Revista Brasileira de Farmacognosia 27 (2017) 663–672

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Review

Vouacapane diterpenoids isolated from *Pterodon* and their biological activities

Leandra A.R. Oliveira[a,b], Gerlon A.R. Oliveira[c], Leonardo L. Borges[d,e], Maria Teresa F. Bara[b,*], Dâmaris Silveira[a]

[a] *Faculdade de Ciências da Saúde, Universidade de Brasília, Campus Darcy Ribeiro, Brasília, DF, Brazil*
[b] *Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil*
[c] *Instituto de Química, Universidade Federal de Goiás, Campus Samambaia, Goiânia, GO, Brazil*
[d] *Escola de Ciências Médicas, Farmacêuticas e Biomédicas, Pontifícia Universidade Católica de Goiás, Goiânia, GO, Brazil*
[e] *Campus Anápolis de Ciências Exatas e Tecnológicas Henrique Santillo, Universidade Estadual de Goiás, Anápolis, GO, Brazil*

ARTICLE INFO

Article history:
Received 7 April 2017
Accepted 30 May 2017
Available online 30 August 2017

Keywords:
Biological activity
Phytochemistry
Sucupira
Vouacapane diterpenoids

ABSTRACT

The *Pterodon* genus comprises two native species in Brazil, known as "sucupira-branca" or "faveira". Their fruits have long been used in Brazilian natural medicine, mainly for the treatment of infections and inflammations. The pharmacological properties of these fruits have often been linked with vouacapane diterpenoids. This review evaluated the scientific research in the period from 1973 to February 2017, aiming to answer how difficult it still is to develop a scientifically supported product based on *Pterodon* vouacapanes. Therefore, this paper reviews purification, identification, and quantification methods applied to vouacapane diterpenoids from *Pterodon*, as well as the performance of these phytochemicals in pharmacological tests described in the literature. Data analysis results support conventional notions that suggest vouacapane diterpenoids from *Pterodon* have anti-inflammatory properties. However, the studies carried out so far still represent partial assessment of the vouacapane activities and further studies need to be completed. *Pterodon* diterpenoids have also been associated with larvicidal, leishmanicidal, cardiovascular, and antitumor activities, which reinforces the genus' potential as a source of phytomedicines. Some remaining gaps about the reviewed activities were mentioned, while trends and perspectives for future research were proposed.

Introduction

The use of medicinal plants has been common since ancient times; the earliest references can be found in Egyptian papyruses, Chinese scriptures, and Sumerian clay tablets (Hamburger and Hostettmann, 1991). Among the medicinal plants used in many countries, the Fabaceae family presents the second largest group of species, with approximately 490 members described in the literature (Gao et al., 2010). This family includes the *Pterodon* genus, which comprises important medicinal plants, particularly in South America.

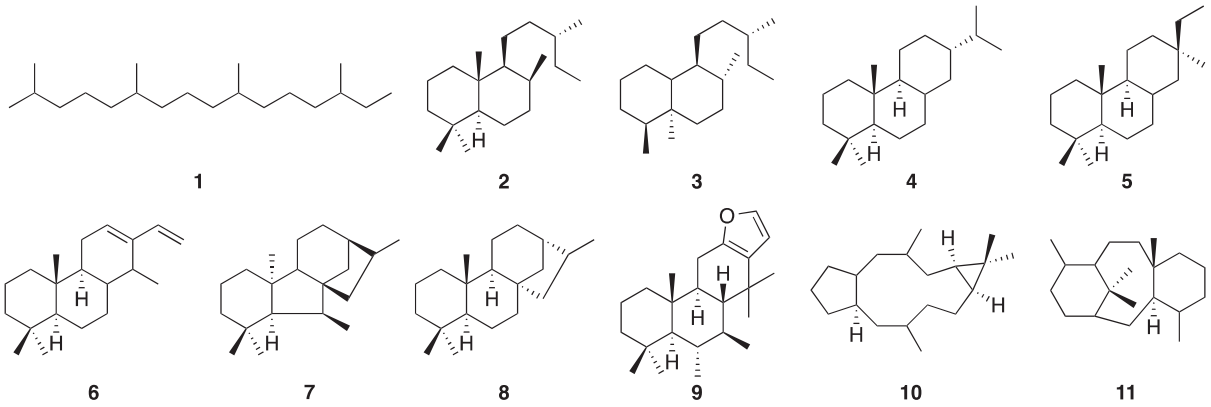
According to the website The Plant List (2013), *Pterodon* has two native Brazilian species, known as "sucupira-branca" or "faveira": *Pterodon abruptus* (Moric.) Benth. (synonym: *Commilobium abruptum* Moric.) and *P. emarginatus* Vogel [synonyms: *Acosmium inornatum* (Mohlenbr.) Yakovlev; *C. polygalaeflorus* Benth.; *C. pubescens* Benth.; *P. apparicioi* Pedersoli.; *P. polygalaeflorus* (Benth.) Benth.; *P. polygaliflorus* (Benth.) Benth.; *P. pubescens* (Benth.) Benth.; and *Sweetia inornata* Mohlenbr.].

Ethnobotanical and ethnopharmacology studies are approaches to find active compounds to deal with health problems. Many relevant medicines were originated from a natural compound isolated from medicinal plants (Rai et al., 2011). An ethnobotanical study conducted in southeastern Brazil indicated that hydroalcoholic macerate of *Pterodon* fruits have been used in popular medicine as anti-inflammatory, mainly in the treatment of rheumatism, sore throat, bronchitis and asthma (Grandi et al., 1989). Other surveys also attribute such properties to *Pterodon* fruits (Corrêa, 1975; Hansen et al., 2010; Raposo et al., 2011; Fagg et al., 2015).

Research on *Pterodon* fruit oil and extract has confirmed several biological activities, *e.g.* anti-inflammatory (Carvalho et al., 1999; Hoscheid et al., 2013; Pascoa et al., 2015), antinociceptive (Silva et al., 2004; Coelho et al., 2005; Oliveira, 2012; Nucci et al., 2012; Martins et al., 2015), effect on arthritis treatment (Sabino et al.,

* Corresponding author.
E-mail: mbara@ufg.br (M.T. Bara).

https://doi.org/10.1016/j.bjp.2017.05.014



1999), antiproliferative (Vieira et al., 2008; Spindola et al., 2011; Pereira et al., 2011; Pereira et al., 2012), antioxidant (Dutra et al., 2008), antimicrobial (Dutra et al., 2009; Toledo et al., 2011), larvicidal against *Aedes aegypti* (Pimenta et al., 2006), and antiparasitic against *Trypanosoma cruzi* (Barreto et al., 2008; Oliveira, 2014), *Leishmania amazonensis* (Dutra et al., 2009; Oliveira et al., 2017) and *L. braziliensis* (Oliveira et al., 2017).

Among the compounds probably related to the biological properties of *Pterodon* are vouacapane diterpenes (Euzébio et al., 2009; Spindola et al., 2010; Galceran et al., 2011; Nucci et al., 2012). Vouacapanes can be found in other genera of the family Fabaceae, beyond *Pterodon*. These diterpenoids are also distributed in the following species: *Bowdichia nitida* (Matsuno et al., 2008), *Caesalpinia bonduc* (L.) Roxb (Balmain et al., 1967; Pudhom et al., 2007), *C. crista* L. (Jadhav et al., 2003; Cheenpracha et al., 2006; Das et al., 2010), *C. echinata* L. (Cota et al., 2011; Mitsui et al., 2015), *C. minax* H. (Jiang et al., 2001; Jiang et al., 2002; Dong et al., 2015; Lian et al., 2015; Zhang et al., 2015), *C. platyloba* S.W. (Hurtado et al., 2013), *C. pulcherrima* (L.) Sw. (Mcpherson et al., 1986; Ragasa et al., 2002; Ragasa et al., 2003; Das et al., 2010), *C. volkenssi* H. (Ochieng et al., 2012), *Dypteryx odorata* (A) W. (Godoy et al., 1989), *D. lacunifera* D. (Mendes and Silveira, 1994), *Stuhlmania moavi* V. (Odalo et al., 2009) and *Vouacapoua americana* A. (Kido et al., 2003).

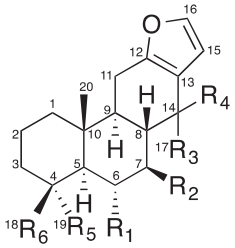
Maurya et al. (2012) reviewed the studies involving natural occurrence of cassane and norcassane diterpenes up to September 2011. This review focused on *Caesalpinia* genus, from which only 12 out of its 322 cited structures were also reported to *Pterodon* genus. Recently, Bao et al. (2016) have reviewed the naturally occurring furanoditerpenoids, and reported only six compounds from *Pterodon*. Therefore, this review summarizes the available data about vouacapane diterpenoids isolated from *Pterodon* aiming to provide an overview of their structural diversity, procedures used in their extraction, isolation, structural elucidation, and quantification, as well as the biological activities reported for these natural products. The assessment spanned the period from 1973 to February 2017. Thus, the present paper aims to encourage future research about this genus and their chemical compounds, indicating trends and trying to answer how difficult it still is to develop a scientifically supported product based on *Pterodon* vouacapanes.

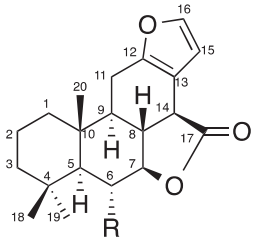
Vouacapane biosynthesis

Diterpenoids constitute a vast class of isoprenoid compounds, biosynthesized from mevalonic acid through 2*E*,6*E*,10*E*-geranylgeranyl pyrophosphate (GGPP) and deoxyxylulose phosphate (Dewick, 2002). Diterpenes' molecular structure contains skeletons with twenty carbon atoms and may reveal acyclic: phytane (**1**), bicyclic: labdane (**2**) and clerodane (**3**), tricyclic: abietane (**4**), pimarane (**5**) and cassane (**6**), tetracyclic: gibberellane (**7**), kaurane (**8**) and vouacapane (**9**), and macrocyclic: lathyrane (**10**) and taxane (**11**) forms (Hanson, 1995; García et al., 2007; Ramawat and Mérillon, 2013).

