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Original Article

Antidepressant, Anxiolytic, and Antioxidant Properties of *Piper Aduncum* Essential Oil from Northern Peru

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ABSTRACT

This investigation aimed to evaluate the antidepressant, anxiolytic, and antioxidant effects of *Piper aduncum* essential oil from northern Peru. The essential oils were obtained through hydro-distillation using a modified Clevenger-type apparatus. The chromatography-mass spectrometry (GC-MS) was used to assess the chemical composition. Behavioral assays in mice were used to evaluate the antidepressant and anxiolytic effects. In addition, the antioxidant capacity was performed through DPPH, ABTS, and FRAP assays. The GC-MS analysis revealed that linalool (29.16%), bicyclogermacrene (13.32%), nerolidol (12.38%), and β-caryophyllene (10.76%) were the principal components. The results demonstrated significant antidepressant and anxiolytic effects comparable to fluoxetine and diazepam, with statistical differences between all groups ($p<0.005$). The IC_{50} values for the DPPH, ABTS, and FRAP assays were 5.9±0.08, 0.20±0.06, and 109.5±1.3, respectively. Consequently, *Piper aduncum* essential oil exhibits antidepressant and anxiolytic-like effects and modest antioxidant properties compared to the controls.

Keywords: *Piper aduncum*, Essential oil, Antidepressant, Anxiolytic, Antioxidant.

INTRODUCTION

Depression and anxiety disorders are leading causes of disability, with an increasing worldwide prevalence [1,2]. At present, approximately 4% and 5% of the world population are affected by anxiety or depression [3].

Pharmacological interventions for depression and anxiety include many drugs, such as selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and benzodiazepines. While these medications are effective, they may also result in side effects and dependency, which underscores the necessity for effective alternatives [4,5].

Medicinal plants may be additional sources of depression and anxiety treatments, providing therapeutic benefits with fewer side effects than conventional medication [6,7]. Essentially, essential oils (EOs) are a helpful alternative for depression and anxiety [8]. EOs have shown promising potential in the treatment of mental illness due to their ability to act on depression and anxiety-related biological receptors with reduced toxicity and side effects [9,10].

Among the essential oils traditionally used for medicinal purposes, oils from the *Piperaceae* family have been characterized, which comprises over 3600 species in tropical and subtropical climates in the northern and southern hemispheres [11]. The most numerous is the *Piper* genus, which has about 2000 species [12]. Much research has been directed into the biological characteristics of essential oils (EOs) from *Piper aduncum* L, encompassing antiprotozoal[13], anthelminthic[14], antioxidant[15], and insecticidal properties[16]. Regarding the neuropharmacological effects of this species, the evidence is not substantial; however, *Piper nigrum* EOs exhibit a dual anxiolytic and antidepressant-like effect through the possible involvement of serotonergic transmission [17].

This investigation aimed to assess the antidepressant, anxiolytic, and antioxidant properties of *Piper aduncum* essential oil from northern Peru in murine models. Furthermore, the chemical composition of essential oils' bioactive components was investigated.

MATERIAL AND METHODS

Plant material and oil isolation

The leaves of *Piper aduncum* L were collected from Cajabamba district, Cajamarca Region, Perú. An expert in botany identified the plant species. One hundred grams of pulverized plant material was put in a round-bottom flask holding 1000 ml of distilled water, which was then linked to a modified Clevenger-type device. Hydro distillation was performed for three hours. The oil was later dried with anhydrous sodium sulfate (Na_2SO_4) and preserved in amber glass vials at + 4°C in a refrigerator for future experimental use.

GC-MS analysis

GC/MS analysis was used to identify the volatile components of the essential oil. The study used a Hewlett-Packard 6890 gas chromatograph with an HP-5MS column (30 m x 0.25 mm inner diameter, 0.25 µm film thickness) and a Hewlett-Packard 5972 mass spectrometer. The ionization voltage of the mass spectrometer in electron impact mode was 70

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eV. The temperature of the ionization source was 250°C. The column's initial temperature was established at 50 °C and sustained for 6 minutes. Following that, the temperature escalated by 3 °C per minute until it attained 240 °C, after which it surged by 15 °C per minute to reach 300 °C, a level that was sustained for 3 minutes. The injector port temperature was maintained at 290°C, employing a helium flow rate of 1.5 mL/min as the carrier gas. The components of essential oils were identified by comparing their mass spectra and retention indices (RI) with real samples from the NIST 2011 mass spectra database and the Wiley and Adams spectra libraries [18].

