern A and smaller than patterns B and C (Figure 4B). For theta oscillations, the animals in the control group were similar to phase 1 (p = 0.9988) and pattern A group (p = 0.5503). The phase 1 group and firing pattern A were similar (p = 0.2806). The power of the recordings in phase 2 was greater than the control, phase 1, and standard A groups. The groups of patterns B and C presented the highest powers recorded in theta oscillations (Figure 4C). For alpha oscillations, the control group was similar to phase 1 (p = 0.999), and it was similar to standard group A (p = 0.5765). Phase 1 was similar to pattern A (0.5068), as was phase 2 (p = 0.3406). Patterns A, B, and C showed an increase in alpha power according to the evolution of the recording (Figure 4D). For beta oscillations, recordings from animals that received EORO showed greater beta wave power in phase 2 than the control and phase 1 groups. Phase 2 was similar to standard group A (p = 0.0867). The control group showed oscillations in the beta band similar to the group in phase 1 (p = 0.999). Standard groups B and

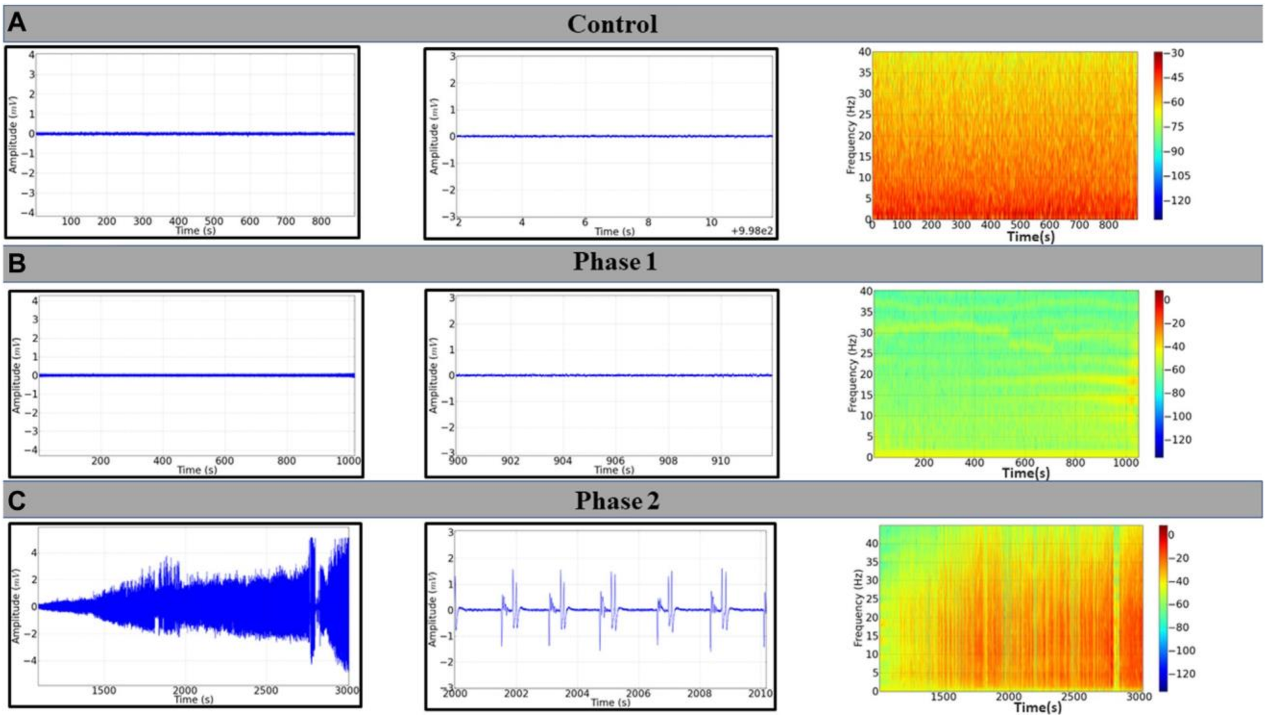

FIGURE 2
Electrocorticographic (ECoG) tracings of Wistar rat after application of 500 mg/kg i.p. of EORO. (A) ECoG traces of the control group; (B) ECoG registration during Phase 1; (C) seizure pattern in Phase 2. Corresponding records are shown on the central panels (10 s); frequency spectrograms are shown on the right.

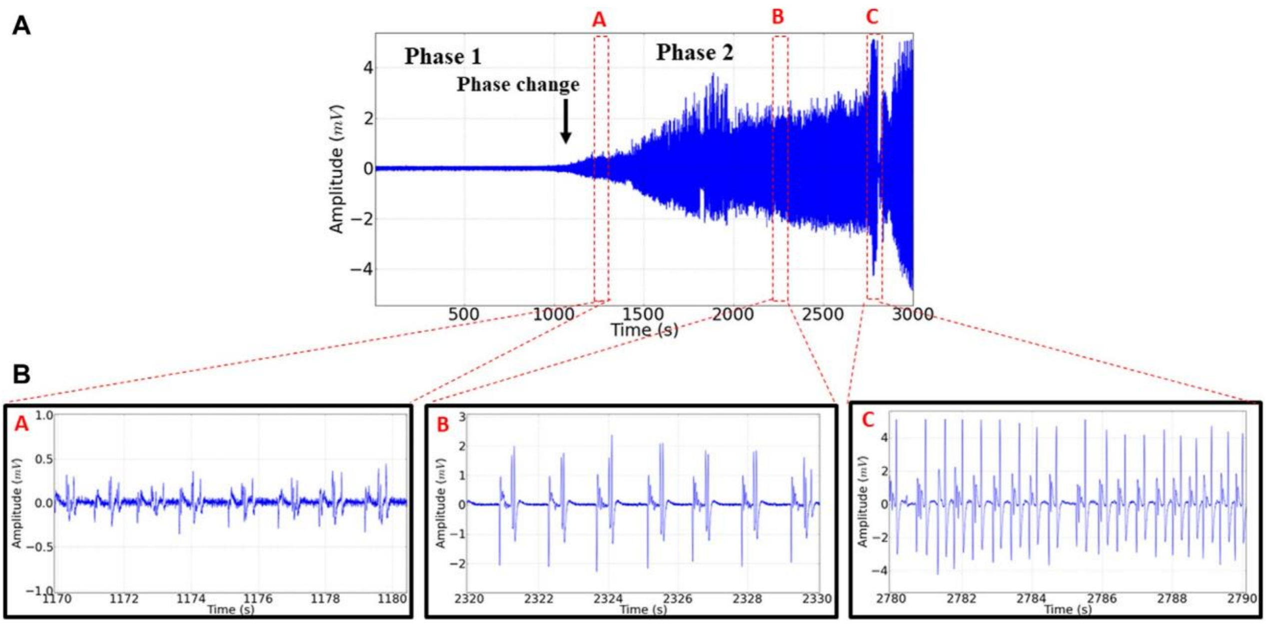

FIGURE 3
(A) Electrocorticographic recording (ECoG) obtained after application of EORO at 500 mg/kg i.p. showing phases 1 and 2. The black arrow indicates the beginning of phase 2. Three ECOG tracing patterns were identified in the second phase, shown as red dots: pattern (A) (1,170–1,180 s), pattern (B) (2,320–2,330 s), and pattern (C) (2,780–2,790 s). All with 10 s recording in phase 2.