The basic cassane skeleton (**6**) may be derived from pimarane (**14**) through methyl migration from C-13 to C-14 (**14'**) in the biosynthetic pathway. Pimaranes are formed through the cyclization of the labdane pyrophosphate (**12**) (Xu et al., 2011; Maurya et al., 2012). Labdane-type diterpene biosynthesis consists of an initial cyclization of GGPP promoted by a class II diTPS (diterpene synthase) to produce a cyclic diphosphate intermediate, followed by conversion of this intermediate (**13**) into the final diterpene skeleton by a class I diTPS (Peters, 2010). Cassanes containing a furan ring are called furanocassanes or vouacapanes (Scheme 1).

Vouacapanes represent an important group of tetracyclic cassanes and their structure is characterized by a skeleton constructed from the fusion of three cyclohexane rings and one furan ring (Jiang et al., 2001). Vouacapanes previously identified in *Pterodon* species are summarized in Table 1, according to structure **15**, in addition to the lactones **37** (Mahajan and Monteiro, 1973) and **38** (Demuner et al., 1996; Omena et al., 2006).

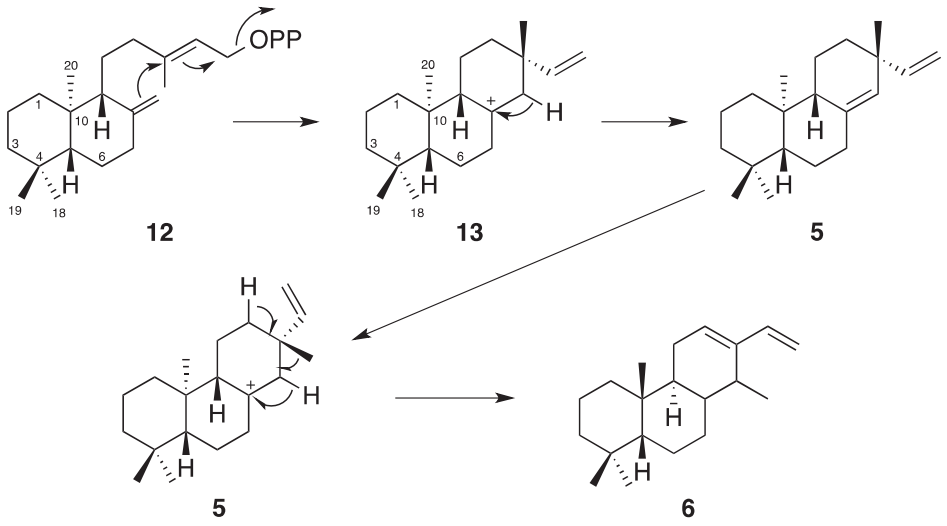




Extraction, quantification and structural elucidation of vouacapanes

Extraction

Vouacapanes are low to medium polarity compounds, thus being mainly soluble in hydrophobic solvents. Vouacapane diterpenes are commonly isolated from the fruits of different *Pterodon* species through similar procedures. Fruits are ground and extracted by the Soxhlet method using various solvents, such as petroleum ether (Mahajan and Monteiro, 1973), hexane (Demuner et al., 1996; Arriaga et al., 2000; Pimenta et al., 2006), and ethanol (Vieira et al.,



Scheme 1. Biosynthetic pathway of the basic cassane skeleton (**6**).

2008). Other extraction procedures include percolation using hexane (Fascio et al., 1976) and ethanol 90% (Omena et al., 2006), heat extraction using ethanol (Campos et al., 1994), and cold extraction using dichloromethane (Spindola et al., 2009, 2010).

Following the Soxhlet extraction, the solvent may be removed and the extract submitted to column chromatography to yield vouacapanes (Mahajan and Monteiro, 1973; Fascio et al., 1976; Demuner et al., 1996; Rubinger et al., 2004; Pimenta et al., 2006; Spindola et al., 2009, 2010; Servat et al., 2012). Moreover, fractionation by liquid–liquid extraction (Omena et al., 2006; Vieira et al., 2008) or acid–base extraction (Mahajan and Monteiro, 1973; Fascio et al., 1976; Campos et al., 1994; Arriaga et al., 2000) may also be performed before purification by column chromatography. The 6α,7β-diacetoxyvouacapane (**18**) was obtained by direct crystallization following extraction (Mahajan and Monteiro, 1973; Fascio et al., 1976).

Chromatographic purification of these compounds is usually performed using silica gel as a stationary phase (Fascio et al., 1976; Rubinger et al., 2004; Vieira et al., 2008). However, Mahajan and Monteiro (1973) used alumina to purify 6α,7β-diacetoxyvouacap14(17)-ene (**16)** and **18**, while Vieira et al. (2008) used florisil column in one of the stages of vouacapane-6α,7β,14β,19-tetraol (**29)** purification.

Open column chromatography is often a challenging and time-consuming technique because fractions have to be analyzed following fractionation, not during it. Furthermore, the various stages of this process may favor the occurrence of chemical reactions involving secondary metabolites, hence leading to the formation of artifacts (Pizzolatti et al., 2002). To avoid these setbacks, Oliveira et al. (2017) proposed a semipreparative high-performance liquid chromatography (HPLC) method for isolating *P. emarginatus* diterpenoids.

Classic chromatographic elutions are commonly monitored by thin layer chromatography, which uses default wavelengths for choosing the fractions containing pure compounds. However, as we have noticed (Oliveira et al., 2017), some wavelengths may not allow to discriminate different vouacapanes in a sample. The UV diode array HPLC detectors enable assessing the chromatogram in a wide wavelength range, enhancing the assurances in the evaluation of purity of the peaks. The assessment of the peaks in their optimal absorbance wavelength, instead of default values, also decreases detection limits, favoring the isolation of minority compounds. In addition, the separation power of high-pressure columns is superior to the ones achieved through open columns. Since just very recently HPLC isolation has been applied to *Pterodon* fruits (Oliveira et al., 2017), it is likely that many vouacapanes were not yet described for this genus.

Structural elucidation

Following extraction and isolation, the next step consists in elucidating the structure of vouacapanes. The structure of vouacapane diterpenes has mainly been determined via nuclear magnetic resonance (NMR) (Campos et al., 1994; Vieira et al., 2008; Euzébio et al., 2009; Galceran et al., 2011). Vouacapanes may be characterized by their furan ring signals. The ^{1}H NMR spectra for vouacapanes show hydrogen furans at approximately δ_H 6.4 ppm (1H, *d*, *J* = 1.9 Hz, H_{15}) and δ_H 7.2 ppm (1H, *d*, *J* = 1.9 Hz, H_{16}), while the ^{13}C NMR spectra show furan ring signals at δ_C 148.2 ppm (C-12), 141.9 ppm (C-16), 123.9 ppm (C-13), and 107.2 ppm (C-15) (Spindola et al., 2009; Hurtado et al., 2013; Oliveira et al., 2017).

Moreover, NMR analysis can be used together with other spectroscopic techniques, such as infrared (IR) (Spindola et al., 2010) and mass spectrometry (MS) (Fascio et al., 1976; Arriaga et al., 2000; Servat et al., 2012; Oliveira et al., 2017), on their own or combined (Demuner et al., 1996; Omena et al., 2006; Spindola et al., 2009).

On the other hand, infrared spectroscopy provides limited information for structural elucidation. However, it is very useful in verifying the identity of compounds by comparing spectra from new samples with those from referenced substances. Omena et al. (2006) and Spindola et al. (2009, 2010) have used IR spectroscopy to obtain structural information about isolated vouacapanes, *e.g.* evidence for hydroxyl (absorption close to 3450 cm^{-1}) and carbonyl functionalities (absorption close to 1710 cm^{-1}). Demuner et al. (1996) have observed that vouacapanes' IR spectrum shows hydroxyl absorption bands at 3560 (sharp, due to free OH) and 3425 (broad, due to hydrogen-bonded OH) cm^{-1} and 1678 and 1510 cm^{-1} (C=C) for a furan ring.

Mass spectrometry has also been used to elucidate the structure of vouacapanes. High-resolution electron impact ionization (HREIMS) has proved to be a useful tool (Arriaga et al., 2000; Spindola et al., 2009; Servat et al., 2012), as well as, more recently, electron spray ionization (Cabral et al., 2013; Oliveira et al., 2017).

Analytical studies and quantification

Accurate and reproducible analytical methods are required to identify and quantify vouacapane diterpenes in *Pterodon* fruits

Table 1
Structure of vouacapanes previously identified in *Pterodon* species, in accordance with the basic structure of vouacapane diterpenes (**15**).