Experimental animals

This study used groups of eight Balb/ mice (25 – 30g) for each essay. The animals were obtained from the Vivarium of Universidad Peruana Cayetano Heredia, Perú. The animals were housed under standard laboratory conditions (room temperature 25.0±2.0°C, relative humidity 55-65%, and 12 h light: dark cycle) and fed with standard rodent pellets and water *ad libitum*. The experimental procedures adopted in this study were under the United States National Research Council Guidelines for the Care and Use of Laboratory Animals[19]. The Ethics Committee of the Medical School of Universidad Cesar Vallejo authorized this study with authorization number 044-CEI-EPM-UCV-2022.

Drug administration

Tween 80 (Sigma-Aldrich, Brazil) was utilized in a saline solution of 0.9% (1:5, v:v) as the drug's vehicle and solvent. In each experiment, rodents were randomly assigned to five groups containing eight mice. Diazepam at 1 mg/kg intraperitoneal (i.p.) and Fluoxetine at 10 mg/kg i.p. were both administered as reference medications (positive controls) for their anxiolytic and antidepressant effects, respectively. The control group administered the vehicle. For acute administration, EOs were administered orally to rodents in 25, 50, or 100 mg/kg concentrations. Following intraperitoneal administration, animals were subjected to behavioral assessments. All solutions were freshly prepared on the test days and given at a dosage of 10 ml/kg based on the animal's body weight.

Elevated plus-maze test (EPM)

The elevated plus maze test was conducted following the methodology outlined by Lister [20]. The EPM apparatus comprised two open arms (30x10 cm) and two closed arms (30x10x25 cm), originating from a central platform (10x10 cm) and elevated to a height of 40 cm. Thirty minutes post oral treatment administration, the animal was positioned at the intersection of the arms, oriented with its head towards one of the open arms. After each test, the maze was meticulously cleaned using wet tissue paper soaked in a 10% ethanol solution. A video camera recorded all test sessions. The percentage of open-arm entries and the time spent in the opened arm were quantified for 5 minutes, using ANY-maze software ® (Stoelting CO, USA).

Light-Dark Box Test (LDBT)

The light and dark box test used the Crawley & Goodwin methodology [21]. The apparatus was a rectangular box (45 × 27 × 27 cm) divided into two sections, one of which was dark (18 × 27 × 27 cm) and the other lighted with a white light (27 × 27 × 27 cm). A 7.5 x 7.5 cm hole was in the wall between each compartment. The percent time spent in the light compartment was quantified for 5 minutes, using ANY-maze software ® (Stoelting CO, USA).

Tail suspension test (TST)

The tail suspension test was conducted according to the established methodology outlined by Steru et al. [22]. The mice were positioned 58 cm above the floor utilizing adhesive tape applied approximately 1 cm from the tips of their tails. The immobility duration during the test phase was recorded as 300 seconds, using ANY-maze software ® (Stoelting CO, USA).

Forced swimming test (FST)

The time spent in immobility was quantified by monitoring how long a mouse stayed floating in the water without attempting to swim. The animals participated in a fitness evaluation 24 hours before the FST experiment, during which they engaged in a 15-minute swimming session following the established protocol. The total duration of immobility was observed and quantified over 300 seconds using ANY-maze software ® (Stoelting CO, USA) [23].

Antioxidant activity

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical assay

The antioxidant experiment using DPPH was conducted in a 96-well plate, according to the methodology outlined by Zhang et al. [24]. 100 µL of a 0.1 mM DPPH solution and 100 µL of the sample at different concentrations were introduced into each well. Control wells contained just 200 µL of methanol. The liquids were gently agitated for one minute and incubated in the dark at 25°C for 30 minutes to facilitate the reaction. The absorbance was measured at 517 nm with a spectrophotometer. BHT and Trolox were used as reference chemicals. All assessments were conducted in triplicate. The percentage of radical inhibition (I%) was calculated using the formula:

$$\text{Scavenging DPPH free radical percentage \%} = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{blank}} x 100)$$

$\text{Abs}_{\text{sample}}$ is the absorbance of the solution containing the sample, and $\text{Abs}_{\text{blank}}$ is the absorbance of the control sample, which contains only methanol. The EC_{50} value was calculated by semi-logarithmic regression analysis. The results were expressed as mean ± standard deviation.

