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In silico analyses of toxicity of the major constituents of essential oils from two *Ipomoea* L. species

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ABSTRACT

Convolvulaceae Juss. is a family of vines and shrubs composed of species of ecological and economic importance. *Ipomoea asarifolia* (Desr.) Roem. & Schult. and *I. setifera* Poir. are ruderal and evergreen weeds that invade pastures and cause intoxication in cattle during the dry season. In the present study, the essential oils (EOs) of the leaves from *I. setifera* (dry season) and *I. asarifolia* (dry and wet seasons) were obtained by steam distillation for 3h. The chemical composition of the EOs was determined using gas chromatography coupled to gas spectrometry (CG/MS) and gas chromatography with flame ionization detector (CG-FID). To correlate the toxicity of the major chemical constituents of *I. setifera* and *I. asarifolia* EOs, we predicted the inhibition activity against the cytochrome P450 (CYP450) and P-glycoprotein 1 (P-gp) using a machine learning-based (ML-based) algorithm. *In silico* analyses were also applied to evaluate the pharmacokinetics properties related to the penetration in the blood-brain barrier (BBB) and gastrointestinal absorption. The chemical composition of the EO of *I. setifera* was characterized by high levels of (*E*)-caryophyllene (36.7%) and β-elemene (20.49%). The *I. asarifolia* EO showed a phytol derivative as the main chemical constituent in the dry season (35.49%), and its content was reduced in the sample collected during the wet season (10.67%). The constituent (*E*)-caryophyllene was also present in the leaves of *I. asarifolia*, but at lower levels (15.93–19.93%) when compared to the EOs of *I. setifera.* Our computational analyses indicated that the constituents caryophyllene oxide, cedroxyde, pentadecanal, and phytol can be related to the toxicity of these weeds. This is the first study to report the chemical composition of *I. asarifolia* and *I. setifera* EOs and correlate their molecular mechanism of toxicity using *in silico* approaches.

1. Introduction

Convolvulaceae family comprises 1660–1880 species, 59 genera, and 12 tribes (Mitchell et al., 2016). The family includes herbs, shrubs, sub-shrubs, lianas, and, seldom, trees (Delgado Júnior et al., 2014; Staples et al., 2012). The genus *Ipomoea* L. is characterized by species with a range of chemical compounds described by different biological activities, such as antibacterial, antifungal, analgesic, anti-inflammatory, antinociceptive, antitumoral, and antioxidant (Chan et al., 2016; Manigauha et al., 2015; Meira et al., 2012; Shamli and Chandra, 2015; Zengin et al., 2017). Nevertheless, several species are toxic for human and animal consumption (Ferreira and Maruo, 2015).

Ipomoea setifera Poir. is widely distributed in South and Central America and Africa (GBIF, 2020a; Plants of the World, 2020a; Tropicos, 2020a), while *I. asarifolia* (Desr.) Roem. & Schult. has confirmed occurrence in South and Central America, tropical Africa, East Asia, and India (GBIF, 2020b; Khaled et al., 2017; Plants of the World, 2020b; Tropicos, 2020b). In Brazil, *I. asarifolia* and *I. setifera* occur in almost all regions, being considered noxious weeds, due to their rapid growth and occurrence in pasture areas (Flora do Brasil, 2020; Timossi and Durigan,

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2006). These ruderal plants invade pastures and cause intoxication in livestock via ingestion of their leaves and flowers mainly during the dry season (Austin and Bianchini, 1998; Carvalho et al., 2014; Costa et al., 2011).

Intoxication caused by the ingestion of *I. asarifolia* leaves have been described in cattle (Carvalho et al., 2014), goats (Carvalho et al., 2014; Freitas et al., 2011; Medeiros et al., 2003), sheep (Araújo et al., 2008; Carvalho et al., 2014; Freitas et al., 2011), and buffaloes (Barbosa et al., 2005). *I. asarifolia* can induce tremorgenic syndrome and neurodegeneration in the intoxicated animals (Carvalho et al., 2014; Freitas et al., 2011; Medeiros et al., 2003). Moreover, *I. asarifolia* causes acute intoxication, inducing symptoms in goats within 24 h after the ingestion, whereas *I. setifera* may cause chronic intoxication (Pinheiro and Rosa, 2010).