Diterpenes	R1	R2	R3	R4	R5	R6	Ref.
6α,7β-Diacetoxyvouacap14(17)-ene (**16**)	(α) OAc	(β) OAc	C=CH_2		(α) Me	(β) Me	Mahajan and Monteiro (1973)
7β-Acetoxyvouacapane (**17**)	(α) H	(β) OAc	(α) Me	(β) H	(α) Me	(β) Me	Mahajan and Monteiro (1973), Fascio et al. (1976), Spindola et al. (2009)
6α,7β-Diacetoxyvouacapane (**18**)	(α) OAc	(β) OAc	(α) Me	(β) H	(α) Me	(β) Me	Mahajan and Monteiro (1973), Fascio et al. (1976), Spindola et al. (2009)
Vouacapane-6α,7β,14β-triol (**19**)	(α) OH	(β) OH	(α) Me	(β) OH	(α) Me	(β) Me	Mahajan and Monteiro (1973), Fascio et al. (1976)
6α,7β-Dihydroxyvouacapan-17β-oate sodium (**20**)	(α) OH	(β) OH	(β) COONa	(α) H	(α) Me	(β) Me	Duarte et al. (1992, 1996)
6α,7β-Dihydroxyvouacapan-17β-oic acid (**21**)	(α) OH	(β) OH	(β) COOH	(α) H	(α) Me	(β) Me	Mahajan and Monteiro (1973), Fascio et al. (1976), Campos et al. (1994), Demuner et al. (1996), Belinelo et al. (2002), Rubinger et al. (2004), Omena et al. (2006), Díaz et al. (2006, 2010), Branco et al. (2008), Santos et al. (2008), Euzébio et al. (2009, 2010), Galceran et al. (2011)
Methyl 6α,7β-dihydroxyvouacapan-17β-oate (**22**)	(α) OH	(β) OH	(β) COOMe	(α) H	(α) Me	(β) Me	Mahajan and Monteiro (1973), Fascio et al. (1976), Arriaga et al. (2000), Rubinger et al. (2004), Omena et al., 2006, Santos et al. (2008), Spindola et al. (2009, 2010, 2011), Díaz et al. (2010)
Methyl 7β-acetoxy-6α-hydroxyvouacapan-17β-oate (**23**)	(α) OH	(β) OAc	(β) COOMe	(α) H	(α) Me	(β) Me	Mahajan and Monteiro (1973), Servat et al. (2012)
6α,7β-diacetoxyvouacapan-14β-al (**24**)	(α) OAc	(β) OAc	(α) H	(β) CHO	(α) Me	(β) Me	Fascio et al. (1976)
6α,7β-Diacetoxyvouacapan-14β-oate (**25**)	(α) OAc	(β) OAc	(α) H	(β) COOMe	(α) Me	(β) Me	Fascio et al. (1976)
Methyl 6α-acetoxy-7β-hydroxyvouacapan-17β-oate (**26**)	(α) OAc	(β) OH	(β) COOMe	(α) H	(α) Me	(β) Me	Fascio et al. (1976), Campos et al. (1994), Hoscheid et al. (2012), Servat et al. (2012), Oliveira et al. (2017)
Methyl 6α-hydroxy-7β-acetoxyvouacapan-17β-oate (**27**)	(α) OH	(β) OAc	(β) COOMe	(α) H	(α) Me	(β) Me	Fascio et al. (1976), Hoscheid et al. (2012), Servat et al. (2012)
6α-Hydroxy-7β-acetoxyvouacap-14(17)-ene (**28**)	(α) OH	(β) OAc	C = CH_2		(α) Me	(β) Me	Campos et al. (1994)
Vouacapane-6α,7β,14β,19-tetraol (**29**)	(α) OH	(β) OH	(α) Me	(β) OH	(β) CH_2OH	(α) Me	Demuner et al. (1996), Arriaga et al. (2000), Vieira et al. (2008)
6α-Acetoxyvouacapane (**30**)	(α) OAc	(β) H	(α) Me	(β) H	(α) Me	(β) Me	Pimenta et al. (2006)
6α-Hydroxyvouacapane (**31**)	(α) OH	(β) H	(α) Me	(β) H	(α) Me	(β) Me	Arriaga et al. (2000), Pimenta et al. (2006)
Vouacapane (**32**)	(α) H	(β) H	(α) Me	(β) H	(α) Me	(β) Me	Pimenta et al. (2006)
6α,7β-Dihydroxyvouacapan-17β-methylene-ol (**33**)	(α) OH	(β) OH	(β) CH_2OH	(α) H	(α) Me	(β) Me	Spindola et al. (2009)
6α-Acetoxy-7β-hydroxyvouacapan (**34**)	(α) OAc	(β) OH	(α) Me	(β) H	(α) Me	(β) Me	Spindola et al. (2009)
6α,19β-Diacetoxy-7β,14β-dihydroxyvouacapan (**35**)	(α) OAc	(β) OH	(β) OH	(α) Me	(α) Me	(α) OAc	Oliveira et al. (2017)
6α-Acetoxy-7β,14β-dihydroxyvouacapan (**36**)	(α) OAc	(β) OH	(β) OH	(α) Me	(α) Me	(β) Me	Oliveira et al. (2017)

or biological samples. Fingerprinting is indicated for the qualitative distinction of samples with complex chemical composition (Sawaya et al., 2010). It covers the scanning of a vast number of intracellular metabolites detected by a selected analytical technique or by a combination of different techniques (Villas-Boas et al., 2005).

Cabral et al. (2013) used mass spectrometry for fingerprinting *P. emarginatus* fruit oil. The fruit surface or paper imprinted with the oil was directly analyzed by infusion electrospray ionization mass spectrometry (DIESI-MS), as well as by desorption/ionization via easy ambient sonic-spray ionization mass spectrometry (EASI-MS). Typical profiles were obtained from the crude oil via these direct MS techniques. The main advantages of MS over other techniques used for fingerprinting are its high sensitivity and selectivity (Villas-Boas et al., 2005; Dettmer et al., 2007).

Few studies have focused on quantifying *Pterodon* vouacapanes. Hoscheid et al. (2012) developed and validated a gas chromatography method to quantify methyl 6α-acetoxy-7β-hydroxyvouacapan-17β-oate (**26**) and methyl 6α-hydroxy-7β-acetoxyvouacapan-17β-oate (**27**) in a semipurified extract of *P. emarginatus* fruits. Samples were quantified following purification, which included liquid–liquid partitioning and open-column chromatography. Oliveira (2014) proposed an alternative HPLC-PDA method to quantify **26** in *P. emarginatus* fruits. The main advantage of this approach over the previous one is that it does not require prior purification stages.

Pharmacological activities

Authors have suggested that the vouacapane skeleton of furan diterpenes is linked with certain pharmacological properties of extracts from *Pterodon* fruits (Carvalho et al., 1999; Euzébio et al., 2009; Spindola et al., 2010; Galceran et al., 2011; Nucci et al., 2012). This section describes the possible pharmacological activities and action mechanisms of vouacapanes isolated from *Pterodon* fruits, based on *in vitro* and *in vivo* studies.

Anti-inflammatory, antinociceptive and analgesic activities

Due to potential side effects and low efficacy of synthetic and chemical drugs, consumption of other complementary drugs, especially herbal remedies, to control pain is increasing (Bahmani et al., 2014). Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk, 1994). Thus, pain has sensory, affective and a cognitive component associated with the anticipation of future harm (Garland, 2012). The nociception is the sensory component (Loeser and Treede, 2008). Inflammatory responses in the peripheral and central nervous systems play key roles in the development and persistence of many pathological pain states (Zhang and An, 2007).

Various neurotransmitters and receptor systems take part in the modulation of pain processes in the central nervous system and peripheral nervous system, such as the vanilloid, opioid, non-opioids (*i.e.* β-adrenergic, dopaminergic), glutamate, protein kinase C, potassium ion (K^+) channels, and nitric oxide/cyclic guanosine monophosphate pathways (Julius and Basbaum, 2001; Zakaria et al., 2016). The antinociceptive action of the vouacapans isolated from *Pterodon* was assessed in chemical and thermal models of nociception in mice, such as acetic acid-induced abdominal constriction, paw compression, formalin and hot plate test.

The first evidence that vouacapan has an analgesia effect was demonstrated by inhibition of acetic acid writhing response in mice (Duarte et al., 1992). The authors assessed the role of endogenous opioid peptides in the antinociceptive effect induced by 6α,7β-dihydroxyvouacapan-17β-oate sodium (**20**), using acetic acid-induced abdominal contortion and paw compression tests on mice. In both models, compound **20** caused a dose-dependent analgesia when administered through oral (*p.o.*), intraperitoneal (*i.p.*), and subcutaneous (*s.c.*) routes (ranging from 62.5 to 500 μmol/kg). Results showed that opioid antagonists only partially blocked the antinociceptive effect. The authors suggested that endorphin release may be involved in the vouacapane's analgesic effect. In another study, Duarte et al. (1996) assessed the possible involvement of biogenic amines in the antinociceptive effect of **20**, using acetic acid-induced abdominal contortion on mice. This compound exhibited an antinociceptive effect (500 μmol/kg, *i.p.*) when compared with the control group. Results suggested that vouacapane's antinociceptive response may be associated with dopamine.

Spindola et al. (2010) examined the contribution of geranylgeraniol (acyclic diterpene) and methyl 6α,7β-dihydroxyvouacapan-17β-oate (**22**) isolated from *P. emarginatus* fruits in the extract's antinociceptive activity. The open field test was performed to rule out the possibility that the antinociceptive effects of geranylgeraniol and **22** are linked to specific disturbances in animals' locomotion (30 mg/kg or 82.8 μmol/kg, *i.p.*). Compounds reduced acetic acid-induced abdominal contortions on mice treated via *i.p.* and *p.o.* routes, with differences in potency being linked to administration routes (10, 30, 100, and 300 mg/kg). Results suggested that the diterpenes essayed may produce synergistic activity. Following the use of naxolone hydrochloride (1 mg/kg or 2.75 μmol/kg, *p.o.*), a non-specific opioid antagonist, in the hot-plate test, it was concluded that the antinociceptive activity is unrelated to opioidergic routes and can be linked to the involvement of VR1 vanilloid receptors and peripheral glutamate receptors.

In a follow-up to the previous study, Spindola et al. (2011) investigated the possible action mechanisms involved in the antinociceptive activity of geranylgeraniol and **22**. The allodynia test, which assessed the touch response of mice submitted to a subplantar injection of complete Freund's adjuvant (CFA; *Mycobacterium tuberculosis*, 1 mg/ml), showed that compounds tested (30 mg/kg, or 103.3 μmol/kg of geranylgeraniol and 82.8 μmol/kg of compound **22**; *i.p.*) reduced pain sensitivity during the acute phase. In the hyperalgesia test, which verified response to a painful stimulus, mice treated with geranylgeraniol showed a significant reduction in carrageenan-induced hypernociception. Regarding the action mechanism, the antinociceptive activity of geranylgeraniol and **22** during the acetic acid-induced contortion test may be related to serotonergic and imidazole systems.