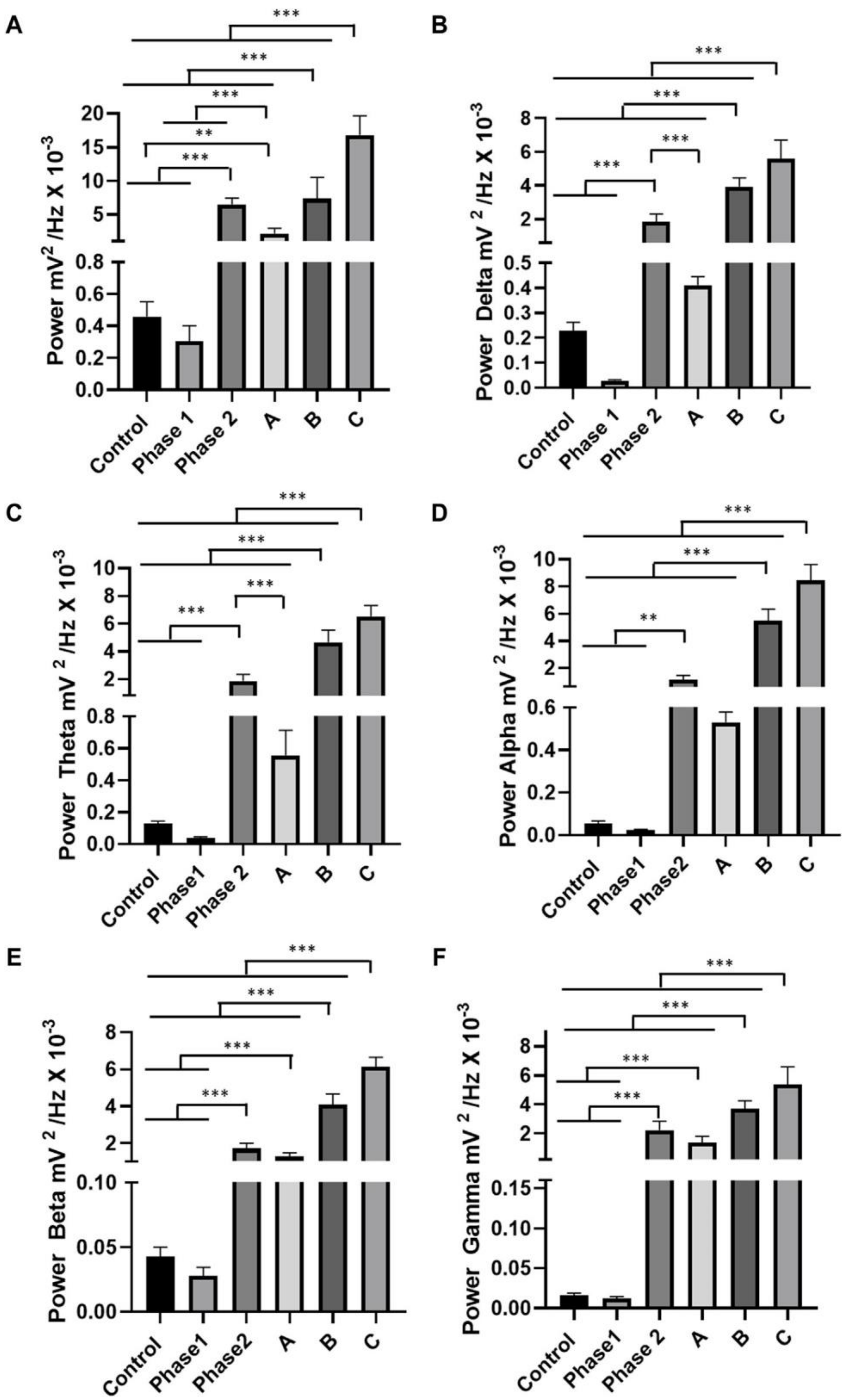

FIGURE 4
Quantitative linear frequency distribution of brain waves: For oscillations of 0–40 Hz in phases 1 and 2 and patterns A, B, and C of the tracings (A); delta oscillations (1–4 Hz) (B); theta oscillations (4–8 Hz) (C); alpha oscillations (8–13 Hz) (D); beta oscillations (13–28 Hz) (E); gamma oscillations (28–40 Hz) (F). The test used was one-way ANOVA. Data expressed as mean ± SD (n = 9 animals per group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

C showed greater beta potency (Figure 4E). For gamma oscillations, the phase 2 group showed greater power in relation to the control and phase 1 groups. The control group and phase 1 were similar ($p = 0.999$). Phase 2 was similar to standard group A, ($p = 0.0632$). Groups B and C presented the highest average power in gamma oscillations (Figure 4F).

3.4 Evaluation of anticonvulsant drugs

To assess the control of the seizures observed in phase 2, anticonvulsants were applied after EORO administration: phenytoin (10 mg/kg i.p.), phenobarbital (10 mg/kg i.p.), and diazepam (10 mg/kg i.p.). The recording patterns obtained with the use of anticonvulsants are shown in Figure 5 A, B, and C. Oscillations in the beta band showed an increase in phase 2 characterized by seizures; thus, the action of anticonvulsant drugs has been tested for oscillations in beta (12–28 Hz). For the control group, the fluctuations in beta were lower than the other groups, except for the group treated with diazepam (p = 0.7758). Registration in phase 2 was higher than the other groups. The group treated with phenytoin was larger than the groups treated with phenobarbital and diazepam. The diazepam-treated group was similar to the phenobarbital-treated group (p = 0.0879) (Figure 5D).