The biosynthesis of secondary metabolites of aromatic and medicinal plants is determined by genetic factors (Li et al., 2020; Verma and Shukla, 2015). However, other factors contribute to the biosynthesis of natural products, such as temperature, humidity, precipitation, solar radiation intensity, seasonality, age, stage of plant development, and inter and intraspecific relationships of the plant, which can cause significant changes in the production of these metabolites and consequently in the chemical composition and activity of essential oils (EOs) (da Silva et al., 2015; Gobbo-Neto and Lopes, 2007; Nascimento et al., 2020; Paulus et al., 2013). Seasonality is a key factor responsible for the variation of the chemical composition and yield of EOs (da Silva et al., 2015; Figueiredo et al., 2018; Silva et al., 2018). The intoxication caused by the bindweeds from the genus *Ipomoea* occurs mainly during the drought when these invasive and drought-resistant species are consumed by livestock due to lack of foraging alternatives (Araújo et al., 2008). However, it is not known which compounds of these species are involved with the intoxication pattern. Proper management of intoxication of livestock requires a better understanding of these plant's chemical profiles.

Several *in vitro* techniques have been used to evaluate the absorption, metabolism and toxicity of chemical substances in human and animal cells (Twarużek et al., 2018). In silico methods are alternatives to experimental approaches to predict toxic outcomes of substances in the environment and humans (Lagunin et al., 2018; Li et al., 2017) as well as to screen for potential bioactivity of compounds (da Costa et al., 2019; Da Costa et al., 2019; Galúcio et al., 2019). The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of a compound represent the pharmacokinetic profile of its structure in living organisms and plays a crucial role to predict their toxicological effects (Atallah et al., 2013). The prediction of ADMET properties of natural or synthetic derivatives compounds have been widely applied in different studies to screen the bioavailability and bioactivity of potential novel drugs (Aouidate et al., 2018; da Costa et al., 2019; Hevener, 2018; Liu et al., 2019; Uba and Yelekçi, 2018; Weako et al., 2020). Based on that, several *in silico* screening methods have been applied to identify drug-like and ADMET properties of compounds using physicochemical calculations as well as to identify toxic or unstable functional groups; and these methods represent a less time-consuming and a cheaper approach, when compared with the experimental techniques (Daina and Zoete, 2016; Dimitri and Lió, 2017; Hutter, 2008).

Plant toxicity is often a defense strategy against herbivory, and it is caused by the presence of a wide variety of secondary metabolites (Moore and Johnson, 2017). Several defense metabolites are found in the EOs of plants, causing episodes of the poisoning of herbivores by the consumption of these oils (Dehghani, 2019; Farhat et al., 2001). The toxicity of an EO may be related to a specific constituent or due to the synergistic effect of several constituents acting together (Izumi et al., 2012; Kim et al., 2012; Pinheiro et al., 2015).

The evaluation of the toxicity of chemical constituents of *I. setifera* and *I. asarifolia* EOs are scarce, and this information could provide important insights into the mechanism of intoxication of these species against the livestock as well as the bioavailability of their chemical compounds (Carvalho et al., 2014; Ferreira and Maruo, 2015). In the present study, we described the chemical composition of the EOs of *I. setifera* and *I. asarifolia*. We also investigated the drug-likeness of the major chemical constituents of EOs using chemical filters to contribute with studies that assess the mechanisms of intoxication associated with these species.

2. Material and methods

2.1. Plant material

The leaves of *I. asarifolia* were collected in the municipality of Belém (Pará/Brazil) at the Parque de Ciência e Tecnologia (Universidade Federal do Pará) in the dry season (August/2019) and wet season (January/2020). Leaves of *I. setifera* were collected in the same locality, but only during the dry season (September/2019). The samples were collected at 0900. Then, the plant material was dried in a forced convection oven at 35 °C for six days and subjected to the EO extraction.

The botanical identification was performed by comparison with an authentic specimen of *I. setifera* and *I. asarifolia* and samples of the plant material (*I. setifera*: MG237128 and *I. asarifolia*: MG237141) were deposited in the Herbarium of the Museu Paraense Emílio Goeldi (Belém, State of Pará, Brazil).

2.2. Extraction of leaves EOs

The EOs of *I. setifera* (522.86 g) and *I. asarifolia* (360.0 g and 382.81 g for the dry and wet season, respectively) were extracted by steam distillation for 3 h. After extraction, the EOs were centrifuged for 5 min at 3000 rpm, dehydrated with Na_2SO_4, and stored at 5 °C.