Servat et al. (2012) assessed the antinociceptive activity of the mixture of isomers methyl 7β-acetoxy-6α-hydroxyvouacapan-17β-oate (**23**) and **26**. The treatment did not significantly change animals' locomotion and reduced acetic acid abdominal contortions in mice in a dose-dependent manner, when compared with the control group, presenting an effective dose 50 (ED_{50}) of 35.6 mg/kg (or 88 μmol/kg). Moreover, the formalin test showed that the antinociceptive activity of the isomer mixture is more closely linked to neuropathic pain than to inflammatory pain. In the allodynia test, the doses tested (30 mg/kg or 74.2 μmol/kg; *i.p.*) proved effective in the first two phases: acute and subacute (4 and 24 h following CFA administration). In the hyperalgesia test, the mixture of vouacapane isomers was not effective in reducing pain, which suggests that the sample shows a higher affinity for neuropathic components.

Galceran et al. (2011) assessed the anti-inflammatory and analgesic potential of 6α,7β-dihydroxyvouacapan-17β-oic acid (**21**). Oral administration of 50 mg/kg (equivalent to 143.5 μmol/kg) of **21** inhibited the inflammatory mechanisms triggered by carrageenan and prostaglandin E2, while not significantly inhibiting the edema produced by dextran. The acetic acid-induced contortion test showed dose-dependent inhibition in mice treated orally with the diterpene (50, 200 or 400 mg/kg *p.o.*). In the formalin test, **21** showed antinociceptive activity on neurogenic and inflammatory pain models in mice given oral treatment (50 and 100 mg/kg *p.o.*). However, in the 100 mg/kg (or 287 μmol/kg) dose (*p.o.*), the compound was not able to increase the latency time during the hot-plate test. Together, these results suggest that **21** has peripheral anti-inflammatory and analgesic effects.

To date, few vouacapane diterpenes from *Pterodon* have been evaluated for anti-inflammatory and antinociceptive activities. Since the tests evaluated only one vouacapane at a time, there is not a comparison regarding the potential of different vouacapanes. The antinociceptive effect of vouacapans may involve multiple mechanisms of action as opioid, catecholaminergic, vanilloid, glutamate, serotonergic and imidazole systems. These data show that vouacapans have a promising effect as antinociceptive substances. It is expected that further studies involving toxicity trials will be carried out in order to ensure a safe use of these substances. Thus, despite significant results, the evaluations made so far have been limited to preliminary tests, involving only five compounds.

Larvicidal activity

Aedes aegypti mosquitoes are vectors for transmitting several arboviruses such as zika (ZIKV), chikungunya (CHIKV), and dengue (DENV). According to the World Health Organization (WHO), DENV, one of the most aggressive re-emerging pathogens worldwide,

causes more than 390 million infections each year (WHO, 2016a). The zika virus is continuing to spread to areas where vectors are present (WHO, 2016b), whereas CHIKV has been identified in over sixty countries (WHO, 2016c). The spread of these viruses is dependent upon the relation of the human host, and the vector and efforts have been placed on strategies to reduce the number of mosquitoes. One such strategy is the search for human-safe compounds that are capable of eliminating mosquito larvae.

Omena et al. (2006) assessed the larvicidal activity of three vouacapane diterpenes against stage 4 *Aedes aegypti* larvae. Compounds **21**, **22**, and 6α-hydroxyvouacapan-7β,17β-lactone (**38**) presented LC_{50} of 14.69 μg/ml (42.2 nmol/ml), 21.76 μg/ml (60 nmol/ml), and 50.08 μg/ml (151.6 nmol/ml), respectively. Given that substances with LC_{50} values lower than 100 μg/ml are considered active against *Aedes aegypti* (Cheng et al., 2003), such results indicated that these compounds are potentially interesting for anti-*Aedes aegypti* products.

Pimenta et al. (2006) tested the larvicidal activity of 6α-acetoxyvouacapane (**30**) against stage 3 *Aedes aegypti* larvae, in concentrations ranging from 12.5 to 500 μg/ml. Compound **30** showed median lethal concentration (LC_{50}) of 186.21 μg/ml (540.5 nmol/ml). Featuring an LC_{50} of 24 μg/ml in this study, the hexanic extract showed to be more promising than the isolated vouacapane. This study did not investigate whether such a remarkable value was due to synergic action of the constituents from the hexanic fraction or to the presence in the oil of some more potent vouacapanes. It seems clear that the choice of a natural product for effectively controlling *Aedes* mosquito should take into account many other factors besides lethal concentration, like resources availability and the costs for obtaining this product. In the study of Pimenta et al. (2006), 1.5 kg of fruits yielded 364 g of hexanic extract and only 181 mg of compound **17** after a chromatographic elution. Therefore, this hexanic extract seems to be more promising than the *Pterodon* vouacapanes assessed until now. In this study, plain oil was not evaluated against the larvae.

In partnership with our group, Oliveira et al. (2016) found that LC_{50} for a nanoemulsion of *Pterodon* oil is about 35 μg/ml, which reinforces the potential of non-purified *Pterodon* products. Thus, further larvicidal studies should involve chemically well-characterized oils instead of isolated vouacapanes, which could also be assessed against other species of mosquito. Surveys on the availability of the plants that produce a suitable oil as well as on the economic feasibility of the approach based on larvae control are required. Due to the low miscibility of *Pterodon* oil in water, nanoemulsions prepared by a low energy and solvent-free method, as optimized by Oliveira et al. (2016), should be considered in further studies.

Leishmanicidal activity

Leishmaniasis is widely distributed across 88 tropical, subtropical and temperate countries, affecting about 12 million people worldwide (Georgiadou et al., 2015). Leishmaniasis is caused by a protozoa parasite from over 20 *Leishmania* species and is transmitted to humans by the bite of infected female phlebotomine sandflies (WHO, 2017a). In 2014, more than 90% of new cases reported to WHO occurred in six countries: Brazil, Ethiopia, India, Somalia, South Sudan and Sudan (WHO, 2017b). Current clinically used drugs against leishmaniasis are related to numerous shortfalls including toxicity, must be administered over prolonged periods and are often associated with serious side effects (Croft and Coombs, 2003). Thus, it is necessary to development new leishmanicidal agents.

Oliveira et al. (2017) tested the leishmanicidal activity of compound **26** against promastigotes of *Leishmania amazonensis* and *L. braziliensis*, in concentrations ranging from 8.0 to 128.0 μg/ml, and showed parasite growth inhibitory concentration 50% (IC_{50}) less than 30 μg/ml (equivalent to 74.16 nmol/ml). Amphotericin B (control) showed an IC_{50} value of 5.41 nmol/l. The amphotericin B is highly active, but its clinical use is limited due to its high toxicity (Croft and Coombs, 2003; Caldeira et al., 2015).

These results indicated that the compound **26** inhibits the growing of promastigotes of *L. amazonensis* and *L. braziliensis*, which are the principal agents of leishmaniasis in Brazil (Ministério da Saúde, 2009). However, besides the determination of the leishmanicidal effect itself, it is important to determine the cytotoxicity of vouacapanes toward a mammalian cell line. Taking both measures into account, the selectivity index (SI) can be calculated by dividing the IC_{50} value of a compound for a mammalian cell line through the IC_{50} for its parasitocidal action. Compounds with high SI values are suitable for *in vivo* studies (Hrckova and Velebny, 2013). Therefore, new studies aiming toward the development of a new drug against leishmaniasis, in addition to including other *Pterodon* vouacapanes, should encompass a cytotoxicity assessment of them.

Cardiovascular-related activity

Cardiovascular diseases cover a range of heart and blood vessel disorders *e.g.* coronary heart, cerebrovascular, peripheral arterial, and rheumatic heart diseases, and WHO estimates that 17.5 million people worldwide die from heart-related diseases each year (WHO, 2016d). Some of these disorders involve one or more risk factors such as hypertension, diabetes, hyperlipidemia or other established diseases. Several natural products have been used to alleviate or prevent some of these disorders or related events, such as cardiac glycosides and reserpine.

Reis et al. (2015) assessed blood vessel relaxation and the possible action mechanisms of compound **26** in isolated mouse aorta preparations. Results suggested that **26** induces endothelium-independent vascular relaxation by blocking the L-type Ca^{2+} channel ($Ca_v1.2$). These results support a possible cardiovascular effect of compound **26** and *Pterodon* oil through vascular dilatation; however, none of the many other *Pterodon* vouacapanes was assessed. Since this class revealed a potential for use as a cardiovascular relaxation agent, other vouacapanes should also be tested in preliminary studies. Further investigations of the most promising substances should include assessments in human tissues and *in vivo* systems.

Cytotoxicity and antitumor activity

Certain substances, such as verapamil or cyclosporine, have been used to overcome multidrug resistance (MDR), an important obstacle to the success of cancer chemotherapy. However, these P-gp modulating agents have not shown significant potential in clinical practice (Meschini et al., 2003), and the identification of new compounds with few side effects is highly desirable. The induction of apoptosis represents a critical factor in cancer therapy and contributed to significant anti-tumor activity for the compounds associated with cytotoxicity properties with potential to inhibit cellular events related to the progression of tumors.

Vieira et al. (2008) assessed the antiproliferative activity of **29** via MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) colorimetric assay by using SK-MEL-37 human melanoma cells, in concentrations ranging from 1.4 to 92 μmol/l, and showed inhibitory concentration 50% (IC_{50}) of 32 μmol/l. Doxorubicin (positive control) showed an IC_{50} value of 35 μmol/L, similar to that of the vouacapane tested. This result showed that the vouacapanes tested displayed cytotoxicity activity against the tested cell line.