4 Discussion

Many substances contained in essential oils have anticonvulsant effects and may benefit people with epilepsy. Compounds such as carvone, citral, eugenol, or linalool are present as promising agents with antiepileptic activity. However, some essential oils are proconvulsant or are even present anticonvulsant and proconvulsant compounds in the same essential oil (Filho et al., 2006; Lopes et al., 2008; Quintans-Júnior et al., 2008; Sousa et al., 2008; Subhan et al., 2008; Bahr et al., 2019; de Oliveira et al., 2020; Mathew et al., 2021; de Oliveira et al., 2022; de Araújo et al., 2023). The behavior of the animals after the application of EORO initially showed depression of the central nervous system with the presence of intense myorelaxation that characterized the first phase of the behavior. However, a phase of excitability was revealed with the appearance of convulsive crises in the second phase, which demonstrated to the same components of the essential oil a decrease of excitability in the initial period and then an increase of excitability. A similar behavior was observed in the ethanolic extract of Nerium oleander, but with different components—in this case oleandrin, which corresponds to a digitaloid (de Melo et al., 2020). Figuêredo et al. (2019) studied the effects of 1,8-cineol (eucalyptol 50 mg/kg)—the key phytochemical component of EORO—in the CNS of mice through the analysis of a behavioral model, finding that the latency of death was significantly prolonged in the groups which were submitted to convulsion induced by PTZ, which is a power stimulant of CNS due to its inhibitory capacity of the receptor GABAA in the control group. This corroborates our phase 1 results that EORO seems to act as a depressor of CNS. A study on the essential oil of *Ocimum basilicum* also suggested the hypnotic and anticonvulsant activities of this oil in the presence of terpene like the 1,8—cineole and linalool (Ismail, 2006). Camphor, the second biggest key phytochemical component of EORO, was studied by Ferreira et al. (2020) through electrocardiographic analysis where moderate neuronal hyperexcitability, fast evolution to tonic-clonic convulsion, and alterations in the electrocardiographic registers presented characters of epileptiform activity with an increase in the total power of the wave, revealing an increase in the delta and theta waves. This corroborated our phase 2 results that the EORO was a CNS exciter, and therefore a proconvulsant. In the phytochemical analysis of the oil used in the study, the compound linalool was identified. This is an acyclic monoterpene that is well-known for its potential in aromatherapy and cosmetics. It is a compound that has anxiolytic potential in animal models capable of potentiating the function of GABA in the GABA A receptor (Milano et al., 2017) and inhibiting the excitatory action of glutamate receptors (Elisabetsky et al., 1999; Kessler et al., 2012, Ohkuma et al., 2002). Linalool derivatives and metabolites including linalool oxide, linalyl acetate, eight-oxolinalyl acetate, 8-carboxylinalyl acetate, and 8-oxolinalool also increase GABAergic activity and may have anticonvulsant effects (Linck et al., 2009; Vatanparast et al., 2017; Bahr et al., 2019) (Table 1). Hydroxylation at C8 of linalyl acetate led to reduced GABAergic responses (Granger et al., 2005; Milanos et al., 2017). The possible oxidation of linalool by P450 system oxidases may have contributed to the formation of different oxygenated byproducts that express distinct affinities and properties regarding the activation of GABA receptors (Boachon et al., 2015). The hydrophobicity of linalool favors greater allosteric interaction with GABA, since these receptors are embedded in the hydrophobic lipid bilayer of neurons (Khom et al., 2007). The oxidation of these compounds results in reduced modulation of the GABAA receptor (Khom et al., 2007). The linalool metabolism can thus directly contribute to the onset of seizures, reducing its effects on GABA, since other substances such as camphor and eucalyptol are found in the essential oil. These are known to promote epileptic events in humans, possibly due to their significant presence (Teis and Koren, 1995; Zibrowski et al., 1998; Pearce, 2008; Culić et al., 2009; Ferreira et al., 2020). This context justifies the two antagonistic phases dependent on the time of contact with the EORO, which can be observed in the electrocorticogram. During phase 2, there are characteristic peaks of asynchronous brain activity (Figure 2) with low amplitude patterns and high frequency, low frequency and high amplitude with polypoint wave, and high amplitude and high frequency with similar morphographic elements between shots with an increase in recorded power, demonstrating the intensity of the convulsive condition (Figures 3A,B). The evaluation of brain band oscillations during phase 1 showed a decrease in delta oscillations (1–4 Hz) in relation to the control group. For phase 2, all oscillations up to 40 Hz showed an increase in potency, but beta oscillations showed greater amplitude during seizures. According to Jalilifar et al. (2018) and Hamoy et al. (2018), who studied different pro-convulsant substances, there is an increased preponderance of beta oscillations in the electrocorticogram . All anticonvulsants tested reduced beta oscillations during seizures, although phenobarbital and diazepam performed better, demonstrating the inhibition of the GABAergic pathway by EORO. Research has already described the ability of camphor to induce seizures (Dubovsky, 1995; Pearce, 2008; Burkhard et al., 1999; Ferreira et al., 2020). EORO has components capable of initially depressing and subsequently causing excitability in the central

