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Cubebin and semisynthetic dibenzyl butyrolactone derivatives: Biological activities

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Lignans are a group of secondary metabolites with a wide variety of chemical structures, formed by coupling two phenylpropanoid units. Currently, there is great interest in lignan due to the wide range of biological activities this class of compounds has demonstrated. This review describes the biological activities of cubebin and semi-synthetic derivatives, focusing primarily on *Piper cubeba* and *Zantoxyllum naranjillo*. The main biological activities reported were: Anti-inflammatory, antitumor, action in erectile dysfunction, trypanocidal, anti-Leishmania and antimicrobial. In this way, it is possible to conclude that cubebin and its derivatives have shown high capacity to become new bioactive molecules obtained by semi-sinthesys, useful to develop medicines for several pathologies, presenting efficacy, toxicology and quality for human use.

Key words: lignans; cubebin, semisynthetic derivatives; biological activities, *Piper cubeba*, Piperaceae, *Zantoxyllum naranjillo*, Rutaceae.

INTRODUCTION

In the 1940s, Haworth introduced the term lignan to refer to a class of chemical plant compounds originating from the biosynthetic pathway of shikimic acid, which are formed by oxidative coupling of cinnamyl alcohols among themselves or cinnamic acids (Barbosa Filho, 2004). The way these phenylpropanoid units connect (C6C3) determines their classification. Lignans are connected by position 8 and 8' of the aliphatic chain (Simões et al., 1999).

Lignans are an important group of secondary metabolites found in many plant species. They are mostly found in nature in its free form, widely distributed in plants. It has been found in species from more than 70 families, isolated from roots, rhizomes, stems, leaves, seeds and fruits (Saleem et al., 2005).

Lignans are formed in response to mechanical injury or invasion of fungi or bacteria. They have several beneficial properties including analgesic, anti-inflammatory (Bastos et al., 2001), antitumor (Yan et al., 2008), trypanocidal (Bastos et al., 1999; de Souza et al., 2005) and anti-

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Leishmania activity (Bodiwala et al., 2007).

Cubebin is a lignan dibenzyl butyrolactone found in a wide variety of plant families around the world (Rao, 1978). For example Aristolochiaceae (De Pascoli et al., 2006), Rutaceae (Bastos et al., 2001), Myristicaceae (Blumenthal et al., 1997) and Piperaceae (Saraiva et al., 2007) have been investigated for different activities of cubebin, especially *P. cubeba* and *Z. naranjillo.*

Zantoxyllum naranjillo Griseb (Rutaceae) has been used to treat inflammation (Reitz, 1960). In this species cubebin was isolated from a hexane leaves. *Piper cubeba* Linn (Piperaceae) is a popular spice used in Europe and countries such as Saudi Arabia, India, Indonesia and Morocco (Junqueira et al., 2007). Pepper has been used since the Middle Ages, both as a spice and in traditional medicine to treat various diseases. The genus Piper has more than 1000 species that grow as herbs, shrubs or trees (Junqueira et al., 2007). Cubebin has been isolated from the *P. cubeba* dry seeds.

Non clinical studies such as Rodrigues (2002) indicated that cubebin has no toxic effects when administered orally. Therefore, it is important to evaluate this compound and its derivatives for therapeutic purposes.

In most articles reviewed the cubebin was isolated from *P. cubeba* seeds. The reason for the preference should possibly have been because the isolation from the leaves of *Z. naranjillo* could put the species on risk of extinction.

Research on natural products contributes effectively to the discovery of new drugs. It can occur by introducing new chemical structures and/or mechanisms of action.

Principles derived from natural products assets are extremely important in expanding the therapeutic arsenal as well as improving patients' quality of life, aiming at the development of effective drugs with high clinical potency and reduced side effects.

Cubebin is a very active substance in several pathological conditions as described previously. It is well known that some minor changes in the structure of the molecule can greatly improve their activity by interfering in its mechanism of action or even important pharmacokinetic parameters such as partition coefficient and dissociation coefficient.