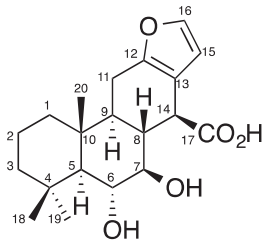
Spindola et al. (2009) tested the activity of 7β-acetoxyvouacapane (**17**), **18**, **22**, 6α,7β-dihydroxyvouacapan-17β-methylene-ol (**33**), and 6α-acetoxy-7β-hydroxyvouacapan

(**34**) against human tumor cell lines UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung), OVCAR-03 (ovary), PC-3 (prostate), HT-29 (colon), 786-0 (kidney), K562 (leukemia), and NCI-ADR/RES (ovary with multidrug resistance phenotype). Cell proliferation was determined by spectrophotometric quantification of cell protein content via sulphorhodamine B. Compound **34** was 26 times more potent in inhibiting 50% growth (GI_{50}) of PC-3 (prostate), 15 times more cytostatic (total growth inhibition – TGI), and six times less toxic than the concentration leading to 50% cell death (LC_{50}) when compared with the control (doxorubicin).

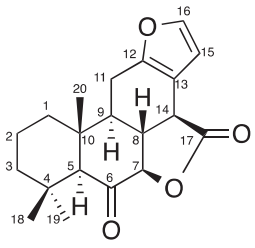
This study also assessed the cytotoxicity of compounds **22**, **33**, and **34** against normal murine cell line (3T3) using the MTT method, in concentrations ranging from 0.25 to 250 μg/ml. Regarding cytotoxicity against 3T3, **34** had less toxicity (IC_{50} of 34.33 μg/ml, equivalent to 95.2 nmol/ml), followed by **33** (IC_{50} of 23.55 μg/ml, equivalent to 70.4 nmol/ml) and **22** (IC_{50} of 22.83 μg/ml, equivalent to 63.0 nmol/ml). Compounds **22**, **33**, and **34** presented high selectivity for prostate cancer. The selective activity against prostate cancer cells and the lower cytotoxicity over normal cells show *Pterodon* vouacapanes merit further investigations in an effort to develop selective medicines that treat cancer with greater patient safety in the future.

For more information on the antiproliferative activity of furanoditerpenes against different cell lines, new active constituents were synthesized from **21** isolated from *P. emarginatus* fruits.

Euzébio et al. (2009) synthesized three lactones derivatives from **21**: 6α-hydroxyvouacapan-7β,17β-lactone (**38**), 6α-acetoxyvouacapan-7β,17β-lactone (**37**), and 6-oxovouacapan-7β,17β-lactone (**39**). The antiproliferative activity of **21** and its derived lactones was assessed against the same human cancer cell lines used by Spindola et al. (2009). Compound **38** was the most active of the four furanoditerpenes, as well as more potent in inhibiting 50% growth (GI_{50}) of adriamycin-resistant ovary cancer cells (NCI-ADR/RES) and erythromyeloblastoid leukemia (K562) when compared with doxorubicin. Compound **37** only showed a growth inhibition effect against erythromyeloblastoid leukemia cells (GI_{50} of 27.4 μg/ml, or 73.6 nmol/ml). Results indicated **38** as the most promising derivative for future studies, in addition to the importance of 7β, of 17β-lactone ring, and of the C-6 hydroxyl group for the antiproliferative activity of **38**.



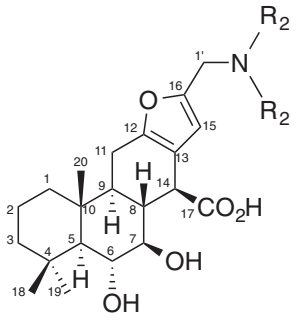
21



39

In a follow-up to the previous study, Euzébio et al. (2010) synthesized six new derived amines from lactone derivative **38**: 16-(*N*,*N*-diethylaminomethyl)-6α-hydroxyvouacapan-7β,17β-lactone (**40**); 16-(*N*,*N*-dipropylaminomethyl)-6α-hydroxyvouacapan-7β,17β-lactone (**41**); 16-(*N*,*N*-diisobutylaminomethyl)-6α-hydroxyvouacapan-7β,17β-lactone (**42**); 16-(1-pyrrolidinylmethyl)-6α-hydroxyvouacapan-7β,17β-lactone (**43**); 16-(1-piperidinylmethyl)-6α-hydroxyvouacapan-7β,17β-lactone (**44**); 16-(4-morpholinylmethyl)-6α-hydroxyvouacapan-7β,17β-lactone (**45**). These compounds were tested on the same cancer cell lines from the previous study and were more potent against most of them, showing lower GI_{50} values than those obtained for **38** (Euzébio et al., 2009). Compounds **40–45** were, like doxorubicin (control), potent growth inhibitors of adriamycin-resistant ovary cancer cells (NCI-ADR/RES), lung cancer cells (NCI-H460), and erythromyeloblastoid leukemia (K562). Theoretical calculations showed that C-16 amino groups may be crucial to the antiproliferative activity of vouacapane derivatives.

The results presented in this topic contributed to show the potential of the cited molecules to obtain prototypes for antitumor drugs. The usual substances employed nowadays in antitumor therapy present several side effects, and in this context the search for new structures with more specificity could be a good strategy in future investigations regarding cytotoxicity effects.



1" 2" 3"

41 R_1=R_2= -CH_2-CH_3

42 R_1=R_2= -CH_2-CH_2-CH_3

43 R_1=R_2= -CH_2-CH_2-$(CH_3)_2$

1" 2" 2" 1"

44 R_1 and R_2= -CH_2-CH_2-CH_2-CH_2

1" 2" 3" 2" 1"

45 R_1 and R_2= -CH_2-CH_2-CH_2-CH_2-CH_2

2" 1" 1" 2"

46 R_1 and R_2= -CH_2-CH_2-O-CH_2-CH_2

Technological applications

Vouacapanes can be used as phytochemical marker for quality control and standardization of the products derived from *Pterodon* due to their pharmacological activities. Aiming to mask the taste of the extracts standardized in vouacapans, some technological alternatives have been developed for the oils of *Pterodon* species, such as microcapsules in polymeric systems (alginate/medium-molecular-weight chitosan (F1-MC), alginate/chitosan with greater than 75% deacetylation (F2-MC), and alginate/low-molecular-weight chitosan (F3-MC)) (Reinas et al., 2014).

Nanoemulsions are an alternative to drug delivery for lipophilic compounds, which can be administrated via oral, ocular, and intravenous in order to reduce side effects and to improve pharmacological properties (Solans et al., 2005; Horman and Zimmer, 2016; Singh et al., 2017). Hoscheid et al. (2017) optimized a nanoemulsion with *P. pubescens* oil, which provided faster injections during intramuscular administration, comparing to conventional formulation. In this study, the nanoemulsion system was evaluated for anti-inflammatory activity through peritonitis model, after preparation and after 365 days of storage at 25 °C. The authors demonstrated that proper storage (25 °C) was capable to preserve the characteristics of the nanoemulsion containing 7.5% PEG-40H castor oil, 5% lecithin, and 5% *P. pubescens* oil, also standardized in vouacapans. In other work from the same authors (Hoscheid et al., 2015), the

nanoemulsions were tested as a potential delivery system for the treatment of rheumatoid arthritis using the intramuscular administration, and chemically stable nanoemulsions were obtained.

Despite the pharmacological potential of the raw material obtained from *Pterodon* genus, there are few studies regarding to technological development of formulations containing such products. Moreover, studies involving formulations containing isolated *Pterodon* vouacapanes were not found. This development and the evaluation of the safety and the efficiency of these formulations must be necessary to start the development of the final products.

Conclusion and perspectives

This review showed that vouacapanes have great potential for medicinal applications, and their presence in plants from the *Pterodon* genus may explain several properties observed in extracts and justify certain ethnomedicinal uses of *Pterodon* species. We expect that other vouacapanes will still be reported, since HPLC only recently has been used in their obtainment. New studies are encouraged to carry out a phytochemical assessment of the raw material and to consider the possibility of purifying minority vouacapanes to be tested, since the studies accomplished so far only encompass a small portion of *Pterodon* vouacapanes. Anti-inflammatory, antinociceptive and analgesic activities were confirmed by scientific studies in animals. However, there is still a lack of scientific support justifying the production of a medicine with these compounds. Toxicity and safety evaluation studies are still needed to assure safety for clinical application. The pharmaceutical technology aiming at suitable delivery of the vouacapanes, in the different applications, is also very scarce. New preliminary studies regarding the cardiovascular and leishmanicidal activities should be carried out with other vouacapanes, before more specific assessments with the ones which prove to be most potent. In our opinion, *Pterodon* oil or oil's fractions are more convenient than vouacapanes for larvicidal activity, and seems to be more promising for future research. As the latest trends and perspectives for future research of *Pterodon* vouacapanes we suggest the need for studies regarding the sustainability of the plant species aiming at the production of medicines.

Authors contributions

LARO, GARO and LLB contributed to the concept, literature search and writing of this review article. DS and MTFB contributed to the concept and critical reading of this review.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank Universidade de Brasília, Universidade Federal de Goiás, Universidade Estadual de Goiás, FAP-DF, Capes and CNPq for the financial support.

References

Arriaga, A.M.C., Castro, M.A.B., Silveira, E.R., Braz-Filho, R., 2000. Further diterpenoids isolated from *Pterodon polygalaeflorus*. J. Braz. Chem. Soc. 11, 187–190.

Bahmani, M., Shirzad, H., Majlesi, M., Shahinfard, N., Kopaei, M.R., 2014. A review study on analgesic applications of Iranian medicinal plants. Asian Pac. J. Trop. Med. 7, 43–53.

