Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
23(3): 527-541, May/Jun. 2013

Studies with *Cissampelos sympodialis*: the search towards the scientific validation of a traditional Brazilian medicine used for the treatment of asthma

Aline C. Cavalcanti,[1] Ingrid C. A. R. Melo,[2] Antonilêni F. D. Medeiros,[2] Michelline V. M. Neves,[2] Ayala N. Pereira,[3] Eduardo J. Oliveira*[,1,2,3]

[1]Programa de Pós-graduação em Desenvolvimento e Inovação Tecnológica em Medicamentos, Universidade Federal do Rio Grande do Norte, Brazil,
[2]Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Universidade Federal da Paraíba, Brazil,
[3]Centro de Biotecnologia, Universidade Federal da Paraíba, Brazil.

Abstract: The paper is a review of the literature on the ethnobotanical, chemical and pharmacological aspects of the species *Cissampelos sympodialis* Eichler, Menispermaceae, in order to assess its potential for the treatment of asthma. The aqueous infusion from the leaves of this Brazilian plant is used in the traditional medicine for the treatment of respiratory conditions, including colds, bronchitis and asthma. A multidisciplinary approach has led to the elucidation of the main chemical biomarkers and of the mechanism of action of the extract and its isolated constituents in animal models of inflammation and asthma. A comprehensive review of the literature on the species and its related chemical constituents was conducted using Pubmed, Web of Sciences, Lilacs, SciFinder, as well as conference proceedings. Retrieved literature data demonstrates that the aqueous fraction of the ethanolic extract from the leaves exerts an immunomodulatory activity in different animal models of asthma. This include an increase in the levels of anti-inflammatory cytokines, a decrease in the production of antigen-specific immunoglobulin, a decrease in mucus production and deposition in the airways, and a direct bronchodilator activity. These preclinical results clearly demonstrate the potential of this species for the treatment of asthma and points to the need for well-designed clinical trials to finally validate the traditional use of this herbal medicine.

Review

Received 1 Dec 2012
Accepted 7 Feb 2013
Available online 26 Mar 2013

Keywords:
asthma
bisbenzylisoquinoline alkaloids
Cissampelos sympodialis
warifteine

ISSN 0102-695X
DOI: 10.1590/S0102-695X2013005000029

Introduction

Asthma is a chronic inflammatory condition of the airways characterized by hyper-responsiveness and different degrees of airflow obstruction (Holgate, 2012). Current therapeutic approaches to asthma have arisen from the realization that it is mainly an inflammatory disease associated with immune system imbalance resulting in the production of proinflammatory cytokines, deposition of mucus, smooth muscle proliferation resulting in airway remodeling, and bronchospasm. The current treatment for mild to severe asthma relies on the use of inhaled corticosteroids to prevent acute episodes of asthma (Society, 2012). While effective and with fewer side effects than treatment with systemic corticosteroids, inhaled corticosteroids are not devoid of systemic effects and associated side-effects, especially when used at higher doses (Petrisko et al., 2008). Thus, the search for alternative treatments is a continuing effort that together with a better understanding of the physiopathology of the disease has led to the introduction of new therapy approaches for asthma, such as leukotrine receptor antagonists (LTRA).

Different classes of naturally-occurring substances have proved useful for the treatment of asthma, including theophylline, a phosphodiestarase inhibitor that is still used therapeutically. Other examples include flavonoids (Yang et al., 2012), coumarins (Xiong et al., 2012), anthraquinones (Chu et al., 2012), triterpenes (Lee et al., 2012), triterpene saponins (Yuan et al., 2011), naphtoquinones (Lee et al., 2010), amongst

others. However, most studies on the effects of plants and associated isolated compounds on asthma have limitations regarding focus on a single particular asthma model, use of non-standardized extracts, lack of knowledge on the active constituents or lack of knowledge on the mechanism of action involved. There is also an urgent need for well controlled clinical trials.

The species *Cissampelos sympodialis* Eichler, Menispermaceae, is endemic to Brazil and is distributed mainly within the northeastern states of Paraíba, Ceará, Alagoas, and Bahia. It is popularly known as "milona", "jarrinha", "orelha-de-onça" and "abuteira". Despite its occurrence in many Brazilian states, there are only scarce reports of the use of this plant species for medicinal purposes. In Paraiba, the aqueous infusion of the plant leaves is used for treatment of several different respiratory diseases such as asthma and bronchitis, as well as other inflammatory diseases such as arthritis and rheumatic disorders (Agra et al., 2007; Barbosa Filho et al., 1997).

This paper reviewed the literature on the ethnobotanical, chemical and pharmacological aspects of the species *C. sympodialis* in order to assess the potential of the plant and its constituents for the treatment of asthma. The review was based on the following databases: ISI Web of Science, NLM Medline (Pubmed), Embase, Lilacs, as well as conference proceedings in the area of natural products. The following search terms were used: *"Cissampelos sympodialis*", "milona", "warifteine", "methylwarifteine", "milonine", "roraimine". In addition, a search using Scifinder® on all substances reported to have been isolated from *C. sympodialis* was also conducted. All searches carried no date or other limits.

There is a wealth of preclinical results pointing to a promising role of the aqueous fraction of the ethanol extract of *C. sympodialis* leaves in the treatment of asthma. These results are based on different animal models of asthma, and different modes of administration of the aqueous fraction of the ethanol extract, including the intraperitonial, oral and most importantly, the inhaled route. Taken together the results pave the way for well-designed controlled clinical trials to establish if these preclinical results can be translated to human use in the treatment of asthma.

Botanical and agronomical aspects

The Menispermaceae family consists of seventy genera and around 420 species. Most species in this family are climbing plants and many of them are used in the folk medicine. They are distributed on tropical and sub-tropical regions of all continents and can be found in different types of soil, vegetation and habitat (Jahan et al., 2010). The genus *Cissampelo*s comprises nineteen species, nine of which occur in Brazil (Rhodes, 1975; Jacques & Bertolino, 2008; Stevens, 2012). In the northeastern Brazilian state of Paraíba three species

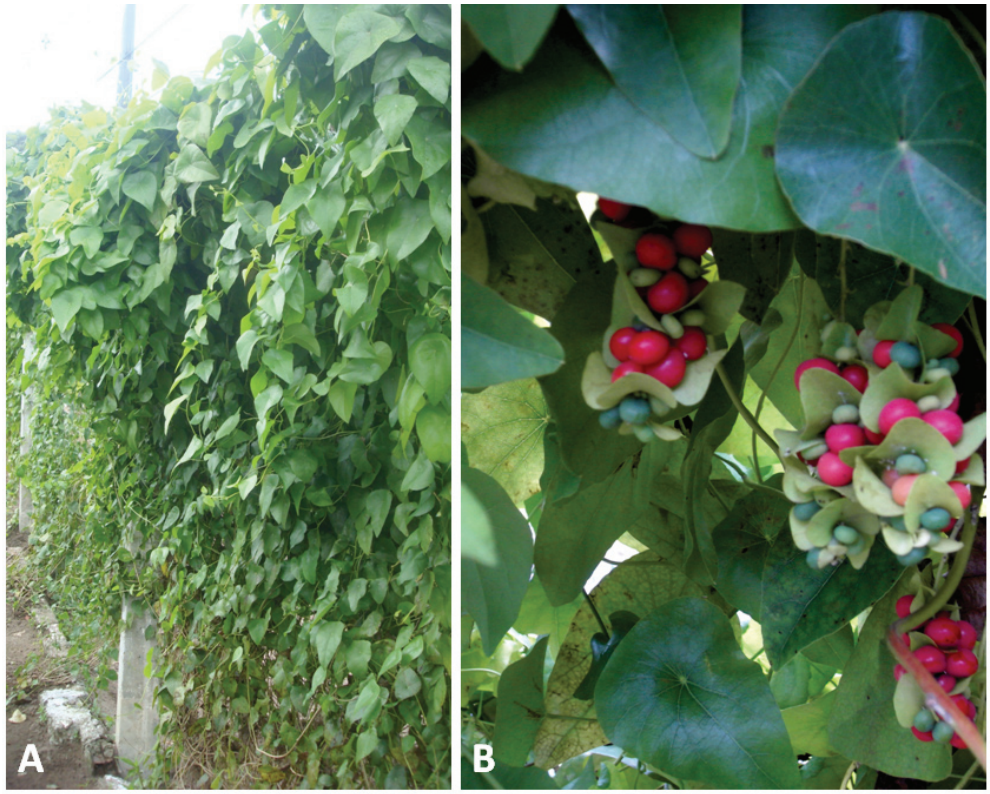


Figure 1. *Cissampelos sympodialis* Eichler: A. Adult plant; B. Detail of deltoids leaves and the ripened fruits (red coloured).

were catalogued: *C. sympodialis, C. glaberrima* and *C. ovalifolia*, which share many morphoanatomical features, but can be distinguished by careful examination of their leaves and by their habitat (Barbosa Filho et al., 1997).

Cissampelos sympodialis is found from the state of Ceará (northeastern Brazil) to the north of Minas Gerais (southeast). It is distinguished mainly by the deltoid shape of its glabrous leaves. Another noteworthy feature is the presence of 3-6 pistillate flowers per fascicle in inflorescence. Its fruit is of drupaceous type, obovate, and from red to orange when ripe (Figure 1) (Eichler, 1864, Rhodes, 1975; Barbosa Filho et al., 1997). Amongst the most important microscopic anatomical characters for the differentiation of *Cissampelos* species are the morphology of the leaf epidermal cells. *C. sympodialis* has epidermal cells with anticlinal walls on the adaxial side and wavy to irregularly sinuous-wave cells in the abaxial leaf side. This, together with the absence of secretory bags serve as diagnostic characters for the species (Porto et al., 2008; 2011). Seed germination occurs in 18 to 25 days, the onset of flowering at around 150 days after planting and the period between flowering and ripening of fruits is approximately 26 days (Fonseca & Figueirêdo, 1999).