2,2′-Azinobis-(3-ethylbenzothiazoline)-6-sulfonic Acid (ABTS•+) Radical Assay

The protocol outlined by Re et al. was adhered to, whereby an ABTS•+ solution was generated by combining ABTS (7 mM) with potassium persulfate (2.45 mM) and allowing it to incubate in the dark for 16 hours to facilitate the creation of the cation radical. The solution was further diluted in PBS to achieve an absorbance of 0.700 (±0.02) at a wavelength of 734 nm. The essential oils were solubilized in methanol to get the desired quantities. Subsequently, 50 µL of the sample solution was amalgamated with 150 µL of the ABTS•+ solution on a 96-well plate. The mixture was incubated in darkness for 30 minutes, after which the absorbance at 734 nm was recorded. A graph depicting the percentage inhibition of the ABTS•+ radical relative to sample concentration was constructed, and the IC_{50} values were determined. BHT and Trolox are used as reference chemicals. All measurements were performed in triplicate [25].

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP test was conducted using the methodology outlined by Xiao et al. [26], with several changes. FRAP stock solutions were formulated using acetate buffer (300 mM, pH 3.4), a 10 mM solution of 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) in 40 mM hydrochloric acid (HCl), and a 20 mM solution of $FeCl_3$ $6H_2O$. Subsequently, 180 µL of the FRAP reagent was combined with 20 µL of successive dilutions of the essential oils (EOs) in a 96-well plate. The mixture was incubated at 37 °C in darkness for 30 minutes. The antioxidant capacity was assessed by measuring the absorbance at 593 nm. The antioxidant activity was quantified as Trolox equivalents (TEAC). All determinations were performed in triplicate.

Statistical analysis

Data for behavioral assays were presented as the mean ± standard error of the mean (S.E.M). Results of antioxidant activity assays (DPPH, ABTS, and FRAP) were presented as IC_{50} values and reported as mean ± standard deviation (S.D.). Data for antidepressant and anxiolytic assays exhibited a normal distribution. Consequently, One-way analysis of variance (ANOVA) was employed, followed by Tukey's post hoc test, with $p < 0.05$ denoting statistical significance. Statistical analysis was conducted utilizing SPSS version 27.0. (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Table 1 lists the 22 identified components of PAEO. The most abundant components were linalool (29.16%), bicyclogermacrene (13.32%), nerolidol (12.38%), β-caryophyllene (10.76%), followed by α-humulene (4.97%), trans-ocimene (4.13%), cis-ocimene (3.86%), germacrene D (3.45%) and limonene (2.32%). Seven compounds were monoterpenes, and fourteen compounds were sesquiterpenes.

In Figure 1A, which presents the immobility time (Mean ± S.E.M) in seconds for the TST assay, the ANOVA test revealed significant differences across all groups ($p<0.05$). The shortest immobility time was observed in the fluoxetine group (64.25 seconds), followed by PAEO 100 mg/kg (120.25±1.79 seconds) and PAEO 50 mg/kg (133.63±1.11 seconds). Tukey's post hoc test confirmed that all groups, including the control, were significantly different from the fluoxetine group ($p<0.05$), except for the PAEO 25 mg/kg group, which did not show significant differences compared to the control ($p>0.05$). Similarly, in Figure 1B, which displays the immobility time for the FST assay, ANOVA indicated significant differences among all groups ($p<0.05$). The Tukey post hoc test showed that, as in the TST assay, the fluoxetine group differed significantly from all other groups ($p<0.05$). In contrast, the control and PAEO 25 mg/kg groups again showed no significant differences ($p>0.05$).

Table 1: Main chemical components (%) of *P. aduncum* essential oil.

N°	Compounds	RI	%
1	α-pinene	938	1.86
2	β-pinene	976	1.75
3	limonene	1034	2.32
4	1,8-cineol	1038	0.25
5	cis-ocimene	1042	3.86
6	trans-ocimene	1055	4.13
7	linalool	1093	29.16
8	(-)-cis-β-Elemene	1170	t
9	copaene	1268	0.14
10	β-elemene	1389	1.18
11	β-caryophyllene	1421	10.76
12	α-humulene	1469	4.97
13	neoalloocimene	1468	t
14	germacrene D	1473	3.45
15	eudesma-3,7-(11)-diene	1482	t
16	bicyclogermacrene	1497	13.32
17	γ-cadinene	1526	0.89
18	δ-cadinene	1535	2.23
19	nerolidol	1564	12.38
20	globulol	1589	0.67
21	bulnesol	1621	0.12
22	α-Bisabolol	1688	1.14
	Total identified (%)		94.58