The purpose of this article is to review the semi-synthetic derivatives that have been produced from cubebin and how they act in relation to the tested activity, using pharmaceutical chemistry to further improve the activity of natural compounds with insertion various substituent's on the molecule.

MATERIALS AND METHODS

Pharmacological activities of cubebin and its derivatives were studied by using the PUBMED search engine, comprising citations for biomedical literature of MEDLINE database, science journals and eBooks. LILACS was another database used, coordinated by BIREME/PAHO/WHO, The Latin American and Caribbean Center on Health Sciences Information, belonging to the Pan American Health Organization. Data were consulted in June 2016, and the following key words were used: Cubebin, *piper cubeba*, cubebin/*piper cubeba*, and cubebin/*zantoxyllum naranjillo.*

RESULTS AND DISCUSSION

Inflammatory and analgesic activities

Inflammation is an immune system coordinated response to noxious stimuli that appear during infection or after a tissue injury. The inflammatory response is the result of several chemical mediators' activity released by immune cells and *in loco* activated biochemical response (Stutz et al., 2009).

Prostaglandins are potent inflammation mediators, and non-steroidal anti-inflammatory drugs act by inhibiting its production. Cyclooxygenase is the pharmacological target of these drugs. It is involved in the first step of arachidonic acid metabolism. Two cyclooxygenase isoforms are known, COX-1 and COX-2. The first is a constitutive isoform found in blood vessels, stomach and kidney, while COX-2 is preferred induced by cytokines in inflammatory context and inflammation mediators. It is desirable that the medicine preferentially inhibit, selectively, COX-2 in order to avoid ulcerogenic side effects typical of these drugs. When used as analgesics, these drugs are generally only effective for mild to moderate pain (Hardman et al., 1996).

Bastos et al. (2001) used in a study the paw edema model induced by carrageenan. Cubebin was isolated from the leaves of *Zanthoxyllum naranjillo*. In that study cubebin was tested and compared to indomethacin, which is a non-steroidal anti-inflammatory, used as a positive control. The researchers observed that carrageenan produced a significant edema in paws of rats, which was more intense in animals treated with 5% Tween (negative control). Cubebin (10 mg/kg) taken 30 minutes before carrageenan injection, inhibited in a very similar way to the same oral dose of indomethacin. In this study, the research group also used other inflammation models as edema induced by dextran, edema induced by histamine, edema induced by serotonin and edema induced by prostaglandin PGE2. Of these, cubebin was able to partially inhibit the edema induced by serotonin and significantly the edema induced by prostaglandin.

Analgesic and anti-inflammatory effects of cubebin and its derivative benzylated, (-) o-benzyl cubebin, were investigated using different models of animal testing. Cubebin was isolated from *P. cubeba* dry seeds.

This study was conducted by Coimbra et al. (2004). The (-) o-benzyl cubebin showed low anti-inflammatory activity and high analgesic activity, producing dose-response correlation in doses of 10, 20 and 40 mg/Kg. In the hot plate test and cell migration, neither cubebin nor its derivative showed activity. In this way, according to the research group, adding benzyl group contributed only for analgesic activity.

Souza et al. (2004) conducted a study in order to obtain

(-)-O-acetyl, (-)-O-methyl, (-)-O-dimethylamylimine cubebin from cubebin (isolated from dry seeds of *P. cubeba L*.) and test their analgesic and anti-inflammatory activities. These compounds were respectively obtained by acetylation, methylation and amination of the cubebin hydroxyl group. According to the results, the researchers concluded that acetylation and amination of cubebin increased analgesic and anti-inflammatory activities. Regarding the hot plate and the cell migration test of rats none of the four compounds showed activity.

Silva et al. (2005) tested cubebin and its derivatives: hinokinin, 6.6-dinitro hinokinin and 6.6-diamino hinokinin isolated from *P. cubeba* dry seeds in different animal models.

