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ADME/Tox Study, Phytochemical Analysis and In Vitro Antifungal Activity of Essential Oil from *Varronia curassavica* Jacq. (Boraginaceae)

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Abstract: *Varronia curassavica* Jacq. is an aromatic species appertaining to the Boraginaceae family and has been mentioned for its numerous traditional uses and pharmacological properties, especially its antimicrobial and anti-inflammatory effects. The aim of the present study was to investigate the phytochemical profile and antifungal activities of the essential oils of *V. curassavica*, in addition to analyzing the ADMET properties of the majority components. The GC-MS analysis of *V. curassavica* essential oil (EOVC) comprised 97.36% of total composition, with α-pinene, β-caryophyllene, and bicyclogermacrene (44.46%, 22.87%, and 13.05%, respectively) as the main constituents among other minor/trace constituents. The antifungal activity of EOVC was evaluated against three *Candida* species and was observed with IC_{50} > 200 µg/mL. Remarkably, the combination of EOVC with fluconazole significantly reduced the IC_{50} required for the drug to inhibit *C. tropicalis* (0.003 µg/mL), *C. albicans* (0.7996 µg/mL), and *C. krusei* (17.73 µg/mL). In addition, ADME/Tox studies using α-pinene revealed that the compound poses no toxicity threats but requires caution due to its high permeability to the blood–brain barrier (BBB). Overall, the obtained results suggest that *Varronia curassavica* essential oil is a potentially good antifungal agent for combating fungal resistance.

Keywords: *Cordia verbenacea*; fungal resistance; α-pinene; antifungal; ethnopharmacology

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1. Introduction

Invasive fungal diseases caused by opportunistic pathogenic fungi represent a significant public health concern due to the high mortality rate among individuals with compromised immune systems [1]. The most common organisms that cause clinical manifestations, such as cutaneous, mucosal, or organ-disseminated candidiasis, belong to the *Candida* genus, especially *C. albicans* (responsible for more than 50% of cases), *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* [2,3]. The main challenges are related to the resistance of these organisms to conventional therapy. Fungal resistance can be influenced by factors such as repetitive therapy and the limited availability of antifungal drugs [4,5].

In recent years, alternative treatment approaches have been investigated based on the therapeutic use of medicinal plants, searching for new bioactive agents with antifungal

action. The use of products of natural origin is considered effective in maintaining health, as well as being easily accessible to the population and having mild adverse effects [6,7]. Antioxidant and antimicrobial activities have clinical relevance and can be attributed to the bioactive compounds present in medicinal plants [8], in addition to medicinal plants being seen as a promising solution for treating conditions such as fungal infections [9].

Medicinal plants are widely used to extract secondary metabolites with pharmacological applications, such as essential oils. Essential oils are volatile and lipophilic components that have been the subject of research due to their promising antimicrobial action, which acts through different mechanisms [10]. In addition, they have diverse biological activities, including antioxidant, antiparasitic, antibacterial, and antifungal properties. These properties represent effective alternatives against microbial resistance, including an efficient action against biofilms [11–13].

The medicinal species *Varronia curassavica* Jacq. (Synonyms *Cordia verbenacea* A. DC. and *C. curassavica* (Jacq) Roemer & Schultes) stands out widely in the field of natural medicine due to its use as an anti-inflammatory, anti-rheumatic, and analgesic agent [14,15]. Current research underscores the therapeutic value of the essential oil, whose chemical composition has been extensively investigated due to the presence of relevant phytochemicals, such as terpenes previously reported as α-pinene and β-caryophyllene, which exhibit diverse activities, including anti-inflammatory, analgesic, antimicrobial, larvicidal, and antiparasitic properties [16–19]. The presence of compounds such as flavonoids contributes to its antibacterial properties, which can influence the biological activity of antibiotics belonging to the class of aminoglycosides, with the absence of substances such as alkaloids [20,21].

Therefore, research into the ethnopharmacological application of *V. curassavica* highlights its antimicrobial action and shows that this plant is effective in combating pathogenic microorganisms, particularly fungal strains. The efficacy of this species and the use of its essential oil do not show any possible toxic effects. The present study reports the phytochemical profile and in vitro antifungal and fluconazole modulatory activity of the essential oil from leaves of *V. curassavica*, in addition to the ADME/Tox studies of its major compound.