Chemistry and bioactive markers

Scientific recognition of many plant species have revealed the presence of phytochemical constituents with important pharmacological activities (Jahan et al., 2010). Menispermaceae species have extensive use in traditional medicine, with twenty-nine of its genera reported to find use for medicinal purposes to treat a variety of diseases all over the world (Barbosa-Filho et al., 2000; De Wet & Van Wyk, 2008).

The species from the family Menispermaceae are marked by the presence of many alkaloid types, especially bisbenzylisoquinoline alkaloids, which are derived from benzyltetrahydroisoquinoline skeleton. Bisbenzylisoquinolinic (BBI) alkaloids are reported to have many pharmacological activities, like acetylcholinesterase (AChE) inhibitors (Ingkaninan et al., 2006; Cometa et al., 2012); antibacterial, antifungal, antiplasmodial, and cytotoxic activities (Lohombo-Ekomba et al., 2004); antinociceptive and anti-arthritic activity (roots of *Cissampelos pareira*) (Amresh et al., 2007); anthelmintic activity (Ayers et al., 2007). Some plant families that are rich in BBI alkaloids and have shown some of the abovementioned activities include species from the families Berberidaceae, Monimiaceae, Ranunculaceae and Lauraceae (Sun et al., 2000).

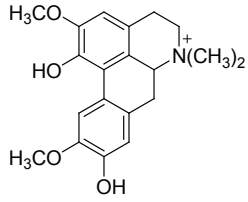
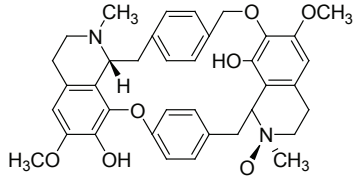
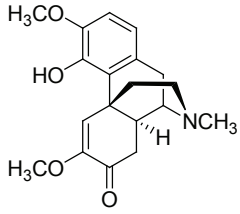
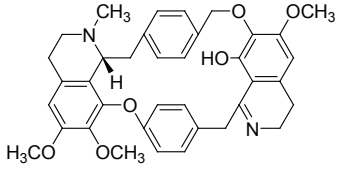
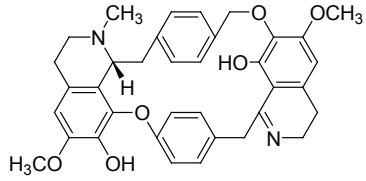
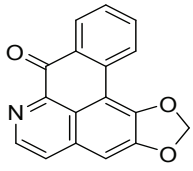
The number of substances that have been isolated over the years from Menispermaceae species is impressive, with 1522 alkaloids isolated between 1970-1997. These alkaloids belong to 23 different structural groups, with bisbenzylisoquinoline alkaloids being the most represented group, followed by aporfinic and protoberberinic alkaloids (Barbosa-Filho et al., 2000).

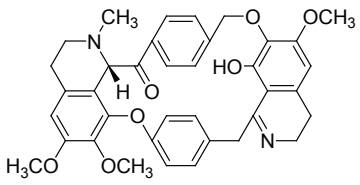
The species of *Cissampelos* that occur in Paraíba are characterized by the presence of isoquinoline-derived alkaloids (Oliveira-Junior et al., 2011). A total of 53 different alkaloids belonging to seven different skeletons are reported in the literature (Barbosa Filho et al., 1997; 2000). The chemical studies with the species *C. sympodialis* has led to the isolation and structural elucidation of the following alkaloids: warifteine (Cortes et al., 1995), methylwarifteine (Barbosa Filho et al., 1997), laurifoline, sympodialine (Alencar, 1994), milonine (Defreitas et al., 1995), roraimine, liriodenine (de Lira et al., 2002), desmethylroraimine (Marinho, 2011) (Chart 1). Methylwarifteine has been shown to be an analog of an alkaloid isolated from *Cissampelos pareira* Linn. cissampereine (Borkakoti & Palmer, 1978).

The main bioactive substance present in *C. sympodialis* leaf ethanol extract identified so far is warifteine, an amorphous yellow BBI alkaloid, melting point 256 °C with decomposition (Aragao et al., 2001), insoluble in polar solvents, but a water soluble salt in acidic conditions (Cerqueira-Lima et al., 2010), that occurs at a concentration of approximately 1% in weight in the concentrated ethanol leaf extract (Thomas et al., 1997b). This substance was first isolated from *Cissampelos ovalifolia* (Gorinsky et al., 1972; Mukherjee & Keifer, 2003). The former studies with *C. ovalifolia* were motivated by its use by South American indians in the preparation of curare. Non-depolarizing neuromuscular blocking activity of warifteine with potency similar to that of tubocurarine and local anesthetic activity was previously described (Gorinsky et al., 1972). Together with warifteine, the other bioactive alkaloids isolated from the ethanol leaf extract of *C. sympodialis* include methylwarifteine, and milonine, a morphinandienone-type alkaloid. These alkaloids are implicated in the pharmacological actions described for the extract of the plant (see the section on "pre-clinical pharmacology" below).

A recent study, aimed at the standardization of the leaf ethanol extract, reported the development of a high performance liquid chromatography method to simultaneously quantify the three main bioactive alkaloid markers in the extract of *C. sympodialis* (Marinho et al., 2012). The developed method was used to study the concentration of warifteine, methylwarifteine and milonine during the vegetative cycle of the plant and the study reported that the concentration of the alkaloids reach a minimum during ripening of the fruits. Since both warifteine and methylwarifteine were found in the extract of the ripened fruits, it seems that the biosynthesis of the alkaloids is diverted from the leaves to the fruits during ripening.

Chart 1. Alkaloids isolated from *Cissampelos sympodialis* Eichler and related pharmacological activities.

Structure/Compound name	Pharmacological activity	Part of the plant
 laurifoline (Alencar, 1994)	Laurifoline considerably inhibited the avian myeloblastosis virus reverse transcriptase, and could be a promising antiretroviral agent (Jucá, 1998)	Leaves of *C. sympodialis*. Also isolated from *Hypserpa nitida*, Menispermaceae (Cheng et al., 2007)
 simpodialine-β-N-oxide (Alencar, 1994)	Not reported	Roots of C. sympodialis
 milonine (Defreitas et al., 1995)	-Hypotensive and vasorelaxant effects in normotensive rats (Cavalcante et al., 2011). -Spasmolitic effect (DeFreitas et al., 1996; Melo et al., 2003) -Cytotoxic effects in culture hepatocytes and V79 fibroblasts (Melo et al., 2003) -May contribute to the anti-allergic effects of *C. sympodialis* extract (Bezerra-Santos et al., 2012a)	Leaves of *C. sympodialis*. Also isolated from *Dehaasia longipedicellaata* (Mukhtar et al., 2004)
 methylwarifteine (Barbosa Filho et al., 1997)	It has shown significant and reproducible inhibitory activity against human carcinoma cells of the nasopharynx in cell culture (Kupchan et al., 1965)	Root, leaves of *C. sympodialis*. Also isolated from *C. ovalifolia* (Borkakoti & Palmer, 1978); *Aristolochia ridicula*, Aristolochiaceae (Machado & Lopes, 2010)
 warifteinne (Barbosa Filho et al., 1997)	-Spasmolytic activity, relaxant effect in both vascular and nonvascular smooth muscle tissues (Cortes et al., 1995; DeFreitas et al., 1996; Melo et al., 2003) -Cytotoxicity in fibroblast cell line (V79); inhibition of the mast cell degranulation and histamine release (Melo et al., 2003). -Antialergic action (Bezerra-Santos et al., 2006) -Selectively inhibits cAMP phosphodiesterase activity (Thomas et al., 1997) Induces muscle relaxation by inhibiting Ca^{++} channels and modifying the intracellular Ca^{++} stores sensitive to noradrenaline (Cortes et al., 1995; DeFreitas, 1994).	Roots, leaves of *C. sympodialis*. Also isolated from *C. ovalifolia* (Borkakoti & Palmer, 1978)
 liriodenine (de Lira et al., 2002)	-Extensive pharmacological activities, such as antitumor, antibacterial, antifungal and trypanocidal activities, as well as anti-Alzheimer's disease (Liu et al., 2011) -Larvicidal activity against the third-instar of *Aedes aegypti* larvae (Feitosa, 2009)	Leaves of *C. sympodialis*. Also isolated from *Annona crassiflora*, Annonaceae (Gonçalves et al., 2009), *Annona foetida*, Annonaceae, (Costa et al., 2011), *Zanthoxylum nitidum*, Rutaceae (Chen et al., 2012), *Rollinia leptopetala*, Annonaceae (Feitosa, 2009)

 roraimine (de Lira et al., 2002)	May contribute to the anti-allergic effects of *C. sympodialis* extract (Bezerra-Santos et al., 2012b)	Roots of *C. sympodialis*

Pre-clinical pharmacology

Toxicological Studies

There are few studies assessing the toxicological effects of *C. simpodialis*, however, research points out to the safety of using the aqueous fraction of the ethanol extract of the leaves (AFL), which is the fraction used in all pharmacological tests with the species.

Acute administration of the ethanol leaf extract at a dose of 5g/kg orally did not cause mortality in mice, rats or dogs. However, changes were observed in liver due to increased activity of the enzyme aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats and γ-glutamyl transferase (GGT) and alkaline phosphatase in dogs. These effects were transient and reversed after discontinuation of treatment (Diniz, 2000).