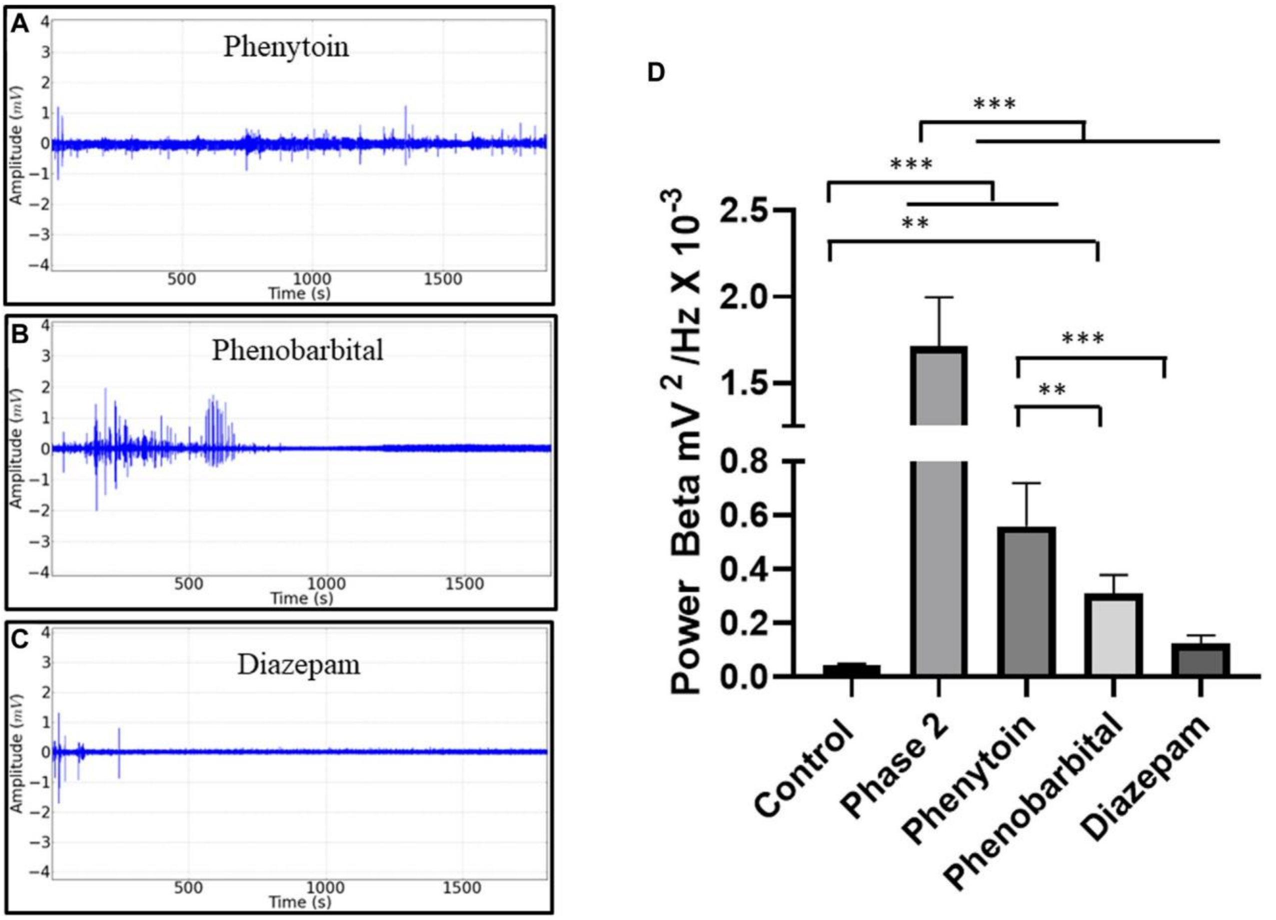

FIGURE 5
Electrocorticographic recordings (ECoG) obtained in phase 2 in beta brain oscillations after anticonvulsant application. Demonstration of the recording pattern found after application of phenytoin 10 mg/kg i.p. (A), phenobarbital at 10 mg/kg ip (B), diazepam at 10 mg/kg ip (C), and mean potency values showing the seizure activity in Phase 2 (D) ANOVA and Tukey's test (n = 9). The p values between the mean amplitudes are represented by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

nervous system with characteristic recording patterns that are repeated during the ECoG tracing. The excitability phase is related to the decrease in GABA activity, which can be reversed more effectively with the use of diazepam. EORO has antagonistic effects that can be observed depending on the time of contact with the body.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials; further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Ethics Committee in Research with Experimental Animals of the Federal University of Pará—CEPAE—UFPA under CUS approval number 6301260821. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

DB: Methodology, writing–original draft, writing–review and editing. YR: Methodology, writing–original draft, writing–review and editing. MO: Methodology, writing–original draft. LV: Methodology, writing–original draft. PP: writing–original draft, writing–review and editing. RG: Methodology, writing–original draft. LS: Methodology, writing–original draf. LL: Methodology, writing–original draf. MF: Methodology, writing–original draf. YS: Methodology, writing–original draf. RC: writing–original draft, writing–review and editing. RV: Methodology, writing–original draft. GB: Methodology, writing–original draft. MH: Methodology, writing–original draft, writing–review and editing, Data curation, Supervision, Conceptualization, Funding acquisition, Resources, Visualization, Software, Formal analysis, Project administration, Validation, Investigation.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by Fundação Amazônia de Amparo a Estudos e Pesquisas do Estado do Pará (FAPESPA) and The APC was funded by Pró-Reitoria de Pesquisa e PósGraduação—PROPESP/UFPA.

Acknowledgments

This study was financed by grants to encourage undergraduate and graduate research from the Federal University of Pará.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher

References

Ahmed, H. M., and Babakir-Mina, M. (2020). Investigation of rosemary herbal extracts (<i>Rosmarinus officinalis</i>) and their potential effects on immunity. *Phytother. Res.* 34 (8), 1829–1837. doi:10.1002/ptr.6648

Allegra, A., Tonacci, A., Pioggia, G., Musolino, C., and Gangemi, S. (2020). Anticancer activity of Rosmarinus officinalis L.: mechanisms of action and therapeutic potentials. *Nutrients* 12 (6), 1739. doi:10.3390/nu12061739

Bahr, T. A., Rodriguez, D., Beaumont, C., and Allred, K. (2019). The effects of various essential oils on epilepsy and acute seizure: a systematic review. *Evid. Based Complement. Altern. Med.* 2019, 1–14. doi:10.1155/2019/6216745

Bao, T. Q., Li, Y., Qu, C., Zheng, Z. G., Yang, H., and Li, P. (2020). Antidiabetic effects and mechanisms of rosemary (Rosmarinus officinalis L.) and its phenolic components. *Am. J. Chin. Med.* 48 (06), 1353–1368. doi:10.1142/S0192415X20500664

Bellumori, M., Innocenti, M., Congiu, F., Cencetti, G., Raio, A., Menicucci, F., et al. (2021). Within-plant variation in Rosmarinus officinalis L. Terpenes and phenols and their antimicrobial activity against the rosemary phytopathogens *Alternaria alternata* and Pseudomonas viridiflava. *Molecules* 26 (11), 3425. doi:10.3390/molecules26113425

Boachon, B., Junker, R. R., Miesch, L., Bassard, J. E., Höfer, R., Caillieaudeaux, R., et al. (2015). CYP76C1 (Cytochrome P450)-mediated linalool metabolism and the formation of volatile and soluble linalool oxides in Arabidopsis flowers: a strategy for defense against floral antagonists. *Plant Cell.* 27 (10), 2972–2990. doi:10.1105/tpc.15.00399

Borges, R. S., Ortiz, B. L. S., Pereira, A. C. M., Keita, H., and Carvalho, J. C. T. (2019). Rosmarinus officinalis essential oil: a review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. *J. Ethnopharmacol.* 229, 29–45. doi:10.1016/j.jep.2018.09.038

Borrás-Linares, I., Stojanović, Z., Quirantes-Piné, R., Arráez-Román, D., Švarc-Gajić, J., Fernández-Gutiérrez, A., et al. (2014). Rosmarinus officinalis leaves as a natural source of bioactive compounds. *Int. J. Mol. Sci.* 15 (11), 20585–20606. doi:10.3390/ijms151120585

Burkhard, P. R., Burkhardt, K., Haenggeli, C. A., and Landis, T. (1999). Plant-induced seizures: reappearance of an old problem. *J. Neurol.* 246, 667–670. doi:10.1007/s004150050429

Chen, X. L., Luo, Q. Y., Hu, W. Y., Chen, J. J., and Zhang, R. P. (2020). Abietane diterpenoids with antioxidative damage activity from Rosmarinus officinalis. *J. Agric. Food Chem.* 68 (20), 5631–5640. doi:10.1021/acs.jafc.0c01347