Balmain, A., Bjamer, K., Connoly, J.D., Ferguson, G., 1967. The constitution and stereochemistry of-C-caesalpin. Tetrahedron Lett. 8, 5027–5031.

Bao, H., Zhang, Q., Ye, Y., Lin, L., 2016. Naturally occurring furanoditerpenoids: distribution, chemistry and their pharmacological activities. Phytochem. Rev. 16, 235–270.

Barreto, R.F.M., Laranja, G.A.T., Silva, M.C.C., Coelho, M.G.P., Paes, M.C., Oliveira, M.M., Castro, S.L., 2008. Anti-*Trypanosoma cruzi* activity of *Pterodon pubescens* seed oil: geranylgeraniol as the major bioactive component. Parasitol. Res. 103, 111–117.

Belinelo, V.J., Reis, G.T., Stefani, G.M., Alves, D.L.F., Veloso, D.P., 2002. Derivatives and its activities on the electrically stimulated guinea-pig ileum preparation. J. Braz. Chem. Soc. 13, 830–837.

Branco, P.A.C., Santos, F.J.L., Rubinger, M.M.M., Alves, D.L.F., Veloso, D.P., Díaz, B.K., Hennsen, B.L., 2008. Inhibition and uncoupling of photosynthetic electron transport by diterpene lactone amide derivatives. J. Biosci. 63, 251–259.

Cabral, E.C., Servat, L., Spindola, H.M., Coelho, M.B., Sousa, I.M.O., Queiroz, N.C.A., Foglio, M.A., Eberlin, M.N., Riveros, J.M., 2013. *Pterodon pubescens* oil: characterisation, certification of origin and quality control via mass spectrometry fingerprinting analysis. Phytochem. Anal. 24, 184–192.

Caldeira, L.R., Fernandes, F.R., Costa, D.F., Frézard, F., Afonso, L.C., Ferreira, L.A., 2015. Nanoemulsion loaded with amphotericin B: a new approach for the treatment of leishmaniasis. Eur. J. Pharm. Sci. 70, 125–131.

Campos, A.M., Silveira, E.R., Braz-Filho, R., Teixeira, T.C., 1994. Diterpenoids from *Pterodon polygalaeflorus*. Phytochemistry 36, 403–406.

Carvalho, J.C.T., Sertié, J.A.A., Barbosa, M.V.J., Patrício, K.C.M., Caputo, L.R.G., Sarti, S.J., Ferreira, L.P., Bastos, J.K., 1999. Anti-inflammatory activity of the crude extract from the fruits of *Pterodon emarginatus* Vog. J. Ethnopharmacol. 64, 127–133.

Cheenpracha, S., Karalai, C., Ponglimanont, C., Chantrapromma, K., Laphookhieo, S., 2006. Cassane-type diterpenes from the seeds of *Caesalpinia crista*. Helv. Chim. Acta 89, 1062–1066.

Cheng, S.S., Chang, H.T., Chang, S.T., Tsai, K.H., Chen, W.J., 2003. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. Bioresour. Technol. 89, 99–102.

Coelho, L.P., Reis, P.A., Castro, F.L., Gayer, C.R.M., Lopes, C.S., Silva, M.C.C., Sabino, K.C.C., Todeschini, A.R., Coelho, M.G.P., 2005. Antinociceptive properties of ethanolic extract and fractions of *Pterodon pubescens* Benth. seeds. J. Ethnopharmacol. 98, 109–116.

Corrêa, M.P., 1975. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro.

Cota, B.B., Oliveira, D.M., Siqueira, E.P., Fagundes, E.M.S., Pimenta, A.M.C., Santos, D.M., Rabello, A., Zani, C.L., 2011. New cassane diterpenes from *Caesalpinia echinata*. Fitoterapia 82, 969–975.

Croft, S.L., Coombs, G.H., 2003. Leishmaniasis – current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol. 19, 502–508.

Das, B., Srinivas, Y., Sudhakar, C., Mahender, I., Laxminarayana, K., Reddy, P.R., Raju, T.V., Jakka, N.M., Rao, J.V., 2010. New diterpenoids from Caesalpinia species and their cytotoxic activity. Bioorg. Med. Chem. Lett. 20, 2847–2850.

Demuner, A.J., Barbosa, L.C.A., Veloso, D.P., Alves, L.D.F., Howarth, O.W., 1996. Structure and plant growth regulatory activity of new diterpenes from *Pterodon polygalaeflorus*. J. Nat. Prod. 59, 770–772.

Dettmer, K., Aronov, P.A., Hammock, B.D., 2007. Mass spectrometry-based metabolomics. Mass Spectrom. Rev. 26, 51–78.

Dewick, M.P., 2002. Medicinal Natural Products: A Biosynthetic Approach. John Wiley & Sons, New York.

Díaz, B.K., Santos, F.J., Rubinger, M.M., Veloso, D.P., Hennsen, B.L., 2006. A diterpene γ-lactone derivative from *Pterodon polygalaeflorus* Benth. as a photosystem II inhibitor and uncoupler of photosynthesis. J. Biosci. 61, 227–233.

Díaz, B.K., Branco, P.A.C., Santos, F.J.L., Rubinger, M.M.M., Alves, D.L.F., Veloso, D.P., Hennsen, B.L., 2010. Furanoditerpene ester and thiocarbonyl deoxy derivatives inhibit photosynthesis. Pest. Biochem. Physiol. 96, 119–126.

Dong, R., Yuan, J., Wu, S., Huang, J., Xu, X., Wu, Z., Gao, H., 2015. Anti-inflammation furanoditerpenoids from *Caesalpinia minax* Hance. Phytochemistry 117, 325–331.

Duarte, I.D.G., Alves, D.L.F., Craig, M.N., 1992. Possible participation of endogenous opioid peptides on the mechanism involved in analgesia induced by vouacapano. Life Sci. 50, 891–897.

Duarte, I.D.G., Alves, D.L.F., Veloso, D.P., Craig, M.N., 1996. Evidence of the involvement of biogenic amines in the antinociceptive effect of a vouacapan extracted from *Pterodon polygalaflorus* Benth. J. Ethnopharmacol. 55, 13–18.

Dutra, R.C., Leite, M.N., Barbosa, N.R., 2008. Quantification of phenolic constituents and antioxidant activity of *Pterodon emarginatus* Vogel seeds. Int. J. Mol. Sci. 9, 606–614.

Dutra, R.C., Braga, F.G., Coimbra, E.S., Silva, A.D., Barbosa, N.R., 2009. Atividade antimicrobiana e leishmanicida das sementes de *Pterodon emarginatus* Vogel. Rev. Bras. Farmacogn. 19, 429–435.

Euzébio, F.P.G., Santos, F.J.L., Veloso, D.P., Ruiz, A.L.T.G., Carvalho, J.E., Alves, D.L.F., Fátima, A., 2009. Effect of 6α,7β-dihydroxyvouacapan-17β-oic acid and its lactone derivatives on the growth of human cancer cells. Bioorg. Chem. 37, 96–100.

Euzébio, F.P.G., Santos, F.J.L., Veloso, D.P., Alcântara, A.F.C., Ruiz, A.L.T.G., Carvalho, J.E., Foglio, M.A., Alves, D.L.F., Fátima, A., 2010. Synthesis, antiproliferative activity in cancer cells and theoretical studies of novel 6α,7β-dihydroxyvouacapan-17β-oic acid Mannich base derivatives. Bioorg. Med. Chem. 18, 8172–8177.

Fagg, C.W., Lughadha, E.M., Milliken, W., Hind, D.J.N., Brandão, M.G.L., 2015. Useful Brazilian plants listed in the manuscripts and publications of the Scottish medic and naturalist George Gardner (1812–1849). J. Ethnopharmacol. 161, 18–29.

Fascio, M., Mors, W.B., Gilbert, B., Mahajan, J.R., Monteiro, M.B., Santos Filho, D., Vichnewski, W., 1976. Diterpenoid furans from *Pterodon* species. Phytochemistry 15, 201–203.

Galceran, C.B., Sertie, J.A.A., Lima, C.S., Carvalho, J.C.T., 2011. Anti-inflammatory and analgesic effects of 6α,7β-dihydroxyvouacapan-17β-oic acid isolated from *Pterodon emarginatus* Vog. fruits. Inflammopharmacology 19, 139–143.