RI, Retention index; t= traces (<0.1%)

Figure 2 displays the effects of PAEO in mice, with EPM results represented as Mean (±S.E.M). In Figure 2A, the group treated with diazepam spent the highest percentage of time in the open arms (66.58%±1.50), followed by the PAEO 100 mg/kg group (52.16%±1.20), PAEO 50 mg/kg group (43.33%±0.81), PAEO 25 mg/kg group (38.46%±0.57), and the control group (31.42%±1.78). ANOVA indicated significant differences among all groups ($p<0.05$). Tukey's post hoc test showed the control group differed significantly from the diazepam, PAEO 50, and PAEO 100 mg/kg groups ($p<0.01$) and from the PAEO 25 mg/kg group ($p<0.05$). The diazepam group also showed significant differences compared to the PAEO groups ($p<0.05$). Figure 2B shows the percentage of open-arm entries, with the diazepam group again having the highest value (56.10%±1.10), followed by PAEO 100 mg/kg (42.32%±0.94), PAEO 50 mg/kg (37.13%±0.67), and PAEO 25 mg/kg (27.77%±0.70). Significant differences were found between the control group and the diazepam, PAEO 50, and PAEO 100 mg/kg groups ($p<0.05$) and with the PAEO 25 mg/kg group ($p<0.005$). Additionally, the diazepam group differed significantly from all PAEO groups ($p<0.01$). In Figure 2C, the LDBT results show the diazepam group spent the most time in the light compartment (53.79%±1.04), followed by PAEO 100 mg/kg (45.92%±0.67), PAEO 50 mg/kg (38.47%±0.64), PAEO 25 mg/kg (32.72%±0.44), and the control group (29.43%±0.96). Significant differences were observed across all groups ($p<0.05$), with the control group showing significant differences compared to the diazepam, PAEO 50, and PAEO 100 mg/kg groups ($p<0.001$), and the PAEO 25 mg/kg group ($p<0.05$). The diazepam group was also significantly different from all PAEO groups ($p<0.01$).

The results in Table 2 reflect the antioxidant activity of Piper aduncum essential oil using the DPPH, ABTS, and FRAP methods, compared to the positive controls BHT and Trolox. In the DPPH assay, the essential oil showed an IC_{50} of 5.9 ± 0.08 mg/mL, significantly higher than the values for BHT (0.007 ± 0.58 mg/mL) and Trolox (0.005 ± 0.35 mg/mL), indicating a lower free radical neutralizing capacity. Similarly, in the ABTS assay, the essential oil's IC50 was 0.20 ± 0.06 mg/mL, higher than BHT (0.005 ± 0.42 mg/mL) and Trolox (0.004 ± 0.28 mg/mL), showing reduced antioxidant efficiency. In the FRAP method, the essential oil reached 109.5 ± 1.3 µmol Trolox × g^{-1}, demonstrating its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Statistically significant differences were observed between the essential oil and the controls ($p<0.05$).

DISCUSSION

We found as the main component linalool, consistent with a study in Brazil where linalool (31.7%) was the principal component found with a significant percentage. However, the content of byclogermacrene, nerolidol, and β-caryophyllene was minor [27]. However, our outcomes contradict other studies conducted in Perú where the main components found were asarone (39.32%) and metileugenol (12.85%) [28]. In addition, Cuban research found piperitone (23.7%) and camphor (17.1%) as the main constituents [11]. Other studies in Brazil found that dillapiole was the major component (86.9%) [29,30]. In another study, apiole (33.49%) and trans-β-caryophyllene (6.67%) were the principal constituents [31]. The variability of essential oil components in *P. aduncum* can be attributed to several factors, including genetic diversity, geographical location, environmental factors, and post-harvest processing methods [32]. Additionally, the drying process can influence the essential oils' yield and chemical composition, as drying tends to increase the yield and alter the proportion of certain compounds[33]. Seasonality is another critical factor; the yield and composition of essential oils can vary with the seasons, as seen in studies on other *Piper* species like *P. cernuum and P. rivinoides*, where specific seasons yielded higher oil percentages and different major components[34]. The presence of multiple elements, including genetics, environment, and processing, highlights the significance of considering them while researching and using essential oils from *P. aduncum*.