2. Materials and Methods

2.1. Plant Material

The leaves of *V. curassavica* (Figure 1) were collected in the municipality of Jardim, Ceará, Brazil, at coordinates: −7.554917 W and −39.306611 S, in the period of January (2022). The sample collected was stored in the Herbário Caririense Dárdano de Andrade-Lima (HCDAL) of the Universidade Regional do Cariri (URCA) under voucher number 15.291. The research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the code AEFC723 and in the Biodiversity Authorization and Information System (SisBio) under the number 82789-1.

Figure 1. *Varronia curassavica* identified in the municipality of Jardim, Ceará, Brazil.

2.2. Essential Oil Extraction

To extract the essential oil of *V. curassavica* (EOVC), the collected leaves were dried, crushed, and added to a 5 L volumetric flask with 2 L of distilled water and subjected to constant boiling for 2 h using a Clevenger-type system. Each extraction used 200 g of leaves, obtaining a yield of 0.83%. After extraction, the oil obtained was stored in amber bottles and refrigerated at 4 °C.

2.3. Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) was used for the phytochemical analysis of EOVC. The analysis was carried out using an Agilent Technologies AutoSystem XL GC-MS system, operating in EI mode at 70 eV. Two capillary columns were used: an HP 5MS (30 m × 0.35 mm; film thickness 0.50 µm) and an HP Innowax (30 m × 0.32 mm; film thickness 0.50 µm). The system was equipped with a split/splitless injector (220 °C) and coupled to an FID detector. The thermal programmer was set from 60 °C (1 min) to 180 °C at a rate of 3 °C/min; the detector temperature was 220 °C. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The injected volume of EOVC was 1 µL, diluted in chloroform (1:10). Two replicate samples were processed in the same way, and the relative concentrations of the components were calculated based on the GC peak areas, without the use of correction factors.

2.4. Antifungal Activity

The antifungal activity of EOVC was tested against *Candida albicans* INCQS 40006, *Candida krusei* INCQS 40095, and *Candida tropicalis* INCQS 40042 obtained from the Collection of Reference Microorganisms in Health Surveillance (CMRVS) of the National Institute for Quality Control in Health (FIOCRUZ-INCQS). The *Candida* strains were grown in Petri dishes containing Sabouraud Dextrose Agar (SDA, Kasvi) and incubated at 37 °C for 24 h. Aliquots were then transferred to test tubes containing 4 mL of saline solution (0.9%) and adjusted to a turbidity of 0.5 McFarland (1.5×10^8 CFU/mL). The EOVC (0.0191 g) was diluted in 0.5% DMSO. It was then diluted in Sabouraud Dextrose Broth (SDB) until it reached the stock concentration (1.024 µg/mL) [22]. Fluconazole (capsule—FLUCOMED) was used as a positive control.

The broth microdilution technique with 96-well plates was used to determine the IC_{50}. EOVC and fluconazole were diluted in SDB (90 µL) (1:1 *v*/*v*) at concentrations ranging from 2 to 1.024 µg/mL, each in quadruplicate. Then, 10 µL of the fungal inoculum was added. Fungal growth and sterility controls were included. The plates were incubated at 37 °C for 24 h, and absorbance was measured at 630 nm using an ELISA reader (Termoplate® Kasuaki, Beijing, China) [23].

2.5. Fluconazole Potentiating Action

In the fluconazole potentiating evaluation, OEVC was tested using the Sub-inhibitory Matrix Concentration (CM/8). Flat-bottomed 96-well plates containing SDB medium with OEVC at its sub-inhibitory concentrations were used. Fluconazole was used in a range of concentrations between 2 and 1.024 µg/mL, following the method described by Coutinho et al. [24] with modifications. The plates prepared for the broth microdilution and serial dilution technique were incubated at 37 °C for 24 h, and the analysis was carried out using an ELISA spectrophotometer (Termoplate® Kasuaki, China) [23].

2.6. In Silico Prediction

The in silico prediction of the major compound (α-pinene, >30%) was evaluated using the ADMETlab 2.0 platform. This platform, recognized for its enhanced functionalities for the analysis of ADME (absorption, distribution, metabolism, excretion), toxicity, physicochemical properties, and medicinal chemistry, is based on more accurate and efficient predictions, relying on general analysis tools such as the toxicological interaction radar [25].