Ćulić, M., Keković, G., Grbić, G., Martać, L., Soković, M., Podgorac, J., et al. (2009). Wavelet and fractal analysis of rat brain activity in seizures evoked by camphor essential oil and 1, 8-cineole. *Gen. Physiol. Biophys.* 28, 33–40.

de Araújo, D. B., do Amaral, A. L. G., da Fonseca, S. M., de Souza, K. R., da Paz, A. P. S., de Mello, V. J., et al. (2023). Lippia origanoides essential oil possesses anticonvulsant effect in pentylenetetrazol-induced seizures in rats: a behavioral, electroencephalographic, and electromyographic study. *Front. Pharmacol.* 14, 1289336. doi:10.3389/fphar.2023.1289336

DeGuzman, P., Jain, A., Tabert, M. H., and Parra, L. C. (2020). Olfaction modulates inter-subject correlation of neural responses. *Front. Neurosci.* 14, 702. doi:10.3389/fnins.2020.00702

de Melo, B. S., de Morais, B. P., Sá, V. S. D. S. F., Lourinho, F. D., Toda, I. P. S. P., do Nascimento, J. L. M., et al. (2020). Behavioural, electrocorticographic, and electromyographic alterations induced by Nerium oleander ethanolic extract: anticonvulsant therapeutics assessment. *Neurotoxicology* 78, 21–28. doi:10.1016/j.neuro.2020.02.001

de Oliveira, F. R., da Silva, N. M., Hamoy, M., Crespo-López, M. E., Ferreira, I. M., da Silva, E. O., et al. (2022). The GABAergic system and endocannabinoids in epilepsy and seizures: what can we expect from plant oils? *Molecules* 27 (11), 3595. doi:10.3390/molecules27113595

de Oliveira, F. R., Rodrigues, K. E., Hamoy, M., Sarquis, Í. R., Hamoy, A. O., Lopez, M. E. C., et al. (2020). Fatty acid amides synthesized from andiroba oil (carapa guianensis aublet.) exhibit anticonvulsant action with modulation on GABA-A receptor in mice: a putative therapeutic option. *Pharm. (Basel)* 13 (3), 43. doi:10.3390/ph13030043

de Oliveira, J. R., Camargo, S. E. A., and de Oliveira, L. D. (2019). Rosmarinus officinalis L.(rosemary) as therapeutic and prophylactic agent. *J. Biomed. Sci.* 26 (1), 5–22. doi:10.1186/s12929-019-0499-8

de Sousa, D. P., Soares Hocayen, P. D. A., Andrade, L. N., and Andreatini, R. (2015). A systematic review of the anxiolytic-like effects of essential oils in animal models. *Molecules* 20 (10), 18620–18660. doi:10.3390/molecules201018620

Dubovsky, S. L. (1995). "Electroconvulsive therapy," in *Comprehensive textbook of psychiatry*. Editors H. I. Kaplan and B. J. Sadock (Baltimore: Williams & Wilkins), 2129.

Elisabetsky, E., Brum, L. S., and Souza, D. O. (1999). Anticonvulsant properties of linalool in glutamate-related seizure models. *Phytomedicine* 6 (2), 107–113. doi:10.1016/ S0944-7113(99)80044-0

Ferreira, L. O., de Souza, R. D., Silva, F. D. S., Costa, F. F. M., Farias, R. A. F., Hamoy, A. O., et al. (2020). Electrocorticographic patterns dominated by low-frequency waves in camphor-induced seizures. *Sci. Rep.* 10 (1), 18222. doi:10.1038/s41598-020-75309-w

Figuêredo, F. R. S. D. N., Monteiro, A. B., Menezes, I. R. A., Sales, V. S., Nascimento, E. P., Rodrigues, C. K. S., et al. (2019). Effects of the Hyptis martiusii Benth. leaf essential oil and 1,8-cineole (eucalyptol) on the central nervous system of mice. *Food Chem. Toxicol.* 133, 110802. doi:10.1016/j.fct.2019.110802

Filho, J. M. B., Medeiros, K. C. P., Diniz, M. D. F. F., Batista, L. M., Athayde-Filho, P. F., Silva, M. S., et al. (2006). Natural products inhibitors of the enzyme acetylcholinesterase. *Rev. Bras. Farmacogn.* 16, 258–285. doi:10.1590/S0102-695X2006000200021

Granger, R. E., Campbell, E. L., and Johnston, G. A. (2005). (+)- and (−)-borneol: efficacious positive modulators of GABA action at human recombinant $\alpha1\beta2\gamma2L$ GABAA receptors. *Biochem. Pharmacol.* 69 (7), 1101–1111. doi:10.1016/j.bcp.2005.01.002

Hamoy, M., Batista, L. D. S., de Mello, V. J., Gomes-Leal, W., Farias, R. A. F., Batista, P. D. S., et al. (2018). Cunaniol-elicited seizures: behavior characterization and electroencephalographic analyses. *Toxicol. Appl. Pharmacol.* 360, 193–200. doi:10.1016/j.taap.2018.10.008

Howes, M. J. R., and Houghton, P. J. (2003). Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacol. Biochem. Behav.* 75 (3), 513–527. doi:10.1016/s0091-3057(03)00128-x

Ismail, M. (2006). Central properties and chemical composition of *Ocimum basilicum* essential oil. *Pharm. Biol.* 44, 619–626. doi:10.1080/13880200600897544

Jalilifar, M., Yadollahpour, A., Moazedi, A. A., and Ghotbeddin, Z. (2018). Quantitative analysis of the antiepileptogenic effects of low frequency stimulation applied prior or after kindling stimulation in rats. *Front. Physiol.* 9, 711. doi:10.3389/ fphys.2018.00711

Kayashima, T., Nagao, K., Umino, M., Kaikiri, H., Shibata, S., and Matsubara, K. (2020). Anti-stress effects of rosemary (Rosmarinus officinalis L.) leaf extract on intestinal goblet cells and immobility of forced-swimming test in BALB/c mice. *Biosci. Biotechnol. Biochem.* 84 (11), 2385–2389. doi:10.1080/09168451.2020.