A subacute study conducted in dogs using an oral dose five times greater than that used in folk medicine (45 mg/kg/dia), demonstrated that AFL did not induce any significant effect on hematological and biochemical parameters. Thus, it was concluded that AFL is devoid of toxic effect, although these results must be confirmed by histological studies, evaluation of its effect on fertility and pregnancy and adverse effects such as teratogenicity and carcinogenicity (Diniz et al., 2002).

A subsequent study has investigated the safety of the AFL of *C. sympodialis* at therapeutic oral doses (9.0, 45.0 and 225.0 mg/kg) during the entire period of pregnancy in female rats. The offspring showed no malformations and no interference on the weight gain of the developing litter was observed, which reflects the absence of adverse effects during pregnancy (Maior et al., 2003).

In a separate study, an anorexic effect of AFL was observed in rats. Thus, chronic administration of AFL in female rats, at doses of 45 and 225 mg/kg, produced a reduction of food consumption and a loss of body weight similar to the effects observed by metamphetamine and mazindol, in a sex-dependent manner, in addition to observed behavioral changes such as significant decrease (p<0.05) on the locomotion frequency, rearing, grooming and defecation. The observed anorexic effect of AFL was attributed to milonine, a morphinadienone aklaloid present in the leaves, but no in the roots of *C. sympodialis* (Almeida et al., 2005).

A preliminary clinical toxicology testing with an aqueous infusion of the leaves of *C. sympodialis*, evaluated the stimulating effect of the species on the neuromotor Hoffmann's reflex in the soleus muscle of adult volunteers. One group received the infusion in a concentration of 1% of the dried leaves of milona and another group a placebo infusion. Thus, intake of the leaf tea a concentration of 10 mg/mL, did not affect the Hoffmann reflex, suggesting that neuromotor activity is not affected (Souza et al., 2000). However, independent, blinded evaluation of the groups is not reported in the study.

A preliminary phase I clinical trial of a syrup of *C. sympodialis* leaves was conducted using 35 healthy volunteers (21 males and 14 females), who were administered 10 mL of syrup at 5.4% (weight/volume of crude leaf ethanol extract) for 30 days and accompanied by clinical examination, electrocardiogram (ECG) and laboratory tests. The syrup proved to be well tolerated and of low toxicity, but lacked proper standardization and quality control (Amaral et al., 2002; Gambarra et al., 2002).

The cytotoxicity of the isolated alkaloids warifteine and milonine was evaluated in primary cultures of rat hepatocytes and fibroblasts from chinese hamster lung (V79 cells). In the viability study of hepatocytes, warifteine showed toxicity (IC50 around 10 mM) similar to the cytotoxic effects determined in the V79 cells. However, milonine was less toxic than warifteine in hepatocytes and V79 cells, also showing similar cytotoxic effects in both cell cultures analyzed. Cimetidine (a cytochrome P450 inhibitor) was unable to protect the cells from the action of warifteine or milonine at the concentration used, which means that toxic effects induced by these compounds are probably independent of the cytochrome P450 system (Melo et al., 2003).

Although the results demonstrated a cytotoxic effect for the alkaloids isolated from *C. sympodialis,* this toxicity is not reproduced during the administration of AFL in the acute and subacute studies in rats and dogs previously mentioned. It is worth mentioning that the alkaloids milonine, warifteine and methylwariteine are found in small amounts in the leaf ethanolic extract, with warifteine occuring at 1% (in weight) in the concentrated extract. However, the concentration of these alkaloids

in the aqueous fraction of the ethanol extract (AFL) is anticipated to be much lower due to their small aqueous solubility. Despite indications of low toxicity, definite chronic toxicological studies are lacking and should be conducted with the AFL at the therapeutic dose intended for human use.

Activity on smooth muscle

One of the earliest reported pharmacological activities of the plant is its action on the tone of smooth muscle. The direct activity of AFL and warifteine on smooth muscle tonus is thought to contribute with the activity of the plant seen on experimental models of asthma. Thus, the extract preparation of *C. sympodialis* is able to relax several smooth muscle tissues with equipotent activity between aqueous fraction of the ethanolic extract of the root (AFR) and leaf (AFL) (DeFreitas, 1994). The AFL also has a relaxing effect in tracheal smooth muscle (Thomas et al., 1997b), reducing in a dose dependent manner the spontaneous tone and relaxing tracheal strips precontracted with capsaicin, carbachol and arachidonic acid. The aqueous fraction of the ethanol leaf extract on trachea is equipotent to aminophylline and seems to involve the intracellular increase in the concentration of cyclic adenosine monophosphate (cAMP), as observed in guinea-pig alveolar macrophages (Thomas et al., 1995). AFL was also able to inhibit both the histamine bronchospasm response to ovalbumim (OVA) in sensitized guinea pigs and the activity of cyclic nucleotide phosphodiesterase (PDE) enzymes IV and V in cultured tracheal muscle (Thomas et al., 1997a). Even though it was reported that warifteine has local anaesthetic activity (Gorinsky et al., 1972) and generally anaesthetic substances contribute to smooth muscle relaxation, this is not the case with warifteine. The effect of warifteine, the plant major alkaloid on smooth muscle has been investigated in detail. Warifteine has smooth muscle relaxing properties, acting both in vascular and nonvascular tissues (Cortes et al., 1995; DeFreitas et al., 1996). The mechanism of the spasmolytic effects of warifteine involves the inhibition of calcium channels and interference with noradrenalin sensitive intracellular calcium stores. The alkaloid is able to inhibit voltage-dependent calcium channels in KCl-induced contractions; also, it inhibits receptor-operated calcium channels, and calcium release from intracellular stores such as those sensitive to IP3 and rhyanodine, showing that there are many ways that warifteine relaxes smooth muscle through calcium-regulatory processes (DeFreitas et al., 1996). Another alkaloid from *C. sympodialis*, milonine, has a vasorelaxant action partly mediated by endothelium, activation of nitric oxide-cGMP pathway and the opening of K+ channels, being able to lower arterial pressure in normotensive rats (Cavalcante et al., 2011). It was shown that AFL induces contraction in rat aortic rings modulated by nitric oxide and cyclooxigenase-derived relaxing factor, which may contribute to the mild hypertensive effect of the plant in normotensive rats, an effect that points to careful usage of the extract in hypertensive individuals (Freitas et al., 2000).

Anti-inflammatory activity

Since asthma can be considered a chronic inflammatory condition, it is important for its treatment that drugs act to control the release and actions of inflammatory mediators. It is widely recognized that there is no experimental model of inflammation that covers all its aspects. The usual methods to determining whether compounds have anti-inflammatory activity are through tests with animal and biochemical models of inflammation (Lewis, 1989; Barbosa-Filho et al., 2006).

The aqueous fraction of the ethanolic extract obtained from the leaves (AFL) of *Cissampelos sympodialis* was evaluated for anti-inflammatory activity and results showed that in mice, the AFL (100 mg/kg, *i.p.*) inhibited both the 12-*O*-tetradecanoylphorbol 13-acetate and capsaicin- induced ear edema by 58 and 37%, respectively. The effective dose of AFL to inhibit the carrageenan-induced rat paw edema was 50 mg/kg (24%). Preliminary results of experiments on cell migration showed that the administration (by subcutaneous route) of AFL at 100 and 200 mg/kg in rats inhibited the carrageenan-induced neutrophil migration measurement by 53 and 50%, respectively after the administration of the irritant (Barbosa-Filho et al., 2006).

To broaden the knowledge on the suitability to use *C. sympodialis* for the treatment of allergic diseases the effect of the intraperitoneal treatment with AFL in ovalbumin-sensitized BALB/c mice was evaluated. The immunological aspects investigated were the anaphylactic shock reaction induced by ovalbumin or compound 48/80, immunoglobulin E production and cell proliferative response. Data showed that sub-acute AFL-treatment inhibited the animal rate mortality by OVA challenge by 50% (200 mg/kg *i.p.*) and 30% (400 mg/kg, *i*.p.), suggesting that the intraperitoneal AFL administration may have an anti-allergic activity through the decrease of IgE production. However AFL-treatment did not inhibit the anaphylactic shock reaction induced by compound 48/80 injection, although the mechanism involved in this anaphylactic response is not receptor dependent unlike IgE-mediated responses (Bezerra-Santos et al., 2005).

Another species of Menispermaceae, *Abuta grandifolia*, also showed anti-inflammatory activity on the carrageenan-induced paw edema model in rats when administered intraperitoneally (Lima et al., 2011).

Immunomodulatory activity

Immunomodulation using medicinal plants can be an alternative to conventional chemotherapy for several diseases, including asthma. The immune response requires multiple interactions between cell types in order to maintain immune homeostasis and many plant derived materials have been demonstrated to be stimulators of the immune system (Kumar et al., 2012).

Neutrophil recruitment to the inflammatory focus is a complex process, highly orchestrated by a huge array of inflammatory mediators (Hebeda et al., 2011). Since neutrophils are key cells in allergy and inflammation by releasing newly synthesized and pre-stored proinflammatory mediators, the role of this cell type in the effects of AFL was investigated. The aqueous fraction of ethanol leaf extract (30-100 mg/mL) was able to inhibit degranulation of cells stimulated by formyl-Met.Phe.Pro, increase the levels of cAMP, and the activity of cAMP-dependent protein kinase A. The inhibition of neutrophil degranulation was greater when the cells were pretreated with the phosphodiesterase (PDE) inhibitor isobutyl methyl xanthine (IBMX) and AFL had a similar effect on inhibition of lysozyme release by dibutyryl cAMP (db-cAMP) and myeloperoxidase release by PGE2, suggesting that the fraction may act by elevating intracellular cAMP levels (Thomas et al., 1999). Considering the deleterious action of neutrophils to the inflamed tissue in case of non-controlled inflammation, blockade of that step is an important therapeutic strategy (Pease & Sabroe, 2002; Yusuf-Makagiansa et al., 2002).