In the paw edema model induced by carrageenan, responses were compared to indomethacin. In this study, carrageenan in rat paws induced a high edematogenic response and treatment of the animals with cubebin and its derivatives variously inhibited, but also significant, edema formation. According to Silva et al. (2005) the introduction of groups -NH_2, 6.6-diamino hinokinin may be beneficial to the activity (82% inhibition) and different substitutions may affect selectivity by COX2. In the writhing test induced by acetic acid in rats, the compounds hinokinin and 6.6-diamino hinokinin showed inhibition levels of 97 and 92%, respectively. Silva et al. (2005) found that polar groups introduction on aromatic rings is advantageous for analgesic and anti-inflammatory activities.

Antitumor activity

Carcinogenesis is a complex process that occurs from the interaction between a carcinogenic agent (or oncogenic) and genes, changing cell characteristics, losing control of cell division and culminating in a uncontrolled growth of neoplastic cells (Mainenti and Rosa, 2008; Vainio et al., 1992).

The oncogenic agents do not necessarily have exogenous origin. The free radicals derived from the very metabolic processes of an aerobic organism generate a cellular oxidative stress. This contributes to aging development, transformation and cell death, which relate to many disease processes, including cancer and other chronic diseases (Vasconcelos et al., 2007). Phenolic substances are known to be holders of pronounced antioxidant properties, acting as free radical scavengers and as metal chelator (Pessuto et al., 2009) for the formation of phenoxyl radical.

In the Aboul-Eneim et al. (2011) study, the antioxidant activity of 16 compounds isolated from *P. cubeba*, among them cubebin, was measured by the ability to eliminate free radicals: Hydroxyl radical (OH^-), superoxide anion radical ($O_2^{\bullet-}$), and 2.2-diphenyl-1-picrylhydrazyl radical (DPPH) in different systems. The results showed that most of the tested compounds acted as free radical scavenger under *in vitro* conditions and can act as antioxidant agents for anti-free radical mechanism.

Rezende et al. (2011) evaluated the genotoxic potential and influence on chromosomal damage induced by doxorubicin in V79 cells and by urethane in somatic cells of *Drosophila melanogaster*. It was concluded that cubebin has antioxidant ability, acting as free radical scavenger at low concentrations, a pro-oxidant at higher concentrations when it interacts with the enzyme system which catalyzes metabolic detoxification of doxorubicin or urethane and/or a DNA repair inductor by recombination. There is a dose-dependent relationship.

These authors continued their studies and analyzed cubebin ability to interact with the enzyme system that catalyzes the urethane metabolic detoxification (carcinogen). This cubebin capacity was proved, by inhibiting the mitochondrial complex I activity, acting as a free radical scavenger. Furthermore, cubebin can modulate the urethane metabolic activation by inhibiting metabolites binding to the DNA (Rezende et al., 2013).

Cubebin can significantly inhibit CYP3A4 (Usia et al., 2005, 2006) and the mitochondrial complex I activity of NADPH oxidase (Saraiva et al., 2009). According to Sinigaglia et al. (2006), co-treatments with oxidants and cubebin reduce the mutation rate, and may be classified as non-mutagenic effect. The cubebin non-mutagenic capacity is not very clear; it may be attributed to the antioxidant capacity and metabolic activity suppression.

In an *in vitro* study held in two different cell lines of human prostate cancer (LNCaP-FGC and PC-3) the anti-cancer potential of an ethanol extract of *P. cubeba* was evaluated, named, in that study, P9605. HPLC analysis revealed that the P9605 had 16.53% of cubebin. The results indicated that the P9605 inhibited proliferation of human LNCaP cells prostate cancer by reducing DNA synthesis and inducing apoptosis. The anti-growth effect was less pronounced in the PC-3 line. The P9605 markedly inhibited the 5α-reductase II activity, which is responsible for converting testosterone to its active form and suppressed PSA secretion in LNCaP cells, thus acting by several mechanisms (Yam et al., 2008).