2.7. Statistical Analysis

The means of the data and their respective standard errors of the mean (SEM) were calculated and subjected to a one-way Analysis of Variance (ANOVA). Subsequently, the data were subjected to a reliability test using the Tukey test at 95% reliability. The results were considered statistically significant when $p < 0.05$. The data were analyzed using GraphPad Prism software version 6 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Phytochemical Composition

The phytochemical analysis of EOVC using GC-MS identified 11 chemical components which represent 97.36% of the oil's total composition. The EOVC is mainly composed of hydrocarbon monoterpenes (47.25%), followed by hydrocarbon sesquiterpenes (43.72%). The major compounds identified were α-pinene (44.46%), β-caryophyllene (22.87%), and bicyclogermacrene (13.05%). As shown in Table 1, α-pinene was identified as the major constituent (>30%) and was therefore selected for the in silico prediction analysis.

Table 1. Chemical composition (%) of the essential oil of *Varronia curassavica*.

Components	RI		(%)
α-pinene	976		44.46
β-Pinene	980		2.79
β-Elemene	1375		1.14
β-Caryophyllene	1428		22.87
α-humulene	1460		2.91
Zingiberene	1492		1.01
Bicyclogermacrene	1496		13.05
cis-α-Bisabolene	1778		2.74
Nerolidol	1961		3.32
Caryophyllene oxide	2023		1.89
Juniper camphor	2205		1.18
Hydrocarbon Monoterpene		47.25	
Oxygenated Monoterpene		1.18	
Hydrocarbon Sesquiterpene		43.72	
Oxygenated Sesquiterpene		5.21	
Total		97.36	

RI: Retention index.

3.2. Antifungal Activity and Fluconazole Potentiating Effect

The results of EOVC antifungal activity are shown in Table 2. Different *Candida* strains exhibited varying levels of susceptibility to EOVC. *Candida albicans* showed the greatest sensitivity to the essential oil, as demonstrated by IC_{50} values reaching concentrations of 254.3 µg/mL, considered clinically relevant. *Candida tropicalis* (IC_{50} = 649.2 µg/mL) showed the second lowest inhibition value, suggesting the potential use of EOVC in antifungal applications against *C. albicans* strains according to their susceptibility.

Table 2. Half-maximal inhibitory concentration (IC_{50}) of *Varronia curassavica* essential oil (EOVC) tested against *Candida* strains and fluconazole (FCZ) potentiating action.

IC50	µg/mL		
	C. albicans	***C. krusei***	***C. tropicalis***
EOVC	254.3	>1024	649.2
FCZ	16.14	52.41	4.775
FCZ + EOVC	0.7996	17.73	0.003

Regarding *C. krusei*, no significant inhibitory action was observed. However, when combined with the drug fluconazole, there was a significant reduction in IC_{50} values

to 17.73 µg/mL, representing a decrease of approximately 65% in the concentration of fluconazole required to inhibit fungal growth. The synergistic activity of EOVC with fluconazole was also noted against strains of *C. albicans* and *C. tropicalis* (Figure 2), with concentration reductions of ~95% and 98%, respectively. These results suggest the potential use of EOVC as a complementary agent in antifungal therapies.