Allergic asthma is associated particularly with a polarized type-2 response modulated by IL-4 and IL-5, and involving up-regulation of IL-10. The up-regulation of IL-10 production during allergic responses may serve to control tissue inflammation that is characterized by leukocytic infiltration. Cytokines, a large group of soluble extracellular proteins or glycoproteins, are key intercellular regulators and mobilizers and they are now seen to be crucial to innate and adaptive inflammatory responses (Oppenheim, 2001; Spelman et al., 2006). Thus, a study tested the hypothesis that the therapeutic effect of the AFL obtained from *C. sympodialis* may be related to a differential control in cytokine secretion. The effect of the aqueous fraction of ethanol leaf extract on cytokine production induced by Con-A in BALB/c spleen cells was investigated, and the results showed that the extract (6.25 to 50 µg/mL) inhibits the proliferative responses of murine spleen cells to Concanavalin-A without inhibiting the production of cytokines by the cells. Additionaly, AFL induces an increase in IL-4 and IL-10 levels, both of these cytokines being potent anti-inflammatory molecules that act by inhibiting synthesis of pro-inflammatory cytokines. The study also found a small decrease in IL-2 secretion despite the major inhibition of cellular proliferation and it is suggested that increased IL-10 production downregulates IFN-gamma secretion and T cell proliferative responses (Piuvezam et al., 1999). In addition to the inhibitory action of IL-10 on most in vitro studies, other studies suggest that recombinant IL-10 might prove useful for the prevention and treatment of certain inflammatory processes *in vivo* (Gérard et al., 1993; Howard et al., 1993; Donckier et al., 1994).

Besides its effect on macrophage function, IL-10 is also an important regulator of immune and inflammatory function (Abrahamsohn, 1998) and a study investigated the effect of AFL from *C. sympodialis* on macrophage function, an additional aspect of the inflammatory response. This was done by studying the effects of AFL on macrophages after *in vitro* infection with the protozoan *Trypanosoma cruzi* DM28c clone. The outcome of *T. cruzi* interaction with macrophages may depend on the activation state of the macrophage (Golden & Tarleton, 1991). The authors observed that AFL (13 to 100 µg/mL) increased *T. cruzi* growth by a 75% reduction in nitric oxide production and this inhibition could be mediated by the stimulation of macrophage interleukin-10 (IL-10) secretion. These results indicate that one of the mechanisms by which AFL exerts an anti-inflammatory effect could be related to a decrease in macrophage activity (Alexandre-Moreira et al., 2003a).

To better characterize the effect of *C. sympodialis* extract on physiological processes relevant to the pathophysiology of asthma, it was important to obtain more detailed knowledge about the modulation of immunological function by the extract. It was observed that AFL inhibited the *in vitro* proliferative response of resting B cells induced by lipopolysaccharide LPS with an IC50 of 17.2 µg/mL, anti-delta-dextran (IC50 13.9 µg/mL) and anti-IgM (IC50 24.3 µg/mL) but did not affect the anti-MHC class II antibody-stimulated proliferative response of B cell blasts obtained by stimulation with IL-4 and anti-IgM. Incubation with the aqueous fraction of ethanol extract used at 50 µg/mL induced an increase in intracellular cAMP levels and IgM secretion by resting B cells and polyclonally activated B cells was inhibited. It was concluded that even activated cells could be inhibited by AFL, depending on the nature of the B cell activator used. This inhibitory effect on B cells could be an additional mechanism for the anti-inflammatory effect of this extract (Alexandre-Moreira et al., 2003b).

The action of the isolated alkaloid warifteine on B lymphocyte function was also studied and the results suggested that it is a potent inhibitor of B cell response both *in vitro* and *in vivo* and that this effect may be due to the induction of increased intracellular cAMP levels, suggesting that this substance may be useful as a modulator of B cell function (Rocha et al., 2010).

Activity on animal models of asthma

Animal models of asthma have been used for over 100 years (Karol, 1994) and there are numerous examples of processes and mediators that have been identified in animal models and are now known to be critical in the development and progression of disease in humans (Zosky & Sly, 2007).

A study in 1995 was the first to suggest a pharmacological basis for the use of the plant in asthma demonstrating that the aqueous fraction of the ethanol extract of the root of *C. sympodialis* reduced the spontaneous tone of trachea, inhibited the contractions induced by submaximal concentrations of carbachol, histamine, prostaglandin, and substance P in guinea-pig tracheal preparations and also increased the intracellular levels of cyclic adenosine monophosphate in guinea-pig bronchoalveolar leucocytes (Thomas et al., 1995).

Based on the spasmolitic activity of the root and leaf extract of *C. sympodialis* and as the leaves are an easily renewable source on which no studies have been reported, the bronchodilator activity of the aqueous fraction of the ethanolic extract of the leaves (AFL) was investigated in the guinea-pig through an in vivo study investigating its ability to protect guinea-pigs against convulsions when exposed to aerosols of histamine in normal animals, or of ovalbumin in sensitized animals, respectively. Thus AFL (100 mg/kg), more effectively by *i.p*. than by *i.g*. route, increased the preconvulsive time (PCT) in the guinea-pigs exposed to an aerosol of histamine to 63.5±5 s 1 h after administration compared to 28±1 s in the untreated group. However it failed to protect the animals against convulsions. These results further suggest that this extract has no specific histamine receptor antagonist property. To the contrary, in the ovalbumin-induced anaphylactic shock in sensitized animals, the extract (100 mg/kg) *i.p.* or *i.g.* not only prolonged the preconvulsion time but also protected the animals against shock during the maximum period of 12 min of challenge with allergen. These positive results obtained in this model reinforced the promising therapeutic potential of the plant observed in previous studies (Thomas et al., 1997a).

Guinea-pigs are useful as models of immediate hypersensitivity to irritants and have pharmacological responses similar to humans (Muccitelli et al., 1987), although care must be taken when extrapolating this to all pathways in the human lung. Other animal models use mice, which are easily sensitized to a number of antigens, to which they are not normally exposed, including ovalbumin (OVA), which is the most popular, and a number of recognized human allergens (Zosky et al., 2004).

The preliminary results with *C. sympodialis* AFL on allergy were expanded by a study of the effects of AFL oral treatment (*p.o*.) in ovalbumin-sensitized mice showing that the extract (200; 400; 600 mg/kg) inhibited both total IgE and OVA-specific IgE production in sera from OVA-sensitized mice. The treatment also decreased OVA-induced paw edema and increased the production of IFN-gamma suggesting AFL may have anti-allergic activity through the decrease of IgE production. This study was crucial towards the scientific validation of the popular use of *C. sympodialis* for the treatment of asthma (Bezerra-Santos et al., 2004).

Another important mediator of inflammatory responses in asthma is the eosinophil. These cells have a critical role in the pathogenesis of allergic inflammatory conditions, like asthma and at present, the most effective pharmacological approach for severe eosinophil-associated reactions is glucocorticoid therapy (Barnes, 1995).

Cissampelos sympodialis AFL as well as its isolated alkaloid warifteine were evaluated in two models of allergic inflammation (asthma and pleurisy models) in actively sensitized mice (Bezerra-Santos et al., 2006). Oral pre-treatment with *C. sympodialis* (4; 40; 400 mg/kg) was able to inhibit, in a dose-dependent manner, both bronchoalveolar lavage (BAL) eosinophil and its influx to the pleura caused by allergic challenge in actively sensitized animals. A pre-treatment with *C. sympodialis* AFL also inhibited the secretion of cysteinyl leukotrienes and the formation of new cytoplasmic lipid bodies within recruited eosinophils during allergic reaction. The mechanism of *C. sympodialis* driven inhibitory effect does not involve a direct effect on the locomotory functions of eosinophils, but the control of the production of eosinophilotactic mediators during allergic inflammation. Similar effects were observed by pre-treatment with warifteine (50 µg/animal). This alkaloid was able to reduce both eosinophil influx and the pleural levels of cysteinyl leukotrienes observed 24 h after allergic challenge. Similarly, pre-treatment with warifteine also reduced BAL eosinophil influx, production of cysteinyl leukotrienes and lipid body formation observed in the asthma model. Similarly to the pre-treatment, oral post-treatment with either *C. sympodialis* extract or warifteine 1 h after the last OVA challenge was able to significantly inhibit eosinophil influx observed 24 h in both models of allergic inflammation. These post-treatments also inhibited the production of cysLT caused by allergic challenge. Taken together these effects demonstrate the potential of *C. sympodialis* to interfere in the pathophysiology of asthma and inflammatory processes as well as establishes warifteine as an important bioactive marker for *C. sympodialis*. AFL fraction (Bezerra-Santos et al., 2006). Since a phytochemical marker is always preferablly the active substance, warifteine is thus central for plant identification, quality control, planning and monitoring of the technological transformation and stability studies (Maciel et al., 2002; Braga et al., 2003;

Toledo et al., 2003).

The mast cell exocytose, after cross-linking between allergen and its high affinity IgE receptors, delivers an array of inflammatory mediators. The major sources of mast cells are the skin, gastrointestinal tract and lung, which may explain the clinical symptoms of immediate allergic reactions such as dermatitis, food allergy and asthma (Galli et al., 2005) .The effect of warifteine isolated from *C. sympodialis* was investigated on the allergic response in a murine model of asthma, its IgE-dependent mechanisms, OVA-specific cell proliferation and NO production in peritoneal macrophages. The *in vivo* treatment with warifteine (0.4-10 mg/kg) inhibited OVA-stimulated spleen cell proliferative responses and also decreased the paw withdrawal latency in both IgE sensitized and histamine/5HT-challenged rats. Warifteine was capable to downregulate allergen-evoked histamine release from isolated peritoneal rat mast cells, suggesting that the protective antiallergic effects raised by warifteine may result, at least in part, from its ability to stabilize mast cells. The antihyperalgesic (4.0 mg/kg) and anti-edema effects of warifteine suggest a decrease of the inflammatory process by interfering with NO production and also in the pre-stored and neo-generated mediators. These findings suggest that warifteine may represent a new strategy to improve the therapeutic arsenal available for controlling immediate allergic reactions, like asthma (Costa et al., 2008).