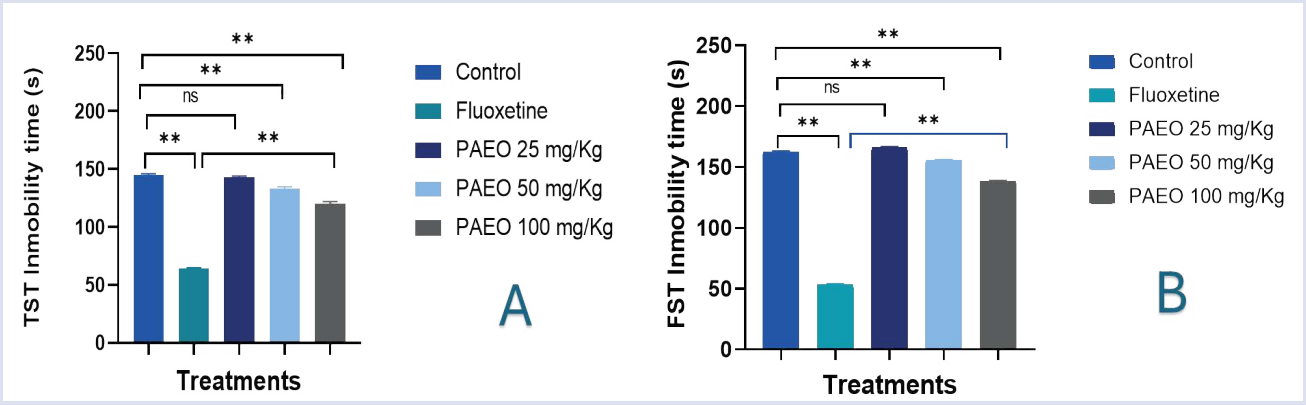

Figure 1. Antidepressant-like effects of PAEO in mice. A: Mean ±S.E.M immobility time in TST. B: Mean ±S.E.M. Immobility time in FST. Differences between groups were analyzed using ANOVA with Tukey's post hoc test for multiple comparisons. Statistical significance compared to the control group indicated by *p<0.05, **p<0.01.