Gao, T., Yao, H., Song, J., Liu, C., Zhu, Y., Ma, X., Pang, X., Xu, H., Chen, S., 2010. Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. J. Ethnopharmacol. 130, 116–121.
García, P.A., Oliveira, A.B., Batista, R., 2007. Occurrence, biological activities and synthesis of kaurane diterpenes and their glycosides. Molecules 12, 455–483.
Garland, E.L., 2012. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. Prim. Care Clin. Office Pract. 39, 561–571.
Georgiadou, S.R., Makaritsis, K.P., Dalekos, G.N., 2015. Leishmaniasis revisited: current aspects on epidemiology, diagnosis and treatment. J. Transl. Int. Med. 3, 43–50.
Godoy, R.L., Lima, P.D.D.B., Pinto, A.C., Aquino Neto, F.R., 1989. Diterpenoids from *Dypterix odorata*. Phytochemistry 28, 642–644.
Grandi, T.S.M., Trindade, J.A., Pinto, M.J.F., et al., Ferreira, L.L., Catella, A.C., 1989. Plantas Medicinais de Minas Gerais, Brasil. Acta Bot. Bras. 3, 185–224.
Hamburger, M., Hostettmann, K., 1991. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry 30, 3864–3874.
Hansen, D., Haraguchi, M., Alonso, A., 2010. Pharmaceutical properties of "sucupira" (*Pterodon* spp). Braz. J. Pharm. Sci. 46, 607–616.
Hanson, J.R., 1995. Diterpenoids. Nat. Prod. Rep. 12, 207.
Horman, K., Zimmer, A., 2016. Drug delivery and drug targeting with parenteral lipid nanoemulsions – a review. J. Control. Release 223, 85–98.
Hoscheid, J., Reinas, A., Cortez, D.A.G., Costa, W.F., Cardoso, M.L.C., 2012. Determination by GC–MS-SIM of furanoditerpenes in *Pterodon pubescens* Benth.: development and validation. Talanta 100, 372–376.
Hoscheid, J., Amado, C.A.B., Rocha, B.A., Outuki, P.M., Silva, M.A.R.C.P., Froehlich, D.L., Cardoso, M.L.C., 2013. Inhibitory effect of the hexane fraction of the ethanolic extract of the fruits of *Pterodon pubescens* Benth. in acute and chronic inflammation. Evid. Based Complement. Alternat. Med., http://dx.doi.org/10.1155/2013/272795.
Hoscheid, J., Outuki, P.M., Kleinubing, S.A., Silva, M.F., Bruschi, M.L., Cardoso, M.L.C., 2015. Development and characterization of *Pterodon pubescens* oil nanoemulsions as a possible delivery system for the treatment of rheumatoid arthritis. Colloids Surf. A 484, 19–27.
Hoscheid, J., Outuki, P.M., Kleinubing, S.A., Goes, P.R.N., Lima, M.M.S., Cuman, R.K.N., Cardoso, M.L.C., 2017. *Pterodon pubescens* oil nanoemulsion: physiochemical and microbiological characterization and *in vivo* anti-inflammatory efficacy studies. Rev. Bras. Farmacogn. 27, 375–383.
Hrckova, G., Velebny, S., 2013. Pharmacological Potential of Selected Natural Compounds in the Control of Parasitic Diseases. Springer, New York.
Hurtado, M.G., Esquivel, F.E.A., García, G.R., Pacheco, M.M.M., Madrigal, R.M.E., Bolaños, T.P., Hernández, J.L.S., Gutiérrez, H.A.G., Rojas, C.M.C.G., Nathan, P.J., Rio, R.E., 2013. Cassane diterpenes from *Caesalpinia platyloba*. Phytochemistry 96, 397–403.
Jadhav, A.N., Kaur, N., Bhutani, K.K., 2003. A new furanoditerpenoid marker for the distinction between the seeds of two species of *Caesalpinia*. Phytochem. Anal. 14, 315–318.
Jiang, R.W., Ma, S.C., But, P.P.H., Mak, C.W., 2001. Isolation and characterization of spirocaesalmin, a novel rearranged vouacapane diterpenoid from *Caesalpinia minax* Hance. J. Chem. Soc. Perkin Trans., http://dx.doi.org/10.1039/B107473N.
Jiang, R.W., Ma, S.C., He, Z.D., Huang, X.S., But, P.P.H., Wang, H., Chan, S.P., Ooi, V.E.C., Xu, H.X., Mak, T.C.W., 2002. Molecular structures and antiviral activities of naturally occurring and modified cassane furanoditerpenoids and friedelane triterpenoids from *Caesalpinia minax*. Bioorg. Med. Chem. 10, 2161–2170.
Julius, D., Basbaum, A.I., 2001. Molecular mechanisms of nociception. Nature 413, 203–210.
Kido, T., Taniguchi, M., Baba, K., 2003. Diterpenoids from Amazonian crude drug of Fabaceae. Chem. Pharm. Bull. 51, 207–208.
Lian, L., Li, X.B., Yuan, J.Z., Cheng, L., Wu, Z.H., Gao, H.Y., 2015. Two new diterpenes from the seeds of *Caesalpinia minax* Hance. J. Asian Nat. Prod. Res. 17, 893–899.
Loeser, J.D., Treede, R.D., 2008. The Kyoto protocol of IASP basic pain terminology. Pain 137, 473–477.
Mahajan, J.R., Monteiro, M.B., 1973. New diterpenoids from *Pterodon emarginatus* Vog. J. Braz. Chem. Soc. 42, 520–525.
Martins, C.N., Martins, D.F., Nascimento, L.F., Venzke, D., Oliveira, A.S., Frederico, M.J.S., Silva, F.R.M.B., Brighente, I.M.C., Pizzolatti, M.G., Santos, A.R.S., 2015. Ameliorative potential of standardized fruit extract of *Pterodon pubescens* Benth. on neuropathic pain in mice: evidence for the mechanism of action. J. Ethnopharmacol. 175, 273–286.
Matsuno, Y., Deguchi, J., Hirasawa, Y., Ohyama, K., Toyoda, H., Hirobe, C., Ekasari, W., Widyawaruyanti, A., Zaini, N.C., Morita, H., 2008. Sucutiniranes A and B, new cassane-type diterpenes from *Bowdichia nitida*. Bioorg. Med. Chem. Lett. 18, 3774–3777.
Maurya, R., Ravi, M., Singh, S., Yadav, P.P., 2012. A review on cassane and norcassane diterpenes and their pharmacological studies. Fitoterapia 83, 272–280.
Mcpherson, D.D., Che, C.T., Cordell, G., Soejarto, D.D., Pezzuto, J.M., Fong, H.H.S., 1986. Diterpenoids from *Caesalpinia pulcherrima*. Phytochemistry 25, 167–170.
Mendes, F.N., Silveira, E.R., 1994. Fatty acids, sesqui- and diterpenoids from seeds of *Dipteryx lacunifera*. Phytochemistry 35, 1499–1503.
Merskey, H., Bogduk, N., 1994. Classification of Chronic Pain, IASP Task Force on Taxonomy. IASP Press, Seattle.
Meschini, S., Marra, M., Calcabrini, A., Federici, E., Galeffi, C., Arancia, G., 2003. Voacamine, a bisindolic alkaloid from *Peschiera fuchsiaefolia*, enhances the cytotoxic effect of doxorubicin on multidrug-resistant tumor cells. Int. J. Oncol. 23, 1505–1513.
Ministério da Saúde, 2009. Guia de Vigilância Epidemiológica, Secretária de Vigilância as Saúde, 7 ed. Ministério da Saúde, Brasília (DF), 813 p.
Mitsui, T., Ishihara, R., Hayashi, K.I., Matsuura, N., Akashi, H., Nozaki, H., 2015. Cassane-type diterpenoids from *Caesalpinia echinata* (Leguminosae) and their NF-kB signaling inhibition activities. Phytochemistry 116, 349–358.
Nucci, C., Martins, L.M., Stramosk, J., Brethanha, L.C., Pizzolatti, M.G., Santos, A.R.S., Martins, D.F., 2012. Oleaginous extract from the fruits *Pterodon pubescens* Benth. induces antinociception in animal models of acute and chronic pain. J. Ethnopharmacol. 143, 170–178.
Ochieng, C.O., Owuor, P.O., Mang'uro, L.A.O., Akala, H., Ishola, I.O., 2012. Antinociceptive and antiplasmodial activities of cassane furanoditerpenes from *Caesalpinia volkensii* H. root bark. Fitoterapia 83, 74–80.
Odalo, J.O., Joseph, C.C., Nkunya, M.H.H., Sattler, I., Lange, C., Dahse, H.M., Mollman, U., 2009. Cytotoxic, anti-proliferative and antimicrobial furanoditerpenoids from *Stuhlmania moavi*. Phytochemistry 70, 2047–2052.
Oliveira, L.A.R., 2014. Isolamento, quantificação e avaliação das atividades leishmanicida e tripanocida de furanoditerpenos do oleorresina de *Pterodon* spp. Vogel (Fabaceae). Goiânia. Dissertação de Mestrado, Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal de Goiás, 120 p.
Oliveira, L.A.R., Oliveira, G.A.R., Lemes, G.F., Romão, W., Vaz, B.G., Albuquerque, S., Gonçalez, C., Lião, L.M., Bara, M.T.F., 2017. Isolation and structural characterization of two new furanoditerpenes from *Pterodon emarginatus* (Fabaceae). J. Braz. Chem. Soc., http://dx.doi.org/10.21577/0103-5053.20170118.
Oliveira, P.C., 2012. Obtenção e caracterização do extrato seco padronizado dos frutos da sucupira *Pterodon emarginatus* Vogel, Fabaceae. Goiânia. Dissertação de Mestrado, Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal de Goiás, 146p.
Oliveira, A.E.M.F.M., Duarte, J.L., Amado, J.R.R., Cruz, R.A.S., Rocha, C.F., Souto, R.N.P., Ferreira, R.M.A., Santos, K., Conceição, E.C., Oliveira, L.A.R., Kelecom, A., Fernandes, C.P., Carvalho, J.C.T., 2016. Development of a larvicidal nanoemulsion with *Pterodon emarginatus* Vogel oil. PLOS ONE 11, 1–16.
Omena, M.C., Bento, E.S., Paula, J.E., Sant'ana, A.E.G., 2006. Larvicidal diterpenes from *Pterodon polygalaeflorus*. Vector Borne Zoonotic Dis. 6, 216–222.
Pascoa, H., Diniz, D.G.A., Florentino, I.F., Costa, E.A., Bara, M.T.F., 2015. Microemulsion based on *Pterodon emarginatus* oil and its anti-inflammatory potential. Braz. J. Pharm. Sci. 51, 117–126.
Pereira, M.F., Martino, T., Dalmau, S.R., Albano, R.M., Férézou, J.P., Costa, S.S., Coelho, M.G.P., Sabino, K.C.C., 2011. Terpenic subfraction of *Pterodon pubescens* induces apoptosis of K562 leukemic cells by modulating gene expression. Oncol. Rep. 25, 215–221.
Pereira, M.F., Martino, T., Dalmau, S.R., Paes, M.C., Fidalgo, C.B., Albano, R.M., Coelho, M.G.P., Sabino, K.C.C., 2012. Terpenic fraction of *Pterodon pubescens* inhibits nuclear factor kappa B and extracellular signal-regulated protein kinase 1/2 activation and deregulates gene expression in leukemia cells. BMC Complement. Altern. Med. 12, 231–239.
Peters, R.J., 2010. Two rings in them all: the labdane-related diterpenoids. Nat. Prod. Res. 27, 1521–1530.
Pimenta, A.T.A., Santiago, G.M.P., Arriaga, A.M.C., Menezes, G.H.A., Bezerra, S.B., 2006. Estudo fitoquímico e avaliação da atividade larvicida de *Pterodon polygalaeflorus* Benth. (Leguminosae) sobre *Aedes aegypti*. Rev. Bras. Farmacogn. 16, 501–505.
Pizzolatti, M.G., Cristiano, R., Monache, F.D., Branco, A., 2002. Artefatos cumaríncos isolados de *Polygala paniculata* L. (Polygalaceae). Rev. Bras. Farmacogn. 12, 21–26.
Pudhom, K., Sommit, D., Suwankitti, N., Petsom, A., 2007. Cassane furanoditerpenoids from the seed kernels of *Caesalpinia bonduc* from Thailand. J. Nat. Prod. 70, 1542–1544.
Ragasa, C.Y., Hofileña, J.G., Rideout, J.A., 2002. New furanoid diterpenes from *Caesalpinia pulcherrima*. J. Nat. Prod. 65, 1107–1110.
Ragasa, C.Y., Ganzon, J., Hofileña, J., Tamboong, B., Rideout, J.A., 2003. A new furanoid diterpene from *Caesalpinia pulcherrima*. Chem. Pharm. Bull. 51, 1208–1210.
Rai, M., Acharya, D., Rios, J.L., 2011. Ethnomedicinal Plants: Revitalizing of Traditional Knowledge of Herbs. Science Publishers, New Hampshire.
Ramawat, K.G., Mérillon, J.M. (Eds.), 2013. Natural Products. Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes. Springer, Berlin.
Raposo, N.R.B., Dutra, R.C., Ferreira, A.S., 2011. Biological properties of sucupira branca (*Pterodon emarginatus*) seeds and their potential usage in health treatments. In: Preedy, V.R., Watson, R.R., Patel, V.B. (Eds.), Nuts & Seeds in Health and Disease Prevention. Elsevier, New York, pp. 1087–1095.
Reinas, A.E., Hoscheid, J., Outuki, P.M., Cardoso, M.L.C., 2014. Preparation and characterization of microcapsules of *Pterodon pubescens* Benth. by using natural polymers. Braz. J. Pharm. Sci. 50, 919–930.
Reis, F.C., Andrade, D.M.L., Neves, B.J., Oliveira, L.A.R., Pinho, J.F., Silva, L.P., Cruz, J.S., Bara, M.T.F., Andrade, C.H., Rocha, M.L., 2015. Blocking the L-type Ca^{2+} channel (Ca_V 1.2) is the key mechanism for the vascular relaxing effect of *Pterodon* spp. and its isolated diterpene methyl-6α-acetoxy-7β-hydroxyvouacapan-17β-oate. Pharmacol. Res. 100, 242–249.
Rubinger, M.M.M., Branco, P.A.C., Guilardi, S., Souza, E.M.R., Gambardella, M.T.P., Borges, E.E.L., Alves, D.L.F., Veloso, D.P., 2004. Preparation, X-ray structural studies and plant growth regulatory activity of methyl 6α,7β-Thiocarbonydioxyvouacapan-17β-oate. J. Braz. Chem. Soc. 15, 219–223.