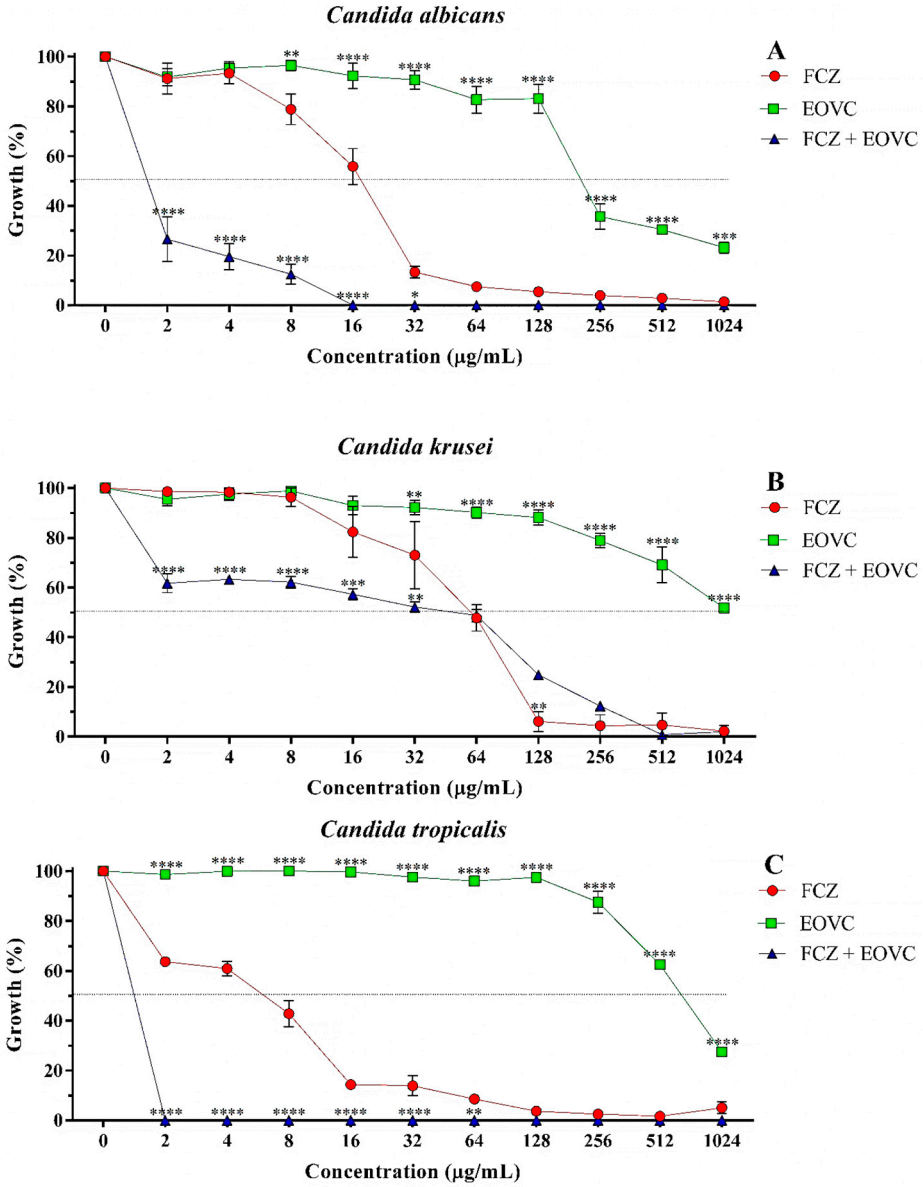

Figure 2. Potentiating activity of fluconazole (FCZ) combined with *V. curassavica* essential oil (EOVC), tested against *C. albicans* (**A**), *C. krusei* (**B**), and *C. tropicalis* (**C**). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. The bars represent the standard error of the mean (n = 3).

3.3. ADME/Tox Prediction with α-Pinene

According to the in silico analysis, the radar of the physicochemical properties of the α-pinene (Figure 3) indicates divergences regarding its application as a drug, since the aqueous partitioning capacity (logP < 3) and the influence on physiological pH (logD < 3) are above the maximum desired limit, for which reason the Pfizer, GSK, and Golden Triangle drug-like rules do not apply (Table 3). However, positive aspects are observed in the solubility (logS) and flexibility (Flexibility = nRot/nRig) parameters.