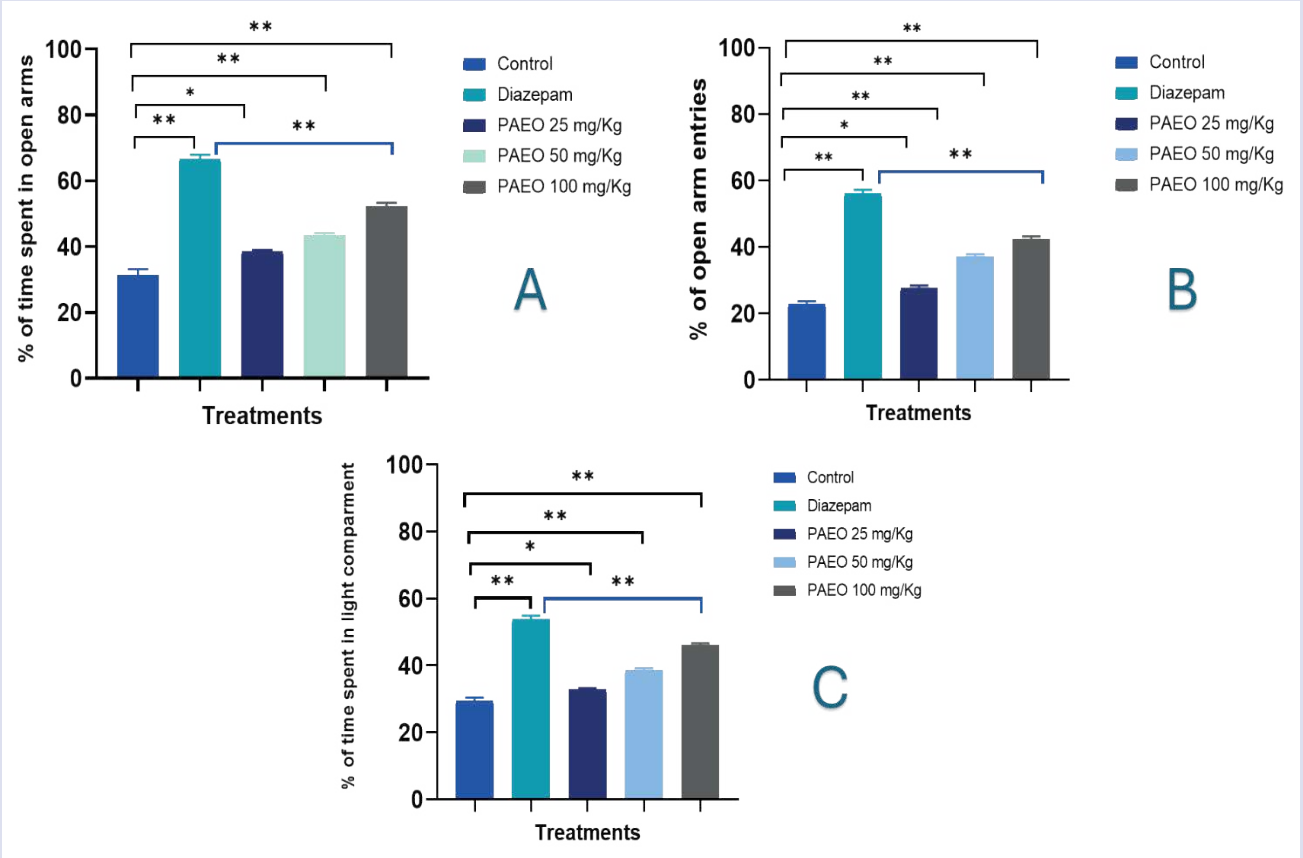

Figure 2. Anxiolytic-like effects of PAEO in mice. A: Mean ± ±S.E.M. percent open arms time in EPM. B: Mean ± S.E.M. percent open-arm entries in EPM. C: Mean ±S.E.M percent time in the light compartment in LDBT. Differences between groups were analyzed using ANOVA with Tukey's post hoc test for multiple comparisons. Statistical significance compared to the control group indicated by *p<0.05, **p<0.01.

Table 2. Radical Scavenging and Ferric Reducing Antioxidant Power Activities of Piper aduncum Essential Oil.

Test Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	FRAP IC_{50} (µmol Trolox× g^{-1})
Essential oil	5.9±0.08[a]	0.20±0.06[a]	109.5 ± 1.3
BHT*	0.007 ± 0.58[b]	0.005 ± 0.42[b]	
Trolox*	0.005± 0.35[c]	0.004± 0.28[c]	

*Positive control. The different superscript letters in a column indicate statistically significant differences ($p < 0.05$).

Research indicates that essential oils from *Piper* species, particularly *Piper nigrum*, possess antidepressant-like properties, likely mediated through serotonergic pathways. *Piper guineense* also shows various CNS activities, like antidepressant effects. The chemical diversity in essential oils from other *Piper* species suggests the potential for multiple biological activities, but specific antidepressant effects remain to be explored [17,35].

Plenty of research suggests that essential oils that are high in linalool have effects that are like those of antidepressants. Because this component affects the central nervous system by altering the monoaminergic and neuroendocrine systems, these effects are accomplished through various methods. In addition, it affects neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), which is an essential factor in developing depression [36]. In studies involving rodents and employing a chronic unpredictable mild stress (CUMS) model, it was observed that the administration of nerolidol reduced depression-like behaviors[37]. In addition, β-caryophyllene has antidepressant effects through the activation of CB2 receptors, which regulate emotional behavior and mood disorders[38]. Also present in our study, although in smaller quantities, α-pinene and β-pinene exhibit antidepressant properties[39]. These compounds could contribute to the overall antidepressant potential of *Piper aduncum* essential oil.

Piper species do exhibit anxiolytic-like effects, as demonstrated by various studies. In this sense, the essential oil of *Piper tuberculatum* (EOPT) has shown notable efficacy in lowering anxiety, suggesting that the monoterpenes may influence the effects of EOPT in the oil that interacts with the GABAergic system [40]. *Piper nigrum* essential oil also showed an anxiolytic-like effect in a dose-dependent manner; nevertheless, the mechanism of action was mediated through a serotonergic but not GABAergic transmission system [17]. In addition, *Piper guineense* essential oil showed significant sedative activity and potent anxiolytic effects [35]. Remarkably, our main component was linalool as in the case of *Piper guineense*; however, the main components for *Piper tuberculatum* were α and β pinene and limonene for *Piper nigrum*. Essential oils are composed of intricate mixtures of diverse compounds that have the potential to interact with specific biological targets, thus leading to variations in the observed mechanisms of action [41].