Hinokinin differs from cubebin because of the presence of a carbonyl group at C-9. Hinokinin has been extensively studied and has shown great therapeutic potential, even in antitumor activity. Barbosa et al. (2014) tested the antigenotoxic and anticarcinogenic potential of hinokinin in preneoplastic lesions in rat colon. According to the authors hinokinin was able to reduce DNA damage induced by 1,2- dimethylhydrazine (DMH) and additionally inhibited the formation of pre-neoplastic lesions.

The antitumor activity of cubebin, isolated from dry seeds of *P. cubeba* and its semisynthetic derivatives (17*)* were tested *in vitro* in six different human tumor cell lines: A549 (human lung carcinoma), KB (human nasopharyngeal carcinoma), K562 (human chronic myeloid leukemia), SiHa (human cervical carcinoma),

HT29 and HCT116 (human colon carcinoma). According to the results, which were expressed as IC50 (concentration able to inhibit cell growth by 50%). Hinokinin and compounds containing group's amide and lactone ring were those who had higher activity on the test cells (Rajalekshmi et al., 2016).

Activity in erectile dysfunction

Erectile dysfunction (ED) is defined as the persistent inability to obtain and/or maintain an adequate penile rigidity which allows a satisfactory sexual intercourse, according to the National Institutes of Health (NIH) on Impotence (Impotence, 1992).

Many plants metabolites produce vessel relaxation, mediated by nitric oxide (NO). Flavonoids, tannins, lignans can directly activate production of NO endothelial synthase or can enhance mediated relaxation by NO and by superoxide anions (Achike and Kwan, 2003).

Carvalho et al. (2013) investigated the vasorelaxant effect produced by cubebin from dry seeds of *P. cubeba* in aortic rings isolated of pre-contracted rats with phenylephrine to evaluate the possible mechanism involved. It has been suggested that cubebin has a vasorelaxant effect dependent on the NO/cGMP pathway. In addition to NO, release of endothelial prostacyclin also contributes to the smooth muscle relaxation via cGMP (Parkington et al., 2004). However, in this case it was concluded that pretreatment with indomethacin failed to modify the relaxation induced by cubebin, suggesting that prostanoids do not contribute to the relaxing effect.

Researchers from the University of Franca (São Paulo, Brazil), have deposited a patent with the number of publication (WO 2011/075801 A1) regarding the use of dibenzyl butyrolactone lignan and its derivatives, as well as other lignans and neolignans, and in particular with cubebin as a vasodilator agent in erectile dysfunction therapy. They conducted *in vivo* tests using Swiss mice. The mice were divided into groups receiving different cubebin doses, negative control groups and groups receiving treatment with sildenafil citrate (positive control). The research group observed that the use of cubebin showed positive effects similar to the drugs currently used for treating male impotence, particularly Viagra, with no tachycardia and the inherent agitation. The suggested mechanism of action was PDE5 blocking (diestarase phosphorus 5).

Trypanocidal activity

Chagas disease is caused by the flagellate protozoan *Trypanosoma cruzi*, which is transmitted to the human host, mainly by the hematophagous vector known as "the barber bug" (*Triatoma infestans, Panstron-gylus megistus*, among others). There are also other possible types of transmission such as blood transfusions, mother-to-child transmission and more rarely by contaminated fresh food (Moncayo and Ortiz Yanine, 2006). This disease affects about 10 million people in Latin America (Rassi et al., 2010).

Drugs available for this disease treatment include benznidazole (Rochagan, Roche) and nifurtimox (Lampit, Bayer), although these drugs cause several side effects. Moreover, there are already *T. cruzi* resistant to treatment (Coura, 2009).

The *T. cruzi* biological cycle is highly complex, involving three different ways: Epimastigote (proliferative form), trypomastigote (infective and bloodstream form) and amastigote (intracellular replication form). This may hinder drug discovery.

Bastos et al. (1999) evaluated some biologically active dibenzyl butyrolactone lignans, isolated from *Zanthoxyllum naranjillo* leaves, which showed cubebin and methylpluviatolide trypanocidal action.