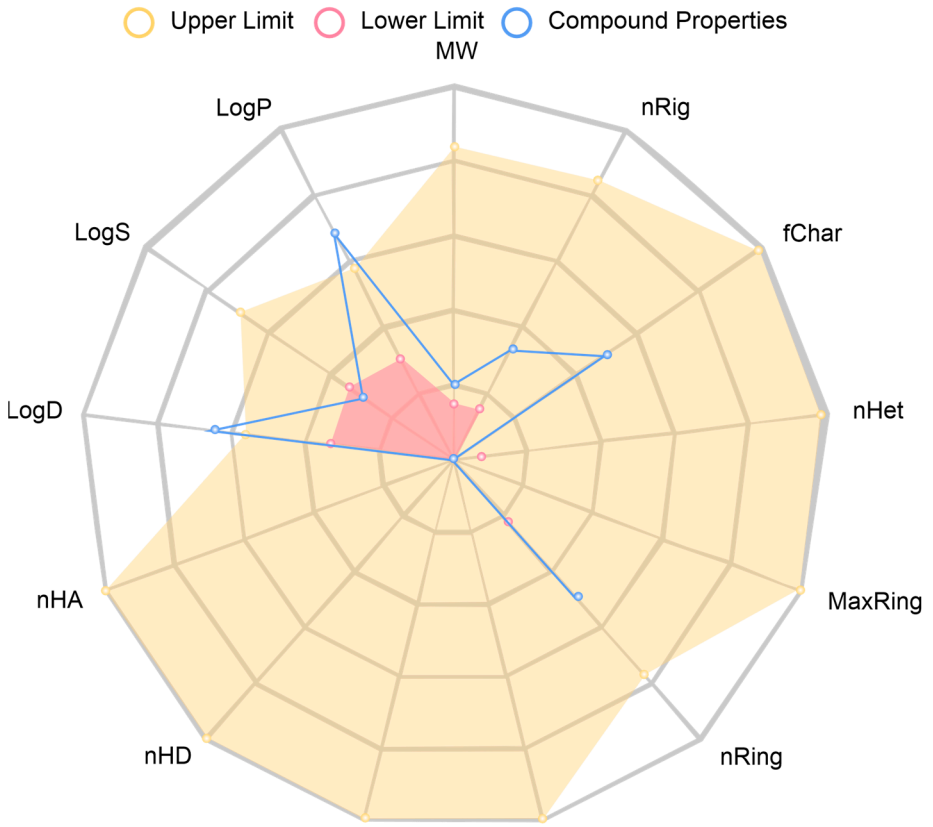

Figure 3. Radar of physicochemical behavior of the compound α-pinene, comparison of minimum (lower) and maximum (upper) desirable measurements.

Table 3. Performance of physicochemical properties and similarity to drugs using α-pinene as a model.

Category	Property	Value
Physicochemical Property	MF	$C_{10}H_{16}$
	MW	136.13 g/mol
	NHA	0
	NHD	0
	NRB	0
	TPSA	0.0
	logS	−4.662
Medicinal Chemistry	Lipinski Rule	Accepted
	Pfizer Rule	Rejected
	GSK Rule	Rejected
	Golden Triangle	Rejected

MF: molecular formula; MW: molecular weight; NRB: number of rotatable bonds; NHA: number of hydrogen acceptors; NHD: number of hydrogen donors; TPSA: Topological Polar Surface Area; LogS: Log of the aqueous solubility.

The ADME/Tox properties of α-pinene are summarized in Table 4, highlighting important data of pharmacological relevance. Absorption is considered promising, since human intestinal absorption activity (HIA) does not present problems, and the compound does not inhibit the permeability glycoprotein (Pgp) responsible for active efflux. However, the distribution of α-pinene and its permeability to the blood–brain barrier (BBB) warns of possible toxic activity. As for the metabolization and toxicity of the compound, there are no significant concerns, except for ophthalmic and pulmonary applications. In addition, Table 5 shows possible routes of toxicity for α-pinene, indicating promising safe aspects.

Table 4. ADME/Tox performance (absorption, distribution, metabolism, excretion, and toxicity) of α-pinene.

Category	Property	Value	Decision
Absorption	Caco-2 Permeability	−4.303	●
	MDCK Permeability	1.8×10^{-5}	●
	Pgp-inhibitor	---	●
	Pgp-substrate	---	●
	HIA	---	●
Distribution	BBB Penetration	++	●
Metabolism	CYP1A2 inhibitor	-	●
	CYP2C19 inhibitor	--	●
	CYP2C9 inhibitor	-	●
	CYP2D6 inhibitor	---	●
	CYP3A4 inhibitor	---	●
Excretion	CL	15.022	●
	$T_{1/2}$	0.114	-
Toxicity	Human Hepatotoxicity	--	●
	Drug-Induced Liver Injury	---	●
	AMES Toxicity	---	●
	Rat Oral Acute Toxicity	---	●
	Skin Sensitization	--	●
	Carcinogenicity	---	●
	Eye Corrosion	+++	●
	Eye Irritation	+++	●
	Respiratory Toxicity	++	●

P-gp; p glycoprotein; HIA: Human intestinal absorption; BBB: Blood–Brain Barrier; CYP: Cytochrome-P; CL: Clearance; $T_{1/2}$: Half-life time. Symbols means 0–0.1 (---), 0.1–0.3 (--), 0.3–0.5 (-),0.7–0.9 (++) e 0.9–1.0 (+++); ●: Non-toxic; ●: high possibility of toxic activity.

Table 5. Possible routes of α-pinene toxicity.

Property	Value	Decision
Androgen receptor	---	●
Androgen receptor ligand-binding domain	---	●
Aryl hydrocarbon receptor	---	●
NR-Aromatase	---	●
Estrogen receptor	---	●
Estrogen receptor ligand-binding domain	+	●
Peroxisome proliferator-activated receptor gamma	---	●
Antioxidant response element	---	●
ATPase family AAA domain-containing protein 5	---	●
Heat shock factor response element	0.032	●
Mitochondrial membrane potential	0.082	●

● and (---): Non-toxic feature; ● and (+): Not very relevant value.