Kessler, A., Villmann, C., Sahin-Nadeem, H., Pischetsrieder, M., and Buettner, A. (2012). GABAA receptor modulation by the volatile fractions of Sideritis species used as 'Greek'or 'Turkish'mountain tea. *Flavour Fragr. J.* 27 (4), 297–303. doi:10.1002/ffj.3099

Khom, S., Baburi, E. U., Timin, E., Hohaus, A., Trauner, G., Kopp, B., et al. (2007). Valerenic acid potentiates and inhibits GABA(A) receptors: molecular mechanism and subunit specificity. *Neuropharmacology* 53, 178–187. doi:10.1016/j.neuropharm.2007.04.018

Linck, V. D. M., da Silva, A. L., Figueiró, M., Piato, A. L., Herrmann, A. P., Birck, F. D., et al. (2009). Inhaled linalool-induced sedation in mice. *Phytomedicine* 16 (4), 303–307. doi:10.1016/j.phymed.2008.08.001

Lopes, E. M. C., Carreira, R. C., Agripino, D. G., Torres, L. M. B., Cordeiro, I., Bolzani, V. D. S., et al. (2008). Screening for antifungal, DNA-damaging and anticholinesterasic activities of Brazilian plants from the atlantic rainforest: ilha do cardoso state park. *Rev. Bras. Farmacogn.* 18, 655–660. doi:10.1590/S0102-695X2008000500002

Mathew, T., John, S. K., Kamath, V., Kumar, R. S., Jadav, R., Swamy, S., et al. (2021). Essential oil related seizures (EORS): a multi-center prospective study on essential oils and seizures in adults. *Epilepsy Res.* 173, 106626. doi:10.1016/j.eplepsyres.2021.106626

Milanos, S., Elsharif, S. A., Janzen, D., Buettener, A., and Villmann, C. (2017). Metabolic products of linalool and modulation of GABAA receptors. *Front. Chem.* 5, 46. doi:10.3389/fchem.2017.00046

Murata, K., Noguchi, K., Kondo, M., Onishi, M., Watanabe, N., Okamura, K., et al. (2013). Promotion of hair growth by Rosmarinus officinalis leaf extract. *Phytother. Res.* 27 (2), 212–217. doi:10.1002/ptr.4712

Olah, N. K., Benedec, D., Socaci, S., Toma, C. C., Filip, L., Morgovan, C., et al. (2017). Terpenic profile of different Rosmarinus officinalis extracts. *Pak J. Pharm. Sci.* 30 (4), 1439–1443.

Ohkuma, M., Kawai, F., and Miyachi, E. (2002). Direct suppression by odorants of ionotropic glutamate receptors in newt retinal neurons. *J Neural Transm (Vienna)*. 109 (11), 1365–71. doi:10.1007/s00702-002-0741-4

Ozdemir, M. D., and Gokturk, D. (2018). The effect of Rosmarinus officinalis and chemotherapeutic etoposide on glioblastoma (U87 MG) cell culture. *Turk Neurosurg.* 28, 853–857. doi:10.5137/1019-5149.JTN.20401-17.3

Paixão, V. L. B., and de Carvalho, J. F. (2021). Essential oil therapy in rheumatic diseases: a systematic review. *Complement. Ther. Clin. Pract.* 43, 101391. doi:10.1016/j.ctcp.2021.101391

Park, S. E., Kim, S., Sapkota, K., and Kim, S. J. (2010). Neuroprotective effect of Rosmarinus officinalis extract on human dopaminergic cell line, SH-SY5Y. *Cell. Mol. Neurobiol.* 30 (5), 759–767. doi:10.1007/s10571-010-9502-3

Pearce, J. M. S. (2008). Leopold Auenbrugger: camphor-induced epilepsy - remedy for manic psychosis. *Eur. Neurol.* 59 (1-2), 105–107. doi:10.1159/000109581

Quintans Júnior, L. J., Almeida, J. R. G. S., Lima, J. T., Nunes, X. P., Siqueira, J. S., Oliveira, L. E. G., et al. (2008). Plants with anticonvulsant properties - a review. *Rev. Bras. Farmacogn.* 18, 798–819. doi:10.1590/S0102-695X2008000500026

Rašković, A., Milanović, I., Pavlović, N., Ćebović, T., Vukmirović, S., and Mikov, M. (2014). Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. *BMC Complement. Altern. Med.* 14, 225. doi:10.1186/1472-6882-14-225

Rodrigues, A. P. S., Souza, B. S. F., Barros, A. S. A., Carvalho, H. O., Duarte, J. L., Boettger, M. L. E., et al. (2020). The effects of Rosmarinus officinalis L. essential oil and its nanoemulsion on dyslipidemic Wistar rats. *J. Appl. Biomed.* 18 (4), 126–135. doi:10.32725/jab.2020.016

Sasaki, K., Omri, A. E., Kondo, S., Han, J., and Isoda, H. (2012). Rosmarinus officinalis polyphenols produce anti-depressant like effect through monoaminergic and cholinergic functions modulation. *Behav. Brain Res.* 238, 86–94. doi:10.1016/j.bbr.2012.10.010

Schriever, V. A., Han, P., Weise, S., Hösel, F., Pellegrino, R., and Hummel, T. (2017). Time frequency analysis of olfactory induced EEG-power change. *PLoS One* 12 (10), e0185596. doi:10.1371/journal.pone.0185596

Sousa, F. C. F., Melo, C. T. V., Citó, M. C. O., Félix, F. H. C., Vasconcelos, S. M. M., Fonteles, M. M. F., et al. (2008). Plantas medicinais e seus constituintes bioativos: uma revisão da bioatividade e potenciais benefícios nos distúrbios da ansiedade em modelos animais. *Rev. Bras. Farmacogn.* 18, 642–654. doi:10.1590/S0102-695X2008000400023

Subhan, N., Alam, M. A., Ahmed, F., Shahid, I. J., Nahar, L., and Sarker, S. D. (2008). Bioactivity of *Excoecaria agallocha*. *Rev. Bras. Farmacogn.* 18, 521–526. doi:10.1590/S0102-695X2008000400004

Theis, J. G., and Koren, G. (1995). Camphorated oil: still endangering the lives of Canadian children. *CMAJ* 152 (11), 1821–1824.

Vatanparast, J., Bazleh, S., and Janahmadi, M. (2017). The effects of linalool on the excitability of central neurons of snail Caucasotachea atrolabiata. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 192, 33–39. doi:10.1016/j.cbpc.2016.12.004

Zappalà, A., Vicario, N., Calabrese, G., Turnaturi, R., Pasquinucci, L., Montenegro, L., et al. (2021). Neuroprotective effects of Rosmarinus officinalis L. extract in oxygen glucose deprivation (OGD)-injured human neural-like cells. *Nat. Prod. Res.* 35 (4), 669–675. doi:10.1080/14786419.2019.1587428

Zibrowski, E. M., Hoh, T. E., and Vanderwolf, C. H. (1998). Fast wave activity in the rat rhinencephalon: elicitation by the odors of phytochemicals, organic solvents, and a rodent predator. *Brain Res.* 800 (2), 207–215. doi:10.1016/s0006-8993(98)00494-6

6. REFERÊNCIAS

1. Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. Journal of agricultural and food chemistry, 61(46), 10835–10847. https://doi.org/10.1021/jf403496k.