Sabino, K.C.C., Castro, F.A., Oliveira, J.C.R., Dalmau, S.R.A., Coelho, M.G.P., 1999. Successful treatment of collagen-induced arthritis in mice with a hydroalcohol extract of seeds of *Pterodon pubescens*. Phytother. Res. 13, 613–615.

Santos, F.J.L., Alcântara, A.F.C., Alves, D.L.F., Veloso, D.P., 2008. Theoretical and experimental NMR studies of the Swern oxidation of methyl 6α,7β-dihydroxyvouacapan-17β-oate. Struct. Chem. 19, 625–631.

Sawaya, A.C.H.F., Abdelnur, P.V., Eberlin, M.N., Kumazawa, S., Ahn, M.R., Bang, K.S., Nagaraja, N., Bankova, V.S., Afrouzan, H., 2010. Fingerprinting of propolis by easy ambiente sonic-spray ionization mass spectrometry. Talanta 81, 100–108.

Servat, L., Spindola, H.M., Rodrigues, R.A.F., Sousa, I.M.O., Ruiz, A.L.T.G., Carvalho, J.E., Foglio, M.A., 2012. *Pterodon pubescens* Benth.: stability study of microencapsulated extract and isolated compounds monitored by antinociceptive assays. J. Braz. Chem. Soc. 23, 1244–1253.

Silva, M.C.C., Gayer, C.R.M., Lopes, C.S., Calixto, N.O., Reis, P.A., Passaes, C.P.B., Paes, M.C., Dalmau, S.R., Sabino, K.C.C., Todeschini, A.R., Coelho, M.G.P., 2004. Acute and topic anti-edematogenic fractions isolated from the seeds of *Pterodon pubescens*. J. Pharm. Pharmacol. 55, 135–141.

Singh, Y., Meher, J.G., Raval, K., Khan, F.A., Chaurasia, M., Jain, N.K., Chourasia, M.K., 2017. Nanoemulsion: concepts, development and applications in drug delivery. J. Control. Release 252, 28–49.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N., Garcia-Celma, M.J., 2005. Nano-emulsions. Curr. Opin. Colloid Interface Sci. 10, 102–110.

Spindola, H.M., Carvalho, J.E., Ruiz, A.L.T.G., Rodrigues, R.A.F., Denny, C., Sousa, I.M.O., Tamashiro, J.Y., Foglio, M.A., 2009. Furanoditerpenes from *Pterodon pubescens* Benth. with selective in vitro anticancer activity for prostate cell line. J. Braz. Chem. Soc. 20, 569–575.

Spindola, H.M., Servat, L., Denny, C., Rodrigues, R.A.F., Eberlin, M.N., Cabral, E., Souza, I.M.O., Tamashiro, J.Y., Carvalho, J.E., Foglio, M.A., 2010. Antinociceptive effect of geranylgeraniol and 6α,7β-dihydroxyvouacapan-17β-oate methyl ester isolated from *Pterodon pubescens* Benth. BMC Pharm. 10, http://dx.doi.org/10.1186/1471-2210-10-1.

Spindola, H.M., Servat, L., Rodrigues, R.A.F., Sousa, I.M.O., Carvalho, J.E., Foglio, M.A., 2011. Geranylgeraniol and 6α,7β-dihydroxyvouacapan-17β-oate methyl ester isolated from *Pterodon pubescens* Benth.: further investigation on the antinociceptive mechanism of action. Eur. J. Pharmacol. 656, 45–51.

2013. The Plant List. Version 1.1. Global Strategy Plant Conservation (acessed December 2016).

Toledo, C.E.M., Britta, E.A., Ceole, L.F., Silva, E.R., Mello, J.C.P., Dias Filho, B.P., Nakamura, C.V., Nakamura, T.U., 2011. Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado using Brazilian cachaça as extractor liquid. J. Ethnopharmacol. 133, 420–425.

Vieira, C.R., Marques, M.F., Soares, P.R., Matuda, L., Oliveira, C.M.A., Kato, L., Silva, C.C., Guillo, L.A., 2008. Antiproliferative activity of *Pterodon pubescens* Benth. seed oil and its active principle on human melanoma cells. Phytomedicine 15, 528–532.

Villas-Boas, S.G., Mas, S., Akesson, M., Smedsgaard, J., Nielsen, J., 2005. Mass spectrometry in metabolome analysis. Mass Spectrom. Rev. 24, 613–646.

Xu, R., Ye, Y., Zhao, W. (Eds.), 2011. Introduction to Natural Products Chemistry. Science Press, Beijing.

WHO, 2016a. WHO Dengue and Severe Dengue, Fact Sheets. World Health Organization, Geneve, http://www.who.int/mediacentre/factsheets/fs117/en (accessed 23.12.16).

WHO, 2016b. WHO Zika Situation Report, Emergencies. World Health Organization, Geneva, http://www.who.int/emergencies/zika-virus/situation-report/15-december-2016/en (accessed 23.12.16).

WHO, 2016c. WHO Chikungunya, Fact Sheet. World Health Organization, Geneva, http://www.who.int/mediacentre/factsheets/fs327/en (accessed 23.12.16).

WHO, 2016d. WHO Cardiovascular Diseases. World Health Organization, Geneva, http://www.who.int/cardiovascular_diseases/en (accessed 23.12.16).

WHO, 2017a. WHO Leishmaniasis. World Health Organization, Geneva, http://www.who.int/mediacentre/factsheets/fs375/en (accessed 02.05.17).

WHO, 2017b. WHO Leishmaniasis, Epidemiological Situation. World Health Organization, Geneva, http://www.who.int/leishmaniasis/burden/en (accessed 01.03.17).

Zakaria, Z.A., Sani, M.H.M., Kadir, A.A., Kek, T.L., Salleh, M.Z., 2016. Antinociceptive effect of semi-purified petroleum ether partition of *Muntingia calabura* leaves. Rev. Bras. Farmacogn. 26, 408–419.

Zhang, J.L., Chen, Z.H., Xu, J., Li, J., Tan, Y.F., Zhou, J.H., Ye, W.X., Tian, H.Y., Jiang, R.W., 2015. New structures, chemotaxonomic significance and COX-2 inhibitory activities of cassane-type diterpenoids from the seeds of *Caesalpinia minax*. RSC Adv 5, 76567–76574.

Zhang, J.M., An, J., 2007. Cytokines, inflammation and pain. Int. Anesthesiol. Clin. 45, 27–37.