Cissampelos sympodialis AFL and warifteine were also evaluated in an experimental model of respiratory allergy to an extract of the dust mite *Blomia tropicalis* (BtE), a model that is considered to resemble more closely human asthma than OVA-based models (Baqueiro et al., 2010). In the study (Cerqueira-Lima et al., 2010) *Cissampelos sympodialis* AFL (400 mg/kg/ *i.g.*) effectively reduced BtE-induced allergy. The parameters analyzed were: specific IgE production, cell migration and histopathological alterations in the lungs, peroxidase (EPO) production in bronchoalveolar lavage fluid (BAL) and cytokine production. The treatment of the BtE-sensitized mice with AFL decreased the IgE titers, although the decrease was not statistically significant. No difference was found in macrophages, neutrophils and lymphocytes counts among treated and untreated groups by the differential cell counts in the BAF, but a significant increase in eosinophil numbers was found. The treatment also reduced the EPO activity found in the BAL of BtE-sensitized mice, a fact that may reflect the inhibition of eosinophil migration to the lung and promoted a significant increase in the production of the regulatory cytokine IL-10. The induction of this cytokine could be the major effect mediating the anti-allergic activity, by modulating cell influx and degranulation. The principal function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. In addition to these activities, IL-10 regulates growth and/or differentiation of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. The AFL-induced production of IL-10 was attributed to the suppression of IL-5 production. These activities are correlated to the immunomodulatory and anti-inflammatory properties of the isolated alkaloid warifteine (tested at 4 mg/kg/ *i.g.*) but it is probable that the effect of warifteine may show an addictive effect with other components of the extract to account for the potency of the AFL fraction (Cerqueira-Lima et al., 2010). This hypothesis cannot be definitely ruled out because not all *C. sympodialis* isolated compounds were tested and because in general plants usually have analogs with similar effects either sharing the same mechanism of action or by different mechanisms (Yunes et al., 2001).

A recent study (Bezerra-Santos et al., 2012b) evaluated the effectiveness of *C. sympodialis* on airway hyperreactivity, collagen fibers and mucus production. For this purpose, either the standardized hydroalcoholic extract of *C. sympodialis* leaves (0.95% in weight of warifteine), as well as warifteine were tested in OVA-induced lung inflammation in actively sensitized mice. The OVA-sensitized and challenged mice pre-treated i.g. with either *C. sympodialis* extract (40 mg/kg) or warifteine (2 mg/kg) showed a significant reduction in IL-13 levels, comparable to dexamethasone (2 mg/kg) treated animals. Similarly to the pre-treatment, oral post-treatment with warifteine but not with *C. sympodialis* extract was able to inhibit airway hyperreactivity in a significant manner. In addition, post-treatment with warifteine reduced IL-13 broncoalveolar levels detected 6 h after the last OVA challenge in the asthma model. These data indicate that *C. sympodialis*, mostly through the active compound warifteine, is capable to ameliorate an established inflammatory allergic reaction. Oral pre- or post-treatment with *C. sympodialis* or pre-treatment with warifteine reduced OVA-induced mucus accumulation and the percentage of mucus producing cells to values similar to nonallergic controls. Oral pretreatment with *C. sympodialis* or warifteine significantly inhibited OVA-induced eosinophil tissue infiltration similar to pre-treatment with dexamethasone. This data corroborate the folk anti-allergic medicine use of *C. sympodialis* and indicate that the anti-allergic and immunoregulatory properties of *C. sympodialis* occur mostly through the active compound warifteine, to inhibit the airway hyperreactivity and lung remodeling through a mechanism at least partially dependent of IL-13 and eosinophil inhibition (Bezerra-Santos et al., 2012b).

Other pharmacological actions

Pharmacological activities other than those

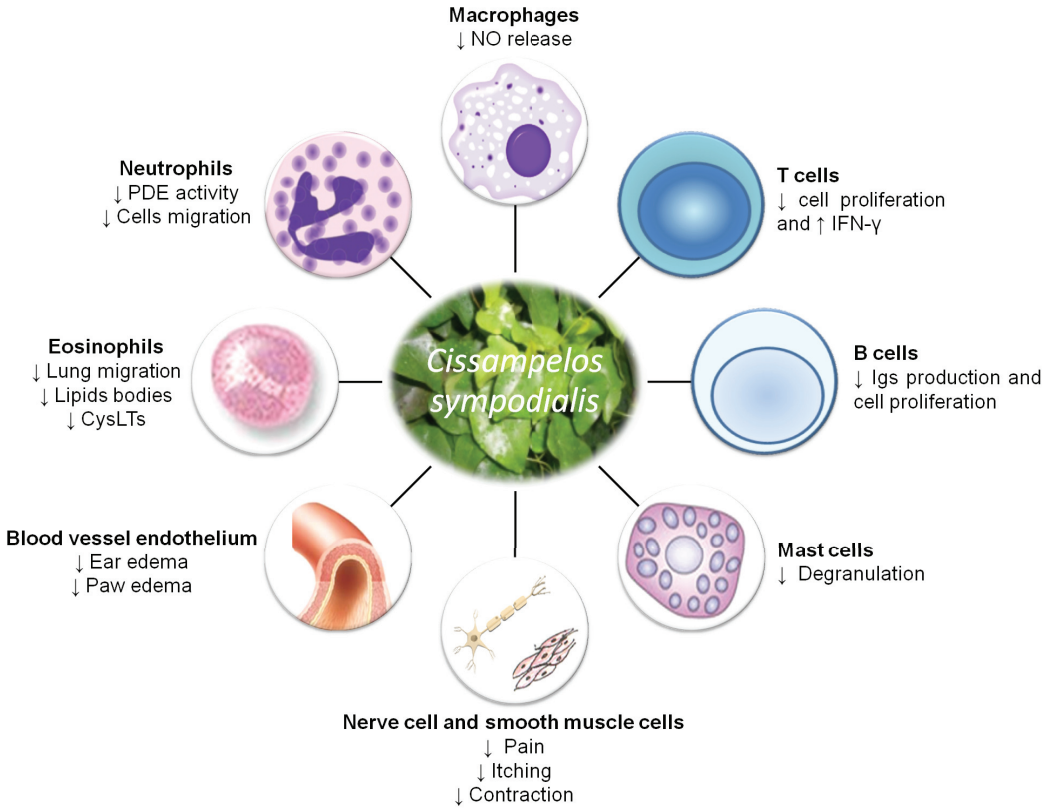


Figure 2. Summary of therapeutic targets for *Cissampelos sympodialis* Eichl. Adapted from (Bezerra-Santos et al., 2012a).

directly related to asthma can be attributed to the mechanisms previously described for *C. sympodialis* extracts and isolated compounds. These activities include antidepressant and CNS stimulant action of *C. sympodialis* extract, possibly related to the inhibition of phosphodiesterase activity (Almeida et al., 1998; Mendonca-Netto et al., 2008). Cardiovascular effects common to phosphodiesterase inhibitors of the methylxanthine class (Knight & Yan, 2012) can also be observed with *C. sympodialis*, such as reversible hypertension as demonstrated in conscious freely-moving rats (Medeiros et al., 1998). In addition, the immunomodulatory activity of AFL led to the suggestion that it can be useful for the treatment of psoriais (Feily & Namazi, 2009). Other activities not directly related to asthma models reported for *C. sympodialis* extracts include antinociceptive (Oliveira-Junior et al., 2011) action similar to the one demonstrated by the hydroalcoholic extract of the roots of *Cissampelos pareira* (Amresh et al., 2007). Finally, warifteine has also demonstrated activity against promastigotes of *Leishmania* (L.) *chagasi* (Silva et al., 2012), thus confirming the studies demonstrating antiparasitic activity against *Leishmania* sp. (Fournet et al., 1993), *Trypanosoma cruzi* (de Arias et al., 1994) and *Plasmodium* sp. (Angerhofer et al., 1999; Wright et al., 2000) for bisbenzilisoquinolinic alkaloids.

Conclusion

The preclinical results reviewed in this paper clearly demonstrate the potential of AFL to interfere with many important targets related to the asthma pathophysiology process. First, there is a direct bronchodilator effect which is at least partly mediated by warifteine acting on voltage-dependend calcium channels and intracellular calcium stores. The anti-inflammatory effect of AFL and warifteine was demonstrated against a variety of flogistic agents and this effect is mediated by its interference with nitric oxide (NO) production by macrophages. The immunomodulatory and anti-allergic effects of AFL and warifteine involve several cell types that participate in the asthmatic process. These effects include: decrease in neutrophil migration and degranulation mediated by phosphodiesterase (PDE) inhibition , decrease in eosinophil infiltration into the lungs mediated by inhibition of the production of chemoattractant cytokines (e.g. eotaxin), decrease in allergen-specific immunoglobulin and cell proliferation by B lymphocytes, increase of production of anti-inflammatory cytokines such as IL-10 and IFN-γ, decrease in both mast cell degranulation and histamine secretion, decrease in mucus production and the

decrease of *cis*-leukotriene (CysLTs) and lipid bodies accumulation by eosinophiles. Figure 2 summarizes the main targets through which the aqueous fraction of the ethanol extract of leaves (AFL) of *Cissampelos sympodialis* exerts its effects on asthma. These effects show that *Cyssampelos sympodialis* AFL exert multiple and synergistic effects in order to decrease the hallmark signs of asthma: bronchospasm, cell migration, mucus accumulation, inflammation and airway remodeling. Although preclinical results do not warrant clinical efficacy, taken together the results clearly point to the urgent need to conducted well-controlled clinical trials with standardized AFL in humans.