Souza et al. (2005) evaluated the cubebin derivatives activity (isolated from the *P. cubeba* dry seeds) against amastigotes forms of *Trypanosoma cruzi* in a cell culture assay. Biological activity was assessed using a colorimetric method and the statistical analyzes were performed by the ANOVA test. According to the article, the most active compound was hinokinin with an IC_{50} value of 0.7 µM. Benzyl cubebin (IC_{50} 5.7 µM) and O-N, N-dimethylaminoethyl-cubebin (IC_{50} 4.7µM) also had significant activity. O-acetyl cubebin was inactive and 6,6-dinitro hinokinin presented IC_{50} 95.3 µM. The researchers concluded that the nitro group was harmful to this activity.

In vitro and *in vivo* studies on cubebin and derivatives activity against *Trypanosoma cruzi* were carried out by Saraiva et al. (2007). Cubebin was isolated from *P. cubeba* dry seeds and its derivatives were obtained by HPLC after partial and purified synthesis.

Cubebin, benzyl cubebin and dinitro hinokinin showed low anti-epimastigote activity in *in vitro* assay. Methyl-cubebin, hinokinin and dimethyl-morelensine showed high activity in that assay. Hinokinin had $IC_{50\ of}$ 0.67 µM and was selected to *in vivo* study. The researchers observed a 70.8% reduction of parasitaemia (amastigotes), while benznidazole reduced parasitaemia in 29.0%, making hinokinin a potential drug for Chagas disease, according to the authors.

In an *in vivo* study, Esperandim et al. (2010) evaluated the trypanocidal activity of cubebin and hinokinin during the chronic Chagas disease phase. Hinokinin was obtained by partial synthesis of cubebin isolated from *P. cubeba* dry seeds.

Albino BALB/c mice were used in that study, they divided into groups according to the drug administration type (oral and intraperitoneal) and dosage (20 and 50 mg/Kg). A negative group was also separated (inoculated with trypomastigotes), treated with the solvent used to prepare the solutions, a positive group treated with benznidazole and an uninfected group. In all cases, the

treatment was initiated 90 days after infection. Parasitism reduction was assessed by the β-galactosidase quantification. Treatment with lignans led to higher reduction of parasitism in all organs evaluated in comparison with benznidazole. Oral treatment was more effective. The data suggested that cubebin and hinokinin can be considered as potential compounds for the development of new drugs against Chagas disease.

Hinokinin and 6,6-dinitro hinokinin were evaluated for their interference with the messenger RNA processing in trypanosomatids. The study performed by Silva et al. (2011) used *T. cruzi* epimastigotes strains Y and BOL (Bolivia). The substances seemed to intervene at some RNA transcription stage, promoting changes in their synthesis. Hinokinin and 6,6-dinitro hinokinin were not able to interfere with RNA processing by trans-splicing in *T. cruzi*, as observed by the RNase protection reaction.

Continuing the 2010 studies, researchers conducted an *in vivo* study in order to verify cubebin and hinokinin activity against *T. cruzi*. Cubebin was obtained in the same way as the previous study. In a study with BALB/c mice, they were inoculated with 2×10^4 trypomastigotes forms 48 h before treatment. The mice were divided into 6 groups: Negative control (5% i.p. injection with 5% DMSO, 2.5% Tween, 5% ethanol); positive control (benznidazole, 20 and 50 mg/Kg *p.o.*); cubebin (20 and 50 mg/kg *p.o.*); hinokinin (20 and 50 mg/Kg *p.o.*); during the acute phase of the *T. cruzi* infection. The animals with acute parasitaemia were investigated by morphometric tissue analysis. There was a significant parasitaemia reduction in animals treated with cubebin and hinokinin compared to the negative control (Esperadim et al., 2013).

Anti-Leishmania activity

Leishmaniasis is a group of tropical diseases caused by trypanosomatidae protozoa, with more than 30 species, of which 11 have medical and veterinary significance (Bates, 2007). Leishmaniasis has a major impact on populations worldwide, particularly in Asia, Africa and Latin America (Chan-Bacab, Pena-Rodriguez, 2001). More than 350 million people live in areas at risk of infection with active parasite (Murray et al., 2005).