4. Discussion

The chemical composition results are supported by several scientific studies that analyzed the essential oil of *V. curassavica*. It was identified that α-pinene and β-caryophyllene are the major phytochemicals in this oil, with percentages ranging from 56.69–25.32% and 21.78–12.52%, respectively [16,19,26–28]. However, different results were observed in which sesquiterpenes are predominant, with β-caryophyllene comprising 25.4–23.26% [29,30] and shyobunol with 27.46–24.24% of the total oil percentage [31].

Variations in the quantity and volatile components of essential oils can be explained by multiple biotic and abiotic factors, such as plant age [28], season and location [27], herbivory [32], and collection during different seasons [14]. Additionally, alterations in phytochemicals may occur due to different essential oil extraction techniques, as observed in

hydrodistillation and microwave extraction methods [31,33]. Changes in phytoconstituents can directly influence the biological activity of the essential oil.

Previous studies indicate that EOVC exhibits antimicrobial activity against parasites that cause human diseases, with the potential to disrupt cell membrane integrity [16,19]. The antifungal activity of EOVC against *C. albicans* can be partially confirmed, since Farias et al. [28] demonstrated an MIC of 1000 µg/mL. Rodrigues et al. [29] also identified the MIC of 512 µg/mL for standard strains of *C. albicans* 40006 and *C. krusei* 653, concluding that EOVC exhibits fungistatic activity.

Terpenoid compounds such as α-pinene have been shown to possess antibacterial, antifungal, anti-*Leishmania*, anti-inflammatory, antioxidant, neuroprotective, and antitumor properties [34–37]. In tests against fungi, α-pinene has been demonstrated to cause organelle damage, induce oxidative stress [38], interrupt enzyme production and biofilm formation, and interfere with fungal membranes [39], specifically by disrupting permeability through binding to ergosterol [40].

Multiple studies demonstrate the anti-*Candida* potential of α-pinene. The Minimum Fungicidal Concentration (MFC) values range from 2 to 4 mg/mL for different strains of *C. albicans* [41], and the MIC ranges from 64 to 128 µg/mL. Additionally, α-pinene can be combined with other antifungal agents, such as boric acid and polyene compounds [42,43]. When tested on 15 strains of *C. albicans*, the MIC values reach 268.13 mg/L [44].

β-caryophyllene is another interesting compound due to its biological activities, including antimicrobial action and the ability to disrupt biofilms by penetrating this protective resistance mechanism [3,45]. Its potential as an antifungal agent has also been evaluated, showing low MIC values [46,47]. When tested against *Candida* strains, β-caryophyllene affects fungal virulence mechanisms [48]. It can be suggested that β-caryophyllene might contribute to synergism with fluconazole, like the synergism observed with other drugs such as ketoconazole [49].

Considering the biological activities of α-pinene, it is also important to investigate its toxic potential. Cytotoxic analyses of α-pinene in fibroblasts [50] and human keratinocytes [42] did not identify any toxic effects. Similarly, toxicity studies using the Zebrafish (*Danio rerio*, Hamilton) model indicated a median lethal concentration (LC_{50}) of 441.360 mg/L [51], which is considered non-toxic as high concentrations are required to achieve toxicity. These results corroborate previous in silico analyses of α-pinene, which indicated milder toxicological effects allowing safer applications [52].

Furthermore, in silico predictions indicate that α-pinene exhibits high rates of BBB penetration. In vivo assays in mice show that inhalation of α-pinene results in significant transport to the brain [53]. Conversely, it has been demonstrated to have a neuroprotective effect by modulating the expression of anti-inflammatory genes, thereby attenuating neuroinflammation, and potentially restoring BBB function in rats [54,55]. Additionally, in vivo models reveal that α-pinene is not a substrate for permeability glycoprotein (Pgp) [56].

5. Conclusions

The essential oil of *Varronia curassavica* leaves is mainly composed of monoterpene and sesquiterpene hydrocarbons, mainly α-pinene and β-caryophyllene. Assays carried out with the essential oil showed the presence of significant antifungal activity against strains of *Candida albicans* and *Candida tropicalis*, revealing potential for possible applications. In addition, a remarkably potentiation of the antifungal action of fluconazole against different strains of *Candida* was observed, indicating possible use as an adjuvant in the treatment of fungal infections. The in silico prediction shows that α-pinene has safe characteristics for relevant clinical applications as an anti-*Candida* agent. However, further research is needed to evaluate the synergistic antifungal action of the major constituents, α-pinene and β-caryophyllene, as well as in vivo toxicity evaluation, in order to establish the safety of using this natural product.

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