2. Almeida, R. N.; Motta, S. C.; Leite, J. R. Óleos essenciais com propriedades anticonvulsivantes (Aceites esenciales con propriedades anticonvulsivantes - Oil essential with anticonvulsivants properties). Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 2, núm. 1, enero, 2003, pp. 3-6.

3. Bacci, L.; Crespo, A. L. B.; Galvan, T. L.; Pereira, E. J. G.; Picanço, M. V. C.; Silva, G. A.; Chediak, M. (2007) Toxicity of insecticides to the sweetpotato whitefly (Hemiptera: Aleyrodidae) and its natural enemies. Pest Management Science 63:699-706.

4. Barreto, Humberto & Lima, Idglan & Kivia, Maria & Resende, Nunes & Coelho, & Luizângela, Reis & Osório, & De, Rafael & Mourão, Almeida & Bernadete, Helena & Cavalcanti, Dos & Santos, & Douglas, Henrique & Coutinho, Henrique & Abreu, Aislan & Das, Maria & Medeiros, Graças & Maria, Antonia & Citó, A.M.G.L. & Lopes, Dantas. (2014). Effect of Lippia origanoides H.B.K. essentialoilintheresistanceto aminoglycosides in methicillinresistant Staphylococcus aureus. European Journal of Integrative Medicine. 6. 560-564. 10.1016/j.eujim.2014.03.011.

5. Cavalheiro, E. A.; Priel, M.R.; Santos, N. F. Developmental aspects of the pilocarpine model of epilepsy. Epilepsy Res 1996;26:115-

6. Carvalho PMM, Macêdo CAF, Ribeiro TF, Silva AA, Da Silva RER, de Morais LP, Kerntopf MR, Menezes IRA, Barbosa R. Effect of the Lippia alba (Mill.) N.E. Brown essential oil and its main constituents, citral and limonene, on the tracheal smooth muscle of rats. Biotechnol Rep (Amst). 2017 Dec 6;17:31-34. doi: 10.1016/j.btre.2017.12.002. PMID: 29619330; PMCID: PMC5881402.

7. Coelho, Angelica & Lima Neto, José & Moura, Arkellau & Sousa, Taciana & Morais, Ilmara & Carvalho, Gabriela & Valmor, Francisco & Cunha, M & Graç, Maria & Medeiros, F & Vasconcelos, Eilika & Oliveira, Aldeídia & Arcanjo, Daniel & Nunes, Lívio & Citó, A.M.G.L.. (2015). Optimization and standardization of extraction method from Lippia origanoides H.B.K.: Focus on potential anti-hypertensive applications. Industrial Crops and Products. 78. 124-130. 10.1016/j.indcrop.2015.10.033.

8. Costa, L. L. O; Brandão, E. C.; Segundo, L. M. B. M. Atualização em epilepsia: revisão de literatura. Rev Med (São Paulo). 2020 mar.-abr.;99(2):170-81.

9. de Albuquerque, U. P., Muniz de Medeiros, P., de Almeida, A. L., Monteiro, J. M., Machado de Freitas Lins Neto, E., Gomes de Melo, J., & dos Santos, J. P. (2007). Medicinal plants of the caatinga

(semi-arid) vegetation of NE Brazil: a quantitative approach. Journal of ethnopharmacology, 114(3), 325–354. https://doi.org/10.1016/j.jep.2007.08.017.

10. Damasceno, Evelyn Ivana T. et al. Antioxidant capacity and larvicidal activity of essential oil and extracts from Lippia grandis. Revista Brasileira de Farmacognosia [online]. 2011, v. 21, n. 1 [Accessed 24 June 2021] , pp. 78-85. Available from: <https://doi.org/10.1590/S0102-695X2011005000013>. Epub 4 Feb 2011. ISSN 1981-528X. https://doi.org/10.1590/S0102-695X2011005000013

11. Fernandes, M. J. S. Epilepsia do lobo temporal: mecanismos e perspectivas. Estud Av. 2013;27(77):85-96.

12. Fluri F, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther. 2015 Jul 2;9:3445-54. doi: 10.2147/DDDT.S56071. PMID: 26170628; PMCID: PMC4494187.

13. Gallucci Neto, J.; Marchetti, R.L. Aspectos epidemiológicos e relevância dos transtornos mentais associados à epilepsia. Rev Bras Psiquiatr. 2005;27:323-8.

14. Garzon, E. Epilepsia Refratária: Conceito e Contribuição das Novas Drogas Antiepilépticas e de outras Modalidades Terapêuticas. Rev. Neurociências 10(2): 66-82, 2002.

15. Gomes, M. M. Bases fisiológicas do eletroencefalograma. Rev Bras Neurol. 51(1):12-7, 2015.

16. Hamoy, M., dos Santos Batista, L., de Mello, V. J., Gomes-Leal, W., Farias, R. A. F., dos Santos Batista, P., … Barbas, L. A. L. (2018). Cunaniol-elicited seizures: Behavior characterization and electroencephalographic analyses. Toxicology and Applied Pharmacology. doi:10.1016/j.taap.2018.10.008 .

17. Hennebelle T, Sahpaz S, Joseph H, Bailleul F. Ethnopharmacology of Lippia alba. J Ethnopharmacol. 2008 Mar 5;116(2):211-22. doi: 10.1016/j.jep.2007.11.044. Epub 2007 Dec 8. PMID: 18207682.

18. Heldwein CG, Silva LL, Reckziegel P, Barros FM, Bürger ME, Baldisserotto B, Mallmann CA, Schmidt D, Caron BO, Heinzmann BM. Participation of the GABAergic system in the anesthetic effect of Lippia alba (Mill.) N.E. Brown essential oil. Braz J Med Biol Res. 2012 May;45(5):436-43. doi: 10.1590/s0100-879x2012007500052. Epub 2012 Apr 5. PMID: 22473320.

19. Hermann, B. P.; Seidenberg, M.; Dow, C.; Jones, J.; Rutecki, P.; Bhattacharya, A.; et al. Cognitive prognosis in chronic temporal Rev Med (São Paulo). 2020 mar.-abr.;99(2):170-81. lobe epilepsy. Ann Neurol. Isman, M.B. (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. The Annual Review of Entomology 51:45-66.

20. Jacoby, A.; Baker, G. A. Quality of life trajectories in epilepsy: a review of the literature. Epilepsy Behav. 2008;12:557-71.

21. Kwan, P.; Brodie, M. J.; Seizure. Refractory epilepsy: a progressive, intractable but preventable condition? Seizure. 2002;11(2):77- 84.

22. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4(8), 118–126. https://doi.org/10.4103/0973-7847.70902.

23.Loscher, W. Mechanisms of drug resistance in status epilepticus. Epilepsia, 48(Suppl. 8):74–77, 2007.