2.3. Chemical analysis

The chemical composition analysis was performed by gas chromatography coupled to mass spectroscopy (GC-MS), using a Shimadzu instrument Model QP 2010 Plus (Shimadzu, Tokyo, Japan), equipped with a DB-5MS column (30 m × 0.25 mm; 0.25 μm film thickness). Helium was used as carrier gas adjusted to 1.2 mL/min; splitless injection of 1 μL of a hexane solution (0.5 mL of hexane and 2 μL of the oil); injector and interface temperature were 250 °C; temperature programmed in the oven: 60–250 °C (3 °C/min). An electron ionization mass spectrometer at 70 eV with the ion source temperature and other parts at 220 °C was used. The quantitative analysis of the chemical constituents was performed by peak-area normalization using a flame ionization detector (FID-Model QP 2010, Shimadzu, Tokyo, Japan) using the same conditions as GC-MS, except that hydrogen was used as a mobile phase. The components were identified based on the retention index (RI), which was calculated using the retention times of a homologous series of n-alkanes (C8–C40, Sigma-Aldrich, St. Louis, MO, USA). The compounds present in the samples were identified by comparison of their mass spectra and retention indices with data present in commercial libraries (Adams, 2007; Mondello, 2011).

2.4. Evaluation of drug-like and ADMET properties

The drug-like and ADMET properties of the major chemical constituents (≥3%) of *I. setifera* and *I. asarifolia* EOs were analyzed using different computational approaches. The inhibition activity of cytochrome P450 (CYP450) and P-glycoprotein 1 (P-gp), enzymes related with the metabolism and absorption of exogenous compounds were analyzed in the SwissADME server which applies a support vector machine, a machine learning-based (ML-based) algorithm that identifies similarities between the analyzed natural compounds with the known inhibitors of these enzymes.

Some pharmacokinetics properties related to the absorption into the biological membranes were investigated using physicochemical and

structural properties of the analyzed compounds and compared with predictive models. The penetration in the blood-brain barrier (BBB) was predicted using the BOILED-Egg model (Daina and Zoete, 2016), and the gastrointestinal absorption was investigated using Lipinski 'Rule of 5' (RO5) (Lipinski et al., 1997), Veber (Veber et al., 2002) chemical filters, and BOILED-EGG model available in the SwissADME server (Daina et al., 2017). Finally, we also investigated the presence of promiscuous fragment of the analyzed compounds using the Rapid Elimination of Swill (REOS) and Brenk filters available in the FAFDrugs (Lagorce et al., 2015) and SwissADME servers (Daina et al., 2017), respectively. REOS combines a set of functional groups related to promiscuous molecules with a set of physicochemical and structural properties of compounds related to the drug-likeness (Walters and Namchuk, 2003). Similarly, Brenk filter contains a set of chemical moieties known by their toxicity and instability (Brenk et al., 2008). Identification of functional groups, as well as, the overall physicochemical properties of compounds related to promiscuity/reactivity is an important task to determine their toxicity (Bruns and Watson, 2012; Walters and Namchuk, 2003; Wang and Greene, 2012). The complete list of the chemical structures of the investigated compounds are available in SDF and SMILES formats in the Supporting information 01.

3. Results and discussion

3.1. Chemical composition of the EOs

In the present study, a total of one hundred chemical constituents were identified in the EOs of *I. setifera* and *I. asarifolia* leaves, representing more than 90% of the chemical composition of the samples (Table 1). The sesquiterpene hydrocarbons were the most abundant compounds, mainly in the sample of *I. setifera* (88.54%), and it could be due to their high molecular weight and reduced volatility that prevents their loss during drying. Conversely, *I. asarifolia* (wet season), was the only sample to present considerable amounts of phenylpropanoids (5.46%).

Analyzing the chemical composition of the EOs of *I. asarifolia,* the phytol derivative was the main chemical constituent identified during the dry season (35.49%) and presented a lower content in the sample collected during the wet season (10.67%). The sesquiterpene hydrocarbon (*E*)-caryophyllene varied between 19.93% and 15.93% in the dry and wet seasons, respectively. The constituents β-elemene (2.3–7.29%) and germacrene D (6.05–9.02%) were also identified in the samples of *I. asarifolia* in both seasons. Safrole was identified only in the EO obtained in the wet season (January/2020) (Table 1).