Acknowledgment

The authors would like to thank CNPq and CAPES for funding.

Authors contributiuons

ACC (PhD student) contributed with data collection, selection of papers, and writing the section related to experimental asthma models and inflammation. ICARM (PhD student) contributed with data collection, selection of papers and writing the section related to toxicological, botanical and agronomical aspects. AFDM (PhD student) contributed with data collection, selection of papers and writing the section related to chemical composition and smooth muscle pharmacology. MVMN (PhD student) and ANP (undergraduate student) contributed with data collection and formatting of the manuscript. EJO (research supervisor) suggested the manuscript outline, participated in the data collection, writing of the abstract and introduction of the manuscript, selection of papers, and final editing of the manuscript.

References

Abrahamsohn IA 1998. Cytokines in innate and acquired immunity to *Trypanosoma cruzi* infection. *Braz J Med Biol Res 31*: 117-121.

Agra MF, Freitas PF, Barbosa-Filho JM 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Rev Bras Farmacogn 17*: 114-140.

Alencar JL 1994. Isolamento e estudos das atividades relaxantes em musculatura lisa e esquelética de novos alcalóides de *Cissampelos sympodialis* Eichl. Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos. In: *Laboratório de Tecnologia Farmacêutica*. Universidade Federal da Paraíba, Brazil.

Alexandre-Moreira MS, Freire-de-Lima CG, Trindade MN, Castro-Faria-Neto HC, Piuvezam MR, Pecanha LMT 2003a. *Cissampelos sympodialis* Eichl (Menispermaceae) leaf extract induces interleukin-10-dependent inhibition of *Trypanosoma cruzi* killing by macrophages. *Braz J Med Biol Res 36*: 199-205.

Alexandre-Moreira MS, Piuvezam MR, Pecanha LMT 2003b. Modulation of B lymphocyte function by an aqueous fraction of the ethanol extract of *Cissampelos sympodialis* Eichl (Menispermaceae). *Braz J Med Biol Res 36*: 1511-1522.

Almeida RN, Melo-Diniz MFF, Medeiros IA, Quintans LJ, Navarro DS, Falcao A, Duarte JC, Barbosa JM 2005. Anorectic and behavioural effects of chronic *Cissampelos sympodialis* treatment in female and male rats. *Phytother Res 19*: 121-124.

Almeida RN, Navarro DS, de Assis TS, de Medeiros IA, Thomas G 1998. Antidepressant effect of an ethanolic extract of the leaves of *Cissampelos sympodialis* in rats and mice. *J Ethnopharmacol 63*: 247-252.

Amaral SM, Diniz MFFM, Medeiros IA, Santos HB 2002. Ensaios toxicológicos clínicos com as folhas de *Cissampelos sympodialis* Eichl (do ponto de vista médico). In: *X Encontro de Iniciação Científica da UFPB*, pp. 100. Universidade Federal da Paraíba, João Pessoa - PB.

Amresh G, Singh PN, Rao CV 2007. Antinociceptive and antiarthritic activity of *Cissampelos pareira* roots. *J Ethnopharmacol 111*: 531-536.

Angerhofer CK, Guinaudeau H, Wongpanich V, Pezzuto JM, Cordell GA 1999. Antiplasmodial and cytotoxic activity of natural bisbenzylisoquinoline alkaloids. *J Nat Prod 62*: 59-66.

Aragao CFS, Filho JMB, Macedo RO 2001. Thermal characterization of warifteine by means of TG and a DSC photovisual system. *J Therm Anal Calorim 64*: 185-191.

Ayers S, Zink DL, Mohn K, Powell JS, Brown CM, Murphy T, Brand R, Pretorius S, Stevenson D, Thompson D, Singh SB 2007. Anthelmintic activity of aporphine alkaloids from *Cissampelos capensis*. *Planta Med 73*: 296-297.

Baqueiro T, Russo M, Silva VM, Meirelles T, Oliveira PR, Gomes E, Barboza R, Cerqueira-Lima AT, Figueiredo CA, Pontes-de-Carvalho L, Alcântara-Neves NM 2010. Respiratory allergy to *Blomia tropicalis*: immune response in four syngeneic mouse strains and assessment of a low allergen-dose, short-term experimental model. *Resp Res 11*: 51-61.

Barbosa-Filho JM, da Cunha EVL, Gray AI 2000. *Alkaloids of the Menispermaceae*. (vol. 54). Illinois: Academic Press.

Barbosa-Filho JM, Piuvezam MR, Moura MD, Silva MS, Lima KVB, da-Cunha EVL, Fechine IM, Takemura OS 2006. Anti-inflammatory activity of alkaloids: a twenty-century review. *Rev Bras Farmacogn 16*: 109-139.

Barbosa Filho JM, Agra MDF, Thomas G 1997. Botanical, chemical and pharmacological investigation on *Cissampelos* species from Paraíba (Brazil). *Journal Braz Assoc Advanc Sci 49*: 386-394.

Barnes PJ 1995. Inhaled glucocorticoids for asthma. *N Engl J Med 332*: 868-875.

Bezerra-Santos CR, Balestieri FMP, Rossi-Bergmann B, Pecanha LMT, Piuvezam MR 2004. *Cissampelos sympodialis* Eichl. (Menispermaceae): oral treatment decreases IgE levels and induces a Th1-skewed cytokine production in ovalbumin-sensitized mice. *J Ethnopharmacol 95*: 191-197.

Bezerra-Santos CR, Peçanha LMT, Piuvezam MR 2005. *Cissampelos sympodialis* Eichl. (Menispermaceae) inhibits anaphylactic shock reaction in murine allergic model. *Rev Bras Farmacogn 15*: 287-291.

Bezerra-Santos CR, Vieira-de-Abreu A, Barbosa JM, Bandeira-Melo C, Piuvezam MR, Bozza PT 2006. Anti-allergic properties of *Cissampelos sympodialis* and its isolated alkaloid warifteine. *Int Immunopharmacol 6*: 1152-1160.

Bezerra-Santos CR, Vieira-de-Abreu A, Vieira GC, Filho JR, Barbosa-Filho JM, Pires AL, Martins MA, Souza HS, Bandeira-Melo C, Bozza PT, Piuvezam MR 2012a. Effectiveness of *Cissampelos sympodialis* and its isolated alkaloid warifteine in airway hyperreactivity and lung remodeling in a mouse model of asthma. *Int Immunopharmacol 13*: 148-155.

Bezerra-Santos CR, Vieira-de-Abreu A, Vieira GC, Filho JR, Barbosa JM, Pires AL, Martins MA, Souza HS, Bandeira-Melo C, Bozza PT, Piuvezam MR 2012b. Effectiveness of *Cissampelos sympodialis* and its isolated alkaloid warifteine in airway hyperreactivity and lung remodeling in a mouse model of asthma. *Int Immunopharmacol 13*: 148-155.

Borkakoti N, Palmer RA 1978. The structure of the bisbenzylisoquinoline alkaloid dimethylwarifteine. *Acta Crystallogr Sect B 34*: 482-489.

Braga FC, Valadares YM, Costa MA, Lombardi JA, Oliveira ABD 2003. Estudo fitoquímico de *Erythraea centaurium, Jacaranda caroba, Remijia ferruginea* e *Solanum paniculatum* visando identificar marcadores químicos para o fitoterápico Ierobina®. *Rev Bras Farmacogn 13*: 28-32.

Cavalcante HMM, Ribeiro TP, Silva DF, Nunes XP, Barbosa-Filho JM, Diniz MFFM, Correia NA, Braga VA, Medeiros IA 2011. Cardiovascular effects elicited by milonine, a new 8,14-dihydromorphinandienone alkaloid. *Basic Clin Pharmacol Toxicol 108*: 122-130.

Cerqueira-Lima AT, Alcantara-Neves NM, de Carvalho LCP, Costa RS, Barbosa JM, Piuvezam M, Russo M, Barboza R, Oliveira ED, Marinho A, Figueiredo CA 2010. Effects of *Cissampelos sympodialis* Eichl. and its alkaloid, warifteine, in an experimental model of respiratory allergy to blomia tropicalis. *Curr Drug Targets 11*: 1458-1467.

Chen Z-F, Liu Y-C, Peng Y, Hong X, Wang H-H, Zhang M-M, Liang H 2012. Synthesis, characterization, and in vitro antitumor properties of gold(III) compounds with the traditional Chinese medicine (TCM) active ingredient liriodenine. *J Biol Inorg Chem 17*: 247-261.

Cheng P, Ma Y-B, Yao S-Y, Zhang Q, Wang E-J, Yan M-H, Zhang X-M, Zhang F-X, Chen J-J 2007. Two new alkaloids and active anti-hepatitis B virus constituents from *Hypserpa nitida. Bioorg Med Chem Lett 17*: 5316-5320.

Chu X, Wei M, Yang X, Cao Q, Xie X, Guan M, Wang D, Deng X 2012. Effects of an anthraquinone derivative from *Rheum officinale* Baill, emodin, on airway responses in a murine model of asthma. *Food Chem Toxicol 50*: 2368-2375.

Cometa M, Fortuna S, Palazzino G, Volpe M, Salgado E, Nicoletti M, Tomassini L 2012. New cholinesterase inhibiting bisbenzylisoquinoline alkaloids from *Abuta grandifolia. Fitoterapia 83*: 476-480.