24.Loup, F.; Fritschy, J. M.; Kiener, T.; Bouilleret, V. GABAergic neurons and GABAA-receptors in temporal lobe epilepsy. Neurochem Int 1999;34:435-45.

25. Menezes, P., Brito, M. C., de Paiva, G. O., Dos Santos, C. O., de Oliveira, L. M., de Araújo Ribeiro, L. A., de Lima, J. T., Lucchese, A. M., & Silva, F. S. (2018). Relaxant effect of Lippia origanoides essential oil in guinea-pig trachea smooth muscle involves potassium channels and soluble guanylyl cyclase. Journal of ethnopharmacology, 220, 16–25. https://doi.org/10.1016/j.jep.2018.03.040

26. Nunes, D. S. (1996). Chemical Approaches to the Study of Ethomedicines In: Medicinal Resources of the Tropical Forest Biodiversity and its Importance to Human Health – Balick, MJl, Elisabetsky, E, Laird, SA, Editors. Columbia University Press, New York, p.46.

27. O'Leary, Nataly & Denham, Silvia & Salimena, Fátima & Múlgura, María. (2012). Species delimitation in Lippia section Goniostachyum (Verbenaceae) using the phylogenetic species concept. Botanical Journal of the Linnean Society. 170. 10.1111/j.1095-8339.2012.01291.x.

28. Oliveira, Danilo & Leitao, Gilda & Bizzo, Humberto & Lopes, Daı´se & Alviano, Daniela & Alviano, Celuta & Leitão, Suzana. (2007). Chemical and Antimicrobial analyses of essential oil of Lippia origanoides H.B.K. Food Chemistry. 101. 236-240. 10.1016/j.foodchem.2006.01.022.

29. Oliveira, Danilo R. et al. Ethnopharmacological studies of Lippia origanoides . Revista Brasileira de Farmacognosia [online]. 2014, v. 24, n. 02 [Accessed 24 June 2021] , pp. 206-214 https://doi.org/10.1016/j.bjp.2014.03.001.

30. Pascual, M. E., Slowing, K., Carretero, E., Sánchez Mata, D., & Villar, A. (2001). Lippia: traditional uses, chemistry and pharmacology: a review. Journal of ethnopharmacology, 76(3), 201–214. https://doi.org/10.1016/s0378-8741(01)00234-3

31. Paxinos, G., Watson, C., 2005. The rat brain in stereotaxic coordinates (5 ed.). New York: Academic Press.

32. Rattan RS (2010) Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection 29:913-920 Rektor, I.; Schachter, S. C.; Arzy, S.; Baloyannis, S. J. Epilepsy, behavior, and art (Epilepsy, Brain, and Mind, part 1). Epilepsy Behav. 2013.

33. Raman, V., Fuentes Lorenzo, J. L., Stashenko, E. E., Levy, M., Levy, M. M., & Camarillo, I. G. (2017). Lippia origanoides extract induces cell cycle arrest and apoptosis and suppresses NF-κB signaling in triple-negative breast cancer cells. International journal of oncology, 51(6), 1801–1808. https://doi.org/10.3892/ijo.2017.4169

34. Sandes, S. S.; et al. Estruturas secretoras foliares em patchouli [Pogostemon cablin (Blanco) Benth.]. Scientia Plena 8, 059902

(2012).

35. Santos, Francisco & Lopes, José & Citó, A.M.G.L. & Oliveira, Evaldo & de Lima, Sidney & Reis, Francisco De Assis. (2004). Composition and Biological Activity of Essential Oils from Lippia origanoides H.B.K.. The Journal of Essential Oil Research. 16. 504-506. 10.1080/10412905.2004.9698782.

36. Sant'ana, T. C. P. et al. Influência do armazenamento de folhas secas no óleo essencial de patchouli (Pogostemon cablin BENTH.) Quim. Nova, Vol. 33, No. 6, 1263-1265, 2010.

37. da Silva LVF, Veras Mourão RH, Manimala J, Lnenicka GA. The essential oil of Lippia alba and its components affect Drosophila behavior and synaptic physiology. J Exp Biol. 2018 Jul 26;221(Pt 14):jeb176909. doi: 10.1242/jeb.176909. PMID: 29880632.

38. da Silva RER, de Morais LP, Silva AA, Bastos CMS, Pereira-Gonçalves Á, Kerntopf MR, Menezes IRA, Leal-Cardoso JH, Barbosa R. Vasorelaxant effect of the Lippia alba essential oil and its major constituent, citral, on the contractility of isolated rat aorta. Biomed Pharmacother. 2018 Dec;108:792-798. doi: 10.1016/j.biopha.2018.09.073. Epub 2018 Sep 22. PMID: 30253371.

39. da Silva, A. P., Silva, N. F., Andrade, E., Gratieri, T., Setzer, W. N., Maia, J., & da Silva, J. (2017). Tyrosinase inhibitory activity, molecular docking studies and antioxidant potential of chemotypes of Lippia origanoides (Verbenaceae) essential oils. PloS one, 12(5), e0175598. https://doi.org/10.1371/journal.pone.0175598.

40. SILVA, LENISE L. et al . S-(+)- and R-(-)-linalool: a comparison of the in vitro anti-Aeromonas hydrophila activity and anesthetic properties in fish. An. Acad. Bras. Ciênc., Rio de Janeiro , v. 89, n. 1, p. 203-212, Mar. 2017 Available from <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S000137652017000100203&lng=en&nrm=iso>. access on 16 Feb. 2021.

41. Teixeira ML, Marcussi S, de C S Rezende DA, Magalhães ML, Nelson DL, das G Cardoso M. Essential Oil from Lippia origanoides (Verbenaceae): Haemostasis and Enzymes Activity Alterations. Med Chem. 2019;15(2):207-214. doi: 10.2174/1573406414666180829150515. PMID: 30160215.

42. FC Terblanché & G. Kornelius (1996) Essential Oil Constituents of the Genus Lippia (Verbenaceae) —A Literature Review, Journal of Essential Oil Research, 8: 5, 471-485, DOI: 10.1080 / 10412905.1996.9700673

43. Toni C, Becker AG, Simões LN, Pinheiro CG, de Lima Silva L, Heinzmann BM, Caron BO, Baldisserotto B. Fish anesthesia: effects of the essential oils of Hesperozygis ringens and Lippia alba on the biochemistry and physiology of silver catfish (Rhamdiaquelen). Fish Physiol Biochem. 2014 Jun;40(3):701-14. doi: 10.1007/s10695-013-9877-4. Epub 2013 Oct 20. PMID: 24141557.

44. Yacubian, E. M. T. Epilepsias em: Nitrine R, Bacheschi LA. A neurologia que todo médico deve saber. 2a ed. São Paulo: Atheneu; 2008. p.235-56.