The chemical composition of the EO of *I. setifera,* obtained from the sample collected in the dry season (September/2019), was characterized by (*E*)-caryophyllene (36.7%), followed by β-elemene (20.49%), germacrene D (9.45%), and α-humulene (8.03%) (Table 1).

Few studies have described the volatile chemical components of the genus *Ipomea* EOs. Marie et al. (2007) obtained the EO of fresh and dried leaves of *I. pescaprae.* According to the reported results, both dry and fresh leaves were characterized by high levels of sesquiterpenoids (70.4% and 42.5%, respectively). The authors also observed a reduction of approximately 20% in the content of monoterpenoids after the drying process, and highlighted that drying can promote the loss of monoterpenoids, which are generally more volatile than sesquiterpenoids. They identified the hydrocarbon sesquiterpenes (*E*)-caryophyllene (36.6%), α-copaene (8.0%), and germacrene D (7.3%) as major compounds in the EO obtained from dried leaves of *I. pescaprae.* In our study, we also observed a predominance of sesquiterpene hydrocarbons compounds, both in the EOs of the leaves of *I. asarifolia* as *I. setifera*, which may be related to the drying process that the leaves were subjected. It is important to highlight that the extraction processes involving drying, dehydration, and storage may also result in loss of some volatile constituents, thus interfering in the presence and final percentages of some volatile chemical constituents.

Table 1
Chemical constituents and its percentages in the essential oils obtained from *Ipomoea setifera* (during dry season) and *I. asariflia* (during the dry and wet season) leaves.

$RI_{(L)}$	$RI_{(C)}$	Constituents name	I. setifera	I. asarifolia	
			Dry Season Area %	Dry season Area %	Wet Season Area %
1095	1100	Linalool			0.57
1186	1190	α-Terpineol			0.04
1197	1199	Safranal			0.03
1285	1292	Safrole			4.90
1299	1302	Theaspirane			0.27
1334	1336	Presilphiperfol-7-ene		0.12	
1335	1339	δ-Elemene	0.43	0.57	0.91
1358	1355	Silphiperfol-4,7(14)-diene			0.04
1345	1346	α-Cubebene	0.03		
1374	1377	α-Copaene	0.78	0.52	0.96
1383	1386	(E)-β-Damascenone	0.33	0.42	0.42
1389	1393	β-Elemene	20.49	2.30	7.29
1403	1406	Methyl eugenol			0.56
1409	1408	α-Gurjunene	1.55		
1410	1415	α-Cedrene		0.36	
1417	1422	(E)-Caryophyllene	36.7	19.93	15.93
1419	1418	β-Ylangene	0.12		
1430	1434	β-Copaene		0.33	0.63
1432	1432	*trans*-α- Bergamotene	0.09		
1434	1436	γ-Elemene	0.59		
1439	1444	Aromadendrene			0.01
1447	1451	*iso*germacrene D	0.08		0.22
1449	1453	Spirolepechinene	0.1		
1452	1454	(E)-β-Farnesene	0.21		
1452	1455	α-Humulene	8.03	2.88	3.58
1452	1461	Cumacrene	0.4		
1458	1462	*allo*Aromadendrene			0.19
1460	1463	Dehydro-aromadendrene	0.16		
1464	1466	9-*epi*-(E)-Caryophyllene	0.93		0.13
1466	1469	Cabreuva oxide C		0.14	
1481	1483	Widdra-2,4(14)-diene			0.07
1484	1486	Germacrene D	9.5	6.05	9.02
1487	1488	*E*-β-ionone			1.42
1489	1492	β-Selinene	1.8	0.11	0.43
1500	1500	*n*-Pentadecane			3.02
1500	1502	Bicyclogermacrene	3.13	1.63	1.46
1505	1504	α-Bulnesene	0.44		
1505	1507	(*E,E*)-α-Farnesene		0.54	0.66
1508	1509	Germacrene A	1.13		
1514	1517	Cubebol			0.34
1522	1524	δ-Cadinene	1.59	1.7	2.21
1542	1540	*cis*-Sesquisabinene hydrate			0.15
1544	1545	α-Calacorene		0.12	0.77
1557	1557	Germacrene B	0.26		
1562	1559	Geranyl butanoate		0.17	
1561	1563	*E*-Nerolidol	0.1	3.59	1.47
1562	1564	*epi*-Longipinanol	0.22		
1564	1567	β-Calacorene			0.45
1570	1575	Dendrolasin		0.24	0.86
1577	1582	Spathulenol	1.28	0.94	1.08
1582	1585	Caryophyllene oxide	4.52	0.94	1.16
1590	1593	Globulol		0.29	
1600	1602	*n*-Hexadecane			0.88
1604	1604	Sesquithuriferol	0.18		
1611	1615	*n*-Tetradecanal			0.26
1608	1612	β-Atlantol		0.13	
1608	1613	Humulene epoxide II	0.63		0.31
1622	1625	α-Colocalene		0.12	0.48
1627	1630	1-epi-Cubenol		0.08	0.23
1630	1631	Muurola-4,10(14)-dien-1β-ol			0.24
1632	1634	α-Acorenol	0.12		
1639	1640	*allo*Aromadendrene epoxide			0.12
1640	1643	*epi*-α-Muurolol		0.5	
1645	1646	Cubenol	0.12		0.1