Cortes SF, deAlencar JL, Thomas G, Barbosa JM 1995. Spasmolytic actions of warifteine, a bisbenzylisoquinoline alkaloid isolated from the root bark of *Cissampelos sympodialis* Eichl (Menispermaceae). *Phytother Res 9*: 579-583.

Costa EM, Pinheiro MLB, Souza ADL, Barison A, Campos FR, Valdez RH, Ueda-Nakamura T, Filho BPD, Nakamura CV 2011. Trypanocidal activity of oxoaporphine and pyrimidine-β-carboline alkaloids from the branches of *Annona foetida* Mart. (Annonaceae). *Molecules 16*: 9714-9720.

Costa HF, Bezerra-Santos CR, Barbosa Filho JM, Martins MA, Piuvezam MR 2008. Warifteine, a bisbenzylisoquinoline alkaloid, decreases immediate allergic and thermal hyperalgesic reactions in sensitized animals. *Int Immunopharmacol 8*: 519-525.

de Arias AR, Inchausti A, Ascurrat M, Fleitas N, Rodriguez E, Fournet A 1994. *In vitro* activity and mutagenicity of bisbenzylisoquinolines and quinones against *Trypanosoma cruzi* trypomastigotes. *Phytother Res 8*: 141-144.

de Lira GA, de Andrade LM, Florencio KC, da Silva MS, Barbosa JM, de-Cunha EVL 2002. Roraimine: a bisbenzylisoquinoline alkaloid from *Cissampelos sympodialis* roots. *Fitoterapia 7*3: 356-358.

De Wet H, Van Wyk BE 2008. An ethnobotanical survey of southern African Menispermaceae. *S Afr J Bot 74*: 2-9.

DeFreitas MR 1994. Alcalóides isolados das folhas de *Cissampelos sympodialis* Eichl. . In: *Programa de Pós graduação em Produtos Naturais e Sintéticos Bioativos, Centro de Ciências da Saúde.* Universidade Federal da Paraíba, João Pessoa.

DeFreitas MR, Cortes SF, Thomas G, Barbosa JM 1996. Modification of Ca^{2+} metabolism in the rabbit aorta as a mechanism of spasmolytic action of warifteine, a bisbenzylisoquinoline alkaloid isolated from the leaves of *Cissampelos sympodialis* Eichl. (Menispermaceae). *J Pharm Pharmacol 48*: 332-336.

Defreitas MR, Dealencar JL, Dacunha EVL, Barbosafilho

JM, Gray AI 1995. Milonine, an 8,14-dihydromorphinandienone alkaloid from leaves of *Cissampelos sympodialis*. *Phytochem 40*: 1553-1555.

Diniz MFFM 2000. Ensaios toxicológicos pré-clínicos com as folhas de *Cissampelos sympodialis* Eichl. (Menispermaceae). Tese: Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos. Universidade Federal da Paraíba, João Pessoa - PB.

Diniz MFFM, Santos HB, Cunha MAL, Duarte JC, Morais MA, Medeiros IA 2002. Subacute toxicology studies on the aqueous fraction of the ethanol extract of the leaves of *Cissampelos sympodialis* Eichl. (Menispermaceae) in dogs. *Rev Bras Farmacogn 12*: 87-89.

Donckier V, Flament V, Gérard C, Abramowicz D, Vandenabeele P, Wissing M, Delvaux A, Fiers W, Leo O, Velu T 1994. Modulation of the release of cytokines and reduction of the shock syndrome induced by anti-CD3 monoclonal antibody in mice by interleukin-10. *Transplantation 57*: 1436-1439.

Eichler AW 1864. Menispermaceae. In: *Flora Brasiliensis* (ed Martius KFPEAW). Monachii, Typographia Regia.

Feily A, Namazi MR 2009. *Cissampelos sympodialis* Eichl (Menispermaceae) leaf extract as a possible novel and safe treatment for psoriasis. *São Paulo Med J 127*: 241-242.

Feitosa EMA 2009. Chemical composition and larvicidal activity of *Rollinia leptopetala* (Annonaceae). *J Braz Chem Soc 20*: 375-378.

Fonseca FV, Figueirêdo CA 1999. Aspectos agronômicos da *Cissampelos sympodialis* Eichl. Nota técnica, pp. 3. Universidade Federal da Paraíba, João Pessoa-PB.

Fournet A, Barrios AA, Muñoz V, Hocquemiller R, Cavé A 1993. Effect of some bisbenzylisoquinoline alkaloids on American *Leishmania* sp. in BALB/c mice. *Phytother Res 7*: 281-284.

Freitas MR, Lemos VS, Queiroga CEG, Thomas G, Medeiros IA, Cortes SF 2000. Mechanisms of the contractile effect of the hydroalcoholic extract of *Cissampelos sympodialis* Eichl. in the rat aorta. *Phytomedicine 7*: 63-67.

Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M 2005. Mast cells as “tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol 23*: 749-786.

Gambarra FF, Medeiros IA, Toscano MGA, Amaral SM, Arruda IG, Diniz MFFM 2002. Ensaios toxicológicos clínicos com as folhas de *Cissampelos sympodialis* Eichl (do ponto de vista farmacêutico). In: *X Encontro de Iniciação Científica da UFPB*, pp. 100. Universidade Federal da Paraíba, João Pessoa - PB.

Gérard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M, Velu T 1993. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J Exp Med 177*: 547-550

Golden JM, Tarleton RL 1991. *Trypanosoma cruzi*: cytokine effects on macrophage trypanocidal activity. *Exp Parasitol 72*: 391-402.

Gonçalves MA, Lara TA, Pimenta LPS 2009. Alcalóides oxaporfínicos da madeira de Annona crassiflora Mart. In: *29ª Reunião Anual da Sociedade Brasileira de Química*. Sociedade Brasileira de Química, Águas de Lindóia.

Gorinsky C, Luscombe DK, Nicholls PJ 1972. Neuromuscular blocking and local anaesthetic activities of warifteine hydrochloride, an alkaloid isolated from *Cissampelos ovalifolia* D. C. *J Pharm Pharmac 24*: 147P.

Hebeda CB, Bolonheis SM, Nakasato A, Belinati K, Souza PD, Gouvea DR, Lopes NP, Farsky SH 2011. Effects of chlorogenic acid on neutrophil locomotion functions in response to inflammatory stimulus. *J Ethnopharmacol 135*: 261-269.

Holgate ST 2012. Innate and adaptive immune responses in asthma. *Nat Med 18*: 673-683.

Howard M, Muchamuel T, Andrade S, Menon S 1993. Interleukin-10 protects mice from lethal endotoxemia. *J Exp Med 177*: 1205-1208.

Ingkaninan K, Phengpa P, Yuenyongsawad S, Khorana N 2006. Acetylcholinesterase inhibitors from *Stephania venosa* tuber. *J Pharm Pharmacol 58*: 695-700.

Jacques FMB, Bertolino P 2008. Molecular and morphological phylogeny of Menispermaceae (Ranunculales). *Plant Syst Evol 274*: 83-97

Jahan R, Khatun A, Nahar N, Jahan FI, Choowdhury AR, Nahar A, Seraj S, Mahal MJ, Khatun Z, Rahmatullah M 2010. Use of Menispermaceae family in folk medicine of Bangladesh. *Adv in Nat Appl Sci 4*: 1-9.

Jucá MB 1998. Inibidores de transcriptase reserva de vírus de mielobrastose de aves. PhD, Programa de Pós-Graduação em Biologia Funcional e Molecular. In: *Instituto de Biologia - Unicamp*. Unicamp, Campinas.

Karol MH 1994. Animal models of occupational asthma. *Eur Respir J 7*: 555-568.

Knight W, Yan C 2012. Cardiac cyclic nucleotide phosphodiesterases: function, regulation, and therapeutic prospects. *Horm Metab Res 44*: 766-775.

Kumar A, Manjunath C, Thaminzhmani T, Y RK, Brahmaiah Y 2012. A review on immunomodulatory activity plants. *Ind J Novel Drug Delivery 4*: 93-103.

Kupchan SM, Patel AC, Fujita E 1965. Tumor inhibitors VI. Cissampareine, new cytotoxic alkaloid from *Cissampelos pareira*. Cytotoxicity of bisbenzylisoquinoline alkaloids. *J Pharm Sci 54*: 580-583.

Lee CC, Wang CN, Lai YT, Kang JJ, Liao JW, Chiang BL, Chen HC, Cheng YW 2010. Shikonin inhibits maturation of bone marrow-derived dendritic cells and suppresses allergic airway inflammation in a murine model of asthma. *Br J Pharmacol 161*: 1496-1511.

Lee MY, Ahn KS, Lim HS, Yuk JE, Kwon OK, Lee KY, Lee

HK, Oh SR 2012. Tiarellic acid attenuates airway hyperresponsiveness and inflammation in a murine model of allergic asthma. *Int Immunopharmacol 12*: 117-124.

Lewis DA 1989. *Anti-inflammatory drugs from marine sources*. Basel: Birkhause Verlag.

Lima GRM, Montenegro CAM, Almeida CLF, Athayde-Filho PF, Barbosa-Filho JM, Batista LM 2011. Database survey of anti-inflammatory plants in south america: A review. *Intern J Mol Sci 12*: 2692-2749.

Liu Y-C, Chen Z-F, Peng Y, Hong L 2011. Research progress on liriodenine. *Lin Chan Hua Xue Yu Gong Ye 31*: 109-116.

Lohombo-Ekomba ML, Okusa PN, Penge O, Kabongo C, Choudhary MI, Kasende OE 2004. Antibacterial, antifungal, antiplasmodial, and cytotoxic activities of *Albertisia villosa. J Ethnopharmacol 93*: 331-335.

Machado MB, Lopes LMX 2010. Dimeric alkaloids and flavonoids from *Aristolochia ridicula. Biochem Syst Ecol 38*: 110-115.