Chemical constituents of PAEO also exert anxiolytic effects, such as linalool, which is effective in reducing anxiety and commonly used in aromatherapy [42,43]. Nerolidol also exhibits anxiolytic properties without altering motor coordination [44,45]. β-Caryophyllene is an agonist for the CB2 receptor, which plays a role in modulating neuropsychiatric disorders, including anxiety [46]. Additionally, it interacts with benzodiazepine-GABAergic receptors, contributing to its anxiolytic effects [47]. Besides, other compounds present in minor quantities, such as limonene, α-pinene, and β-pinene, exert anxiolytic effects [39]. Consequently, some elements of PAEO possess anxiolytic properties. Nonetheless, not all components have been subjected to thorough examination. The observed effects on anxiety may result from the activity of certain elements individually or from the synergistic interplay of several constituents. Further research is needed to comprehensively elucidate the mechanisms of these interactions and identify the molecules responsible for their therapeutic benefits.

Essential oils from *Piper* species have anxiolytic and depressive effects, most likely mediated via interactions with neurotransmitter systems, neuroprotective effects, anti-inflammatory characteristics, and the synergistic activities of their bioactive constituents [48].

The more pronounced anxiolytic properties of PAEO compared to its antidepressant effects may be due to the synergistic interaction of its predominant bioactive compounds or the specificity in therapeutic targets; however, more research is needed to verify these assumptions and fully elucidate the specific mechanisms of action and the therapeutic potential of these natural products for anxiety and depression.

Research suggests that essential oils derived from Piper species, including those from *Piper cubeba* and *Piper nigrum*, exhibit antioxidant properties, demonstrating enhanced radical scavenging capacities [49,50]. The essential oils of *Piper* species are abundant in diverse phytochemicals that improve their capacity to neutralize radicals, including superoxide and hydroxyl radicals, which impede lipid peroxidation [51]. Linalool, the main chemical component discovered via our study, has shown the capacity to protect PC12 cells from the oxidative stress caused by hydrogen peroxide (H_2O_2). To do this, the cell's reactive oxygen species (ROS) levels decrease, and apoptosis is prevented. This demonstrates the compound's neuroprotective, anti-nociceptive, and anti-inflammatory capabilities [52]. Its antioxidant properties also mitigate benzene-induced oxidative stress associated with leukemia and hepatic injury [53]. However, our results indicate that PAEO exhibits lower antioxidant activity than reference compounds such as BHT and Trolox. This is in accordance with a study where *Piper acutifolium* essential oil, rich in α-phellandrene, β-myrcene, and β-phellandrene, has also shown low antioxidant activity in DPPH, ABTS, and FRAP assays [25]. The chemical nature of the predominant compounds in the oil can explain this. Previous studies have shown that non-oxygenated monoterpenes and sesquiterpenes possess some antioxidant capacity and are less effective than oxygenated phenolic compounds or sesquiterpenes in neutralizing free radicals and reducing metal ions [54,55]. The absence of oxygenated compounds or low chemical profile concentration could explain their lower efficacy. This trend is consistent with other essential oils with similar chemical characteristics, showing that oxygenated compounds are vital to achieving higher antioxidant activity because they stabilize free radicals better[56].

While PAEO contains components such as linalool and β-caryophyllene, which possess known antioxidant and neuroprotective properties, the oil's overall antioxidant capacity, as indicated by DPPH, ABTS, and FRAP assays, is relatively modest compared to standard antioxidants like BHT and Trolox. This suggests that the anxiolytic and antidepressant effects of PAEO may not be primarily due to its antioxidant activity. Therefore, while antioxidants generally support neuronal health [57,58], the therapeutic effects of PAEO appear to be more directly linked to its interaction with specific neurotransmitter pathways rather than its antioxidant strength.

CONCLUSION

Piper aduncum essential oil has antidepressant, anxiolytic, and modest antioxidant properties. These therapeutic activities are presumably affected by the individual effects of their key components and possible synergistic interactions among them; however, further study is necessary to validate these associations. Furthermore, more extensive research is required to clarify the specific biochemical pathways and mechanisms of action associated with these effects, especially to ascertain if the antioxidant capabilities significantly contribute to the reported depressive and anxiolytic actions.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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