(*continued on next page*)

Table 1 (*continued*)

$RI_{(L)}$	$RI_{(C)}$	Constituents name	I. setifera	I. asarifolia	
			Dry Season Area %	Dry season Area %	Wet Season Area %
1652	1656	α-Cadinol		0.63	1.03
1668	1667	Intermediol	0.67		
1671	1676	*n*-Tetradecanol			0.65
1671	1677	14-hydroxy-9-epi-(E)-Caryophyllene	0.17		
1675	1678	Cadalene		0.13	
1683	1685	epi-α-Bisabolol		1.98	
1685	1690	α-Bisabolol			1.73
1701	1701	*n*-Heptadecane		0.06	0.72
1706	1708	Melaleucol			0.23
1711	1715	Pentadecanal	1.46	3.32	
1713	1715	Cedroxyde			2.25
1722	1725	2Z,6E-Farnesol		0.08	0.27
1773	1779	*n*-Pentadecanol		0.14	0.33
1801	1801	Octadecane			0.04
1841	1844	Phytone	0.32	1.57	1.19
1864	1865	14-methyl-Hexadec-(8Z)-enal			0.05
1874	1880	*n*-Hexadecanol		0.76	0.72
1891	1891	*n*-Heptadecanal		0.26	0.27
1901	1901	*n*-Nonadecane			0.1
1906	1903	Musk Toray			0.07
1913	1915	(5E,9E)-Farnesyl acetone	0.18	0.95	
1929	1928	Musk ambrette			0.65
1938	1938	Hexadecanolact-16-one			0.02
1942	1947	Phytol	0.62	0.21	0.19
1959	1964	Palmitic acid		2.06	3.79
2026	2030	(*E,E*)-Geranyl linalool	0.13	0.16	0.52
2077	2084	*n*-Octadecanol		1.29	0.68
2102	2102	Heneicosane		0.22	0.24
2129	2129	Phytol derivative		35.49	10.67
2165	2168	Stearic acid		0.09	0.27
2202	2202	*n*-Docosane			0.1
2209	2212	Octadecanol acetate			0.04
2218	2223	*E*-Phytol acetate		0.25	
2291	2291	1-Tricosene		0.52	
2302	2302	*n*-Tricosane		0.5	0.33
2401	2401	*n*-Tetracosane		0.07	0.07
		Sesquiterpene hydrocarbons	88.54	37.41	45.44
		Oxygenated sesquiterpenes	8.01	9.54	11.34
		Phenylpropanoids			5.46
		Oxygenated diterpenes	0.75	35.86	11.38
		Others	2.29	12.65	17.47
		Total	99.59	95.46	91.09

$RI_{(c)}$: Retention index calculated using a *n*-alkane standard solutions (C8-C40) in column DB5-MS.
$RI_{(L)}$: Retention index from literature; (Adams, 2007; Mondello, 2011).

Marie et al. (2007) reported phytol in the *I. pescaprae* EO with levels of 5.8% and 0.3% in the dried and fresh leaves, respectively. In the present study, phytol was quantified with levels below 0.62% in the EOs of *I. setifera* and *I. asarifolia.* However, our results showed that phytol derivative was the main chemical constituent identified in the EO of *I. asarifolia* during the dry season (35.49%), and this result was not previously reported in the literature.