Maciel MAM, Pinto AC, Jr. VFV 2002. Plantas medicinais: a necessidade de estudos multidisciplinares. *Quim Nova 25*: 429-438.

Maior FNS, Siqueira JS, Barbosa MDS, Almeida RN 2003. Desenvolvimento pós-natal da prole exposta ao extrato hidroalcoólico da *Cissampelos sympodialis* Eichl., durante o período gestacional de ratas. *Lat Am J Pharm 22*: 321-325.

Marinho AF 2011. Caracterização dos marcadores, desenvolvimento e validação de método analítico aplicado ao estudo de sazonalidade e identificação de novos alcalóides de *Cissampelos sympodialis.* MSc Thesis, Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos. In: *Laboratório de Tecnologia Farmacêutica*, pp. 192, João Pessoa.

Marinho AF, Barbosa-Filho JM, Oliveira EJ 2012. A validated method for the simultaneous quantitation of bioactive alkaloid markers in the leaf ethanolic extract of *Cissampelos sympodialis* Eichl.: a phenological variation study. *Phytochem Anal 23*: 426-432.

Medeiros IA, Pires SLS, Almeida RN, Thomas G 1998. Cardiovascular effects of an aqueous fraction of the ethanol extract of the leaves of *Cissampelos sympodialis* Eichl. in the rat. *Phyromedicine 5*: 97-102.

Melo PS, Cavalcante HMD, Barbosa JM, Diniz MDFM, de Medeiros IA, Haun M 2003. Warifteine and milonine, alkaloids isolated from *Cissampelos sympodialis* Eichl: cytotoxicity on rat hepatocyte culture and in V79 cells. *Toxicol Lett 142*: 143-151.

Mendonca-Netto S, Varela RWB, Fechine ME, Queiroga MNG, Souto-Maior FN, Almeida RN 2008. Antidepressant effects of total tertiary alkaloid fraction of *Cissampelos sympodialis* Eichler in rodents. *Rev Bras Farmacogn 18*: 165-169.

Muccitelli RM, ucker SS, Hay DW, Torphy TJ, Wasserman MA 1987. Is the guinea pig trachea a good *in vitro* model of human large and central airways? Comparison on leukotriene-, methacholine-, histamine- and antigen-induced contractions. *J Pharmacol Exp Ther 243*: 467-473.

Mukherjee R, Keifer PA 2003. Warifteine and methylwarifteine: H-1 and C-13 assignments by two-dimensional NMR spectroscopy. *Magn Res Chem 41*: 213-218.

Mukhtar MR, Hadi AHA, Litaudon M, Awang K 2004. Morphinandienone alkaloids from *Dehaasia longipedicellata. Fitoterapia 75*: 792-794.

Oliveira-Junior WM, Benedito RB, De Menezes Patricio Santos CC, Dias Rodrigues LT, Marinho AF, DeMorais LC, Diniz MFF, DeAlmeida RN 2011. Analgesic effect of hydroalcoholic extract of *Cissampelos sympodialis* Eichl leaves. *Bol Latinoam Caribe Plant Med Aromat 10*: 333-337.

Oppenheim JJ 2001. Cytokines: past, present, and future. *Int J Hematol 74*: 3-8.

Pease JE, Sabroe I 2002. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. *Am J Respir Crit Care Med 1*: 19-25.

Petrisko MA, Skoner JD, Skoner DP 2008. Safety and efficacy of inhaled corticosteroids (ICS) in children with asthma. *J Asthma 45*: 1-9.

Piuvezam MR, Pecanha LMT, Alexander J, Thomas G 1999. *Cissampelos sympodialis* Eichl. leaf extract increases the production of IL-10 by concanavalin-A-treated BALB/c spleen cells. *J Ethnopharmacol 67*: 93-101.

Porto NM, De Figueiredo RCBQ, Oliveira AFM, Agra MF 2011. Leaf epidermal characteristics of *Cissampelos* L. (Menispermaceae) species from Northeastern Brazil. *Microsc Res Techniq 74*: 370-376.

Porto NM, Lima Diniz Basilio IJ, Agra MF 2008. Pharmacobotanical study of the leaves of *Cissampelos sympodialis* Eichl.,(Menispermaceae). *Rev Bras Farmacogn 18*: 102-107.

Rhodes DG 1975. A revision of the genus *Cissampelos. Phytologia 30*: 415-484.

Rocha JDB, Decote-Ricardo D, Redner P, Lopes UG, Barbosa JM, Piuvezam MR, Arruda LB, Pecanha LMT 2010. Inhibitory effect of the alkaloid warifteine purified from *Cissampelos sympodialis* on B lymphocyte function *in vitro* and *in vivo. Planta Med 76*: 325-330.

Silva ECd, Rayol CD, Medeiros PL, Figueiredo RCBQ, Piuvezan MR, Barbosa-Filho JM, Marinho AF, Silva TG, Militão GCG, Cassilhas APP, Andrade PPd 2012. Antileishmanial activity of warifteine: a bisbenzylisoquinoline alkaloid isolated from *Cissampelos sympodialis* Eichl. (Menispermaceae). *ScientificWorldJournal*, DOI: 10.1100/2012/516408.

Society BT 2012. SIGN 101. *British Guideline on The Management of Asthma. A National Clinical Guideline* Scottish Intercollegiate Guidelines Network.

Souza MST, Ferreira DA, Almeida RN, Carvalho LC, Diniz

MFFM 2000. Ensaios toxicológicos clínicos com as folhas de *C. sympodialis*. Avaliação sobre o reflexo Hoffmann. In: *VIII Encontro de Iniciação Científica da UFPB*. Universidade Federal da Paraíba, João Pessoa - PB.

Spelman K, Burns JJ, Nichols D, Winters N, Ottersberg S, Tenborg M 2006. Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. *Altern Med Rev* 11: 128-150.

Stevens PF 2012. Angiosperm Phylogeny Website v.12 (2001 onwards). http:// www.mobot.org/MOBOT/research/APweb/.

Sun S-W, Kuo C-H, Lee S-S, Chen C-K 2000. Determination of bisbenzylisoquinoline alkaloids by high-performance liquid chromatography (II). *J Chromatogr A 891*: 189-194.

Thomas G, Araujo CC, Agra MD, Diniz MDFM, Bachelet M, Vargaftig BD 1995. Preliminary studies on the hydroalcoholic extract of the root of *Cissampelos sympodialis* Eichl in guinea-pig tracheal strips and bronchoalveolar leukocytes. *Phytother Res 9*: 473-477.

Thomas G, Araújo CC, Duarte JC, De Souza DP 1997a. Bronchodilator activity of an aqueous fraction of an ethanol extract of the leaves of *Cissampelos sympodialis* Eichl. (Menispermaceae) in the guinea pig. *Phytomedicine 4*: 233-238.

Thomas G, Burns F, Pyne S, Pyne NJ 1997b. Characterization of an extract from the leaves of *Cissampelos sympodialis* Eichl. on the spontaneous tone of isolated trachea. *Phytother Res 11*: 496-499.

Thomas G, Selak M, Henson PM 1999. Effects of the aqueous fraction of the ethanol extract of the leaves of *Cissampelos sympodialis* Eichl. in human neutrophils. *Phytother Res 13*: 9-13.

Toledo ACO, Hirata LL, Buffon MDCM, Miguel MD, Miguel OG 2003. Fitoterápicos: uma abordagem farmacotécnica. *Lecta-USF 21*: 7-14.

Wright CW, Marshall SJ, Russell PF, Anderson MM, Phillipson JD, Kirby GC, Warhurst DC, Schiff PL 2000. In vitro antiplasmodial, antiamoebic, and cytotoxic activities of some monomeric isoquinoline alkaloids. *J Nat Prod 63*: 1638-1640.

Xiong YY, Wang JS, Wu FH, Li J, Kong LY 2012. The effects of (+/-)-praeruptorin A on airway inflammation, remodeling and transforming growth factor-beta1/Smad signaling pathway in a murine model of allergic asthma. *Int Immunopharmacol 14*: 392-400.

Yang N, Patil S, Zhuge J, Wen MC, Bolleddula J, Doddaga S, Goldfarb J, Sampson HA, Li XM 2012. *Glycyrrhiza uralensis* flavonoids present in anti-asthma formula, ASHMI(TM) , inhibit memory Th2 responses in vitro and *in vivo*. *Phytother Res Epub doi*: 10.1002/ptr.4862.

Yuan X, Sun S, Wang S, Sun Y 2011. Effects of astragaloside IV on IFN-gamma level and prolonged airway dysfunction in a murine model of chronic asthma. *Planta Med 77*: 328-333.

Yunes RA, Pedrosa RC, Filho C 2001. Fármacos e fitoterápicos: a necessidade do desenvolvimento da indústria de fitoterápicos e fitofármacos no Brasil. *Quim Nova 24*: 147-152.

Yusuf-Makagiansa H, Anderson ME, Yakovleva TV, Murray JS, Siahaan TJ 2002. Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. *Med Res Rev 2*: 146-166.

Zosky GR, Garnier CV, Stumbles PA, Holt PG, Sly PD, Turner DJ 2004. The pattern of methacholine responsiveness in mice is dependent on antigen challenge dose. *Resp Res 5*: 15-25.

Zosky GR, Sly PD 2007. Animal models of asthma. *Clin Exp Allergy 37*: 973-988.

***Correspondence**

Eduardo de Jesus Oliveira
Centro de Biotecnologia, Universidade Federal da Paraíba
Campus I, s/n, Cidade Universitária, 58051-970 João Pessoa-PB, Brazil
eduardo@ltf.ufpb.br
Tel.: +55 83 3216 7342
Fax: +55 83 3216 7365