Another study reported the chemical composition of the EO extracted from *I. potatoes* dried leaves, which was characterized by the hydrocarbon diterpenes bietadiene (8.9%) and abieta-8,11,13-triene (7.1%); the hydrocarbon sesquiterpenes (*E*)-caryophyllene (8.8%), and trans-(*Z*)-α-bergamotol (6.0%); the monoterpene hydrocarbon cis-sabinene (5.5%), and sesquiterpene oxygenated spathulenol (5.3%). The total percentage of sesquiterpene hydrocarbons and oxygenated sesquiterpene were 33.2% and 13.3%, respectively (Ogunmoye et al., 2015). Joshi (2015) reported α-bulnesene (23.8%), α-humulene (13.7%), and seychellene (11.2%), as the main compounds in the EO of *I. obscura* leaves. These authors also found that sesquiterpene hydrocarbon were 78.4% of the total composition. Abdallah et al. (2017) evaluated the EO of the dried leaves of *I. carnea* obtained by hydrodistillation and identified caryophyllene oxide (23.21%), germacrene D (17.73%), and (*E*)-caryophyllene (14.48%). The sesquiterpene hydrocarbons and oxygenated sesquiterpenes were 46.04% and 34.82% of the total composition, respectively.

Similar to the results obtained in the present study, the literature has reported that the chemical compounds of the EOs varies among the species of the *Ipomea* genus (Abdallah et al., 2017; Joshi, 2015; D. E. P Marie et al., 2007; Ogunmoye et al., 2015). However, we would like to highlight that similarly to previous studies of other species of this genus, the sesquiterpene hydrocarbon and oxygenated sesquiterpenes were the predominant chemical classes found in the EOs of *I. setifera* and *I. asarifolia*.

3.2. Analysis of ADMET properties of major constituents of the EOs

According to the computational analyses, the chemical constituents caryophyllene oxide and cedroxyde were classified with high risk of toxicity due to the presence of epoxide in their structure, a functional group highly reactive and toxic (Schramm et al., 2011) (see chemical structures in Supporting information 01 and the structural alerts in Fig. 1 and Supporting information 02). REOS and Brenk filters identify chemical groups that may lead to false positives in high-throughput assays (HTS) due to their reactivity or assay interference (Brenk et al., 2008; Walters and Namchuk, 2003). Some functional groups identified in the compounds are also indicated as toxicophoric, thus are directly related to the toxicity.

Using the ML-based algorithm, caryophyllene oxide and cedroxyde compounds were predicted as inhibitors of CYP450 enzymes (Fig. 1 and Supporting information 03). The penetration of compounds across the BBB is an important characteristic of toxic substances with implications in neurological disorders (Hallier-Vanuxeem et al., 2009). Based on this assumption, we investigated this property in the major compounds ($\geq$3% of the total composition) of *Ipomoea* L. species EOs using the BOILED-EGG model to predict the brain penetration and gastrointestinal absorption and we correlated it with the possible neurological toxicity of the analyzed natural compounds. According to our analyzes, caryophyllene oxide and cedroxyde may be permeable across the BBB and both compounds are inhibitors of CYP2C9 with an area under the curve (AUC) equal to 0.86 and 0.85, respectively. Additionally, both compounds showed oral bioavailability using Lipinski, Veber, and BOILED-EGGS which indicated that both are probably highly absorbed by the gastrointestinal tract (see supporting information 03). Previous studies demonstrated that caryophyllene oxide is the main chemical component in the toxic EO of *Artemisia campestris* (Judzentiene et al., 2010) and it is involved with the inhibition of the mitochondrial electron transport chain (Monzote et al., 2009).

The pentadecanal was also considered to be potentially toxic due to the presence of consecutive alkyl chains and aldehyde in its structure. Different studies have demonstrated that the presence of long aliphatic chains in the compound structures, could lead to their promiscuity (Walters and Namchuk, 2003). Moreover, the aldehyde present in pentadecanal structure is also considered a promiscuous structural moiety that is recognized and metabolized by enzymes of the detoxification system of organisms, such as aldehyde oxidases and CYP450 (Ahmed Laskar and Younus, 2019; Walters and Namchuk, 2003).

Regarding the pharmacokinetic profile of phytol, our analyses identified that this compound probably is not permeable across the BBB. Additionally, no structural alerts related to the promiscuity were found for this compound. Concerning the compound bioavailability, phytol showed at least one violation of Lipinski and Veber chemical filters, and violated the BOILED-EGG model which indicates that its gastrointestinal absorption is limited (see Supporting information 03). Regarding its toxicity, phytol was identified as an inhibitor of P-gp (area under curve

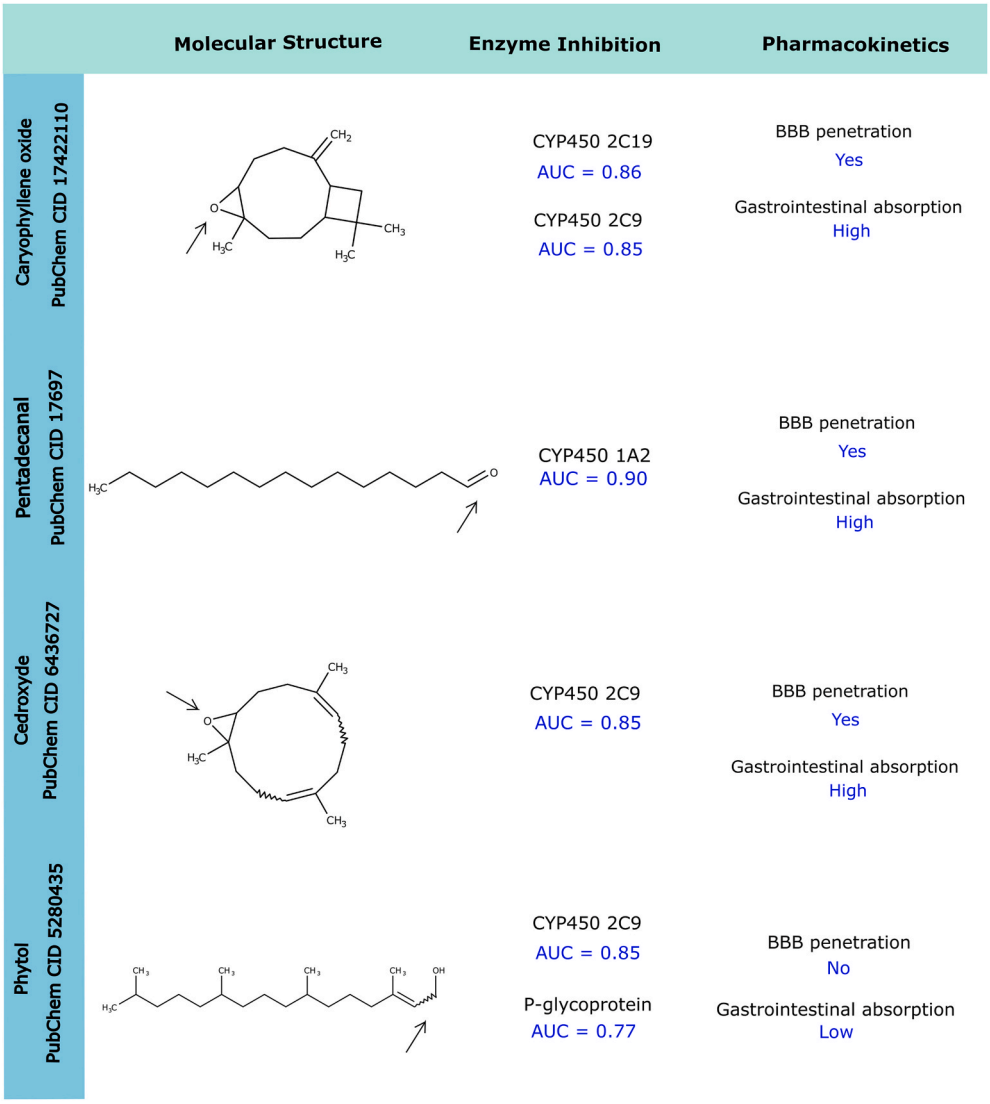

Fig. 1. Schematic overview of molecular structure, enzyme inhibition (CYP450 variants and P-gp), and pharmacokinetic properties (BBB penetration and gastrointestinal absorption) of compounds found in the essential oils possibly related to the toxicity of the *Ipomoea* L. species. The arrows indicate the structural moieties related to the toxicity or/and reactivity.

= 0.77) and CYP2C9 (AUC = 0.85) enzymes, both related with the metabolism of exogenous substances (see Supporting information 03) (Aller et al., 2009; Ferguson and Tyndale, 2011). Computational analysis of the pharmacokinetic profile and toxicity of phytol derivative identified in the EO of *I. asarifolia* could not be performed due to the absence of the compound structure. However, we conjecture that the phytol derivative has similar pharmacokinetic properties to phytol, as both compounds have similar fragmentation patterns (see Supporting information 04).

When absorbed by mammals, phytol is metabolized resulting in phytanic acid (Steinberg et al., 1965a, 1965b; Stoffel and Kahlke, 1965; Van Den Brink and Wanders, 2006), a methyl-branched fatty acid which has been reported to induce apoptosis in astrocytes and causes neurological damage (Roca-Saavedra et al., 2017). Based on this, we also performed the computational pharmacokinetics analysis of this acid and we found that, in contrast to phytol, the phytanic acid is probably highly absorbed by the gastrointestinal tract and the BBB and it is an inhibitor of P-gp and CYP450 enzymes (Supporting information 02 and 03).

According to previous studies, the cytotoxic activity of phytol is related to its dosage (Alencar et al., 2018; Islam et al., 2015). Araújo et al. (2008) evaluated the *in vivo* toxicity of dry plant material of *I. asarifolia* during the dry and wet seasons and they found greater toxicity of leaves during the dry season. Thus, the high content of phytol derivative in the dry season could be related to the increased toxicity of *I. asarifolia* either directly or through its metabolism into phytanic acid.

It is important to highlight that phytol derivative is the main chemical constituent of the *I. asarifolia* EO obtained during the dry season and our analyses identified that phytol and, its metabolized product, the phytanic acid are potentially toxic. In addition, caryophyllene oxide and pentadecanal found in *I. setifera* EO have high gastrointestinal and blood-brain barrier absorption and both compounds are inhibitors of enzymes involved with detoxification system of organisms.

4. Conclusion

The neurological disorders related to the ingestion of the aerial parts of *I. asarifolia* and *I. setifera* by cattle, sheep, and goats is a recurrent problem in the pastures, causing economic consequences to livestock. Therefore, evaluating the chemical composition and toxicity of those species may shed light on the mechanism of intoxication of these plants. Here, we report that the phytol derivative content of *I. asarifolia* EO in

the dry season, is three times higher than that observed in the wet season, and it could be correlated with the occurrence of neurological diseases in the livestock, previously reported in the literature (Araújo et al., 2008; Barbosa et al., 2005; Carvalho et al., 2014; Tortelli et al., 2008). In addition, our computational analyses identified that the caryophyllene oxide and cedroxyde are probably related to the toxicity of the EO due to the presence of epoxide in their structures. Moreover, both compounds are inhibitors of CYP450 activities also showing permeability to the BBB and gastrointestinal tract.

Regarding the phytol, we also found that this compound is an inhibitor of P-gp and CYP450 enzymes which may increase absorption of some compounds and limit the metabolism of other co-ingested chemicals components. Moreover, the metabolized product of phytol, the phytanic acid has an increased probability to be absorbed by the gastrointestinal tract and the BBB. Our results represent an important contribution to understand the toxicity, pharmacokinetics, and molecular mechanism of action of the chemical constituents of *Ipomoea* L. species EOs.

Author contributions

Conceptualization: Lidiane Diniz do Nascimento, Oseias Souza da Silva Júnior, and Eloisa Helena de Aguiar Andrade. Investigation: Oseias Souza da Silva Júnior (experimental analyses), Celeste de Jesus Pereira Franco (experimental analyses), Angelo Antonio Barbosa de Moraes (experimental analyses), Kauê Santana da Costa (in silico analyses), Jorddy Neves Cruz (in silico analyses); Data curation: Kauê Santana da Costa, Lidiane Diniz do Nascimento, Jorddy Neves Cruz, and Eloisa Helena de Aguiar Andrade; Writing—original draft preparation: Oseias Souza da Silva Júnior, Kauê Santana da Costa, and Lidiane Diniz do Nascimento. Writing—review and editing: Angelo Antonio Barbosa de Moraes, Jorddy Neves Cruz , and Kauê Santana da Costa; Supervision: Eloisa Helena de Aguiar Andrade , Kauê Santana da Costa, and Lidiane Diniz do Nascimento. All authors have read and agreed to the published version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxicon.2021.02.015.

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