Leishmania spp. infection can lead to different clinical manifestations depending on the species of *Leishmania* and host immune response. There are three main types: Cutaneous, mucocutaneous and visceral. Visceral leishmaniasis is caused by *L. donovani*, the most severe form of leishmaniasis and when left untreated can be fatal (Ready, 2014). Cutaneous and mucocutaneous leishmaniasis are more common and usually appear as an ulcer that can take months or years to heal and may become chronic (Salman et al., 1999).

Leishmania can be considered an opportunistic pathogen in HIV-positive patients, immunocompromised, and stimulate virus replication in these patients (Carvalho and Ferreira, 2001).

Drugs of choice for leishmaniasis treatment are pentavalent antimonials, which have high renal and cardiac toxicity. Therefore, there is the need for other drugs that are safe and effective.

Bodiwala et al. (2007) investigated amides and lignans of *P. cubeba* and *Piper retrofractum.* Two lignans were isolated: Cubebin and hinokinin of hexane extract of *P. cubeba*, and sesamin lignan and two amides, pellitorine and piplartine, of methanol and hexane extracts of *P. retrofractum*. *In vitro* cytotoxicity assays were performed to assess activity against promastigotes, using the MTT colorimetric method [3- (4.5-dimethylthiazol-2-yl) -2.5-diphenylterazolium bromide] and *in vivo* assays against amastigotes using infected hamsters. In both tests the species *L. donovani was used.*

According to the results of the research group, cubebin and piplartine showed high *in vitro* activity at 100 µM concentrations and were tested *in vivo* in a visceral leishmaniasis model in hamsters. Piplartine showed *in vivo* activity at 30 mg/Kg.

Antibacterial activity against oral pathogens

Tooth cavity is a multifactorial, chronic infectious disease, which puts people at risk not only in childhood and adolescence, but throughout life. It is the most common cause of pain in the oral cavity and tooth loss (Edelstein, 2006; Featherstone, 2004). Tooth cavity develops an imbalance in the oral microflora and may be triggered by the presence of bacteria, genetic and immunological factors and behavioral aspects which interact, allowing the initiation and development of tooth cavities (Selwitz et al., 2007; Aas et al., 2008). Studies point to *Streptococcus mutans* as the primary pathogen in the etiology of tooth cavity (Hoiby et al., 2011; Tanzer et al., 2001).

Silva et al. (2007) investigated ethanol extract activity of *P. cubeba*, cubebin and semisynthetic derivatives against oral pathogens. Pathogens chosen to participate in the survey were: *Streptococcus salivaris*, *Streptococcus mitis*, *Enterococcus faecalis*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguinis* and *Candida albicans*. Microdilution was the method used in the evaluation. MIC (minimal inhibitory concentration) was defined as the lowest concentration that did not allow any growth on blood agar, and lack of growth of viable cells indicated a bactericidal effect. The extract of *P. cubeba*, cubebin and its derivatives showed activity against all the microorganisms tested. According to the research group analysis, introducing polar groups in aromatic ring was beneficial for antimicrobial activity.

PERSPECTIVES AND CONCLUSION

The *Piper* genus has been very studied by scientists

worldwide. It a genus that can be found in all continents, with several species. In this concept, the main compounds isolated from two species have shown potential to become new molecules, in its natural or semi-synthetic forms (O-benzyl cubebin, 6,6'-dinitrohinokinin, amide derived from hinokinin), for the therapeutical proposes. Researchers have been studying cubebin and its derivatives by semisynthesis and have found great therapeutic perspectives. Substitutions on the cubebin aromatic ring, according to the desired derivative, specifically influence each biological activity studied. Cubebin has proven *in vivo* efficacy in most of the activities described in the article. Therefore, more detailed studies is necessary about its activity in living organisms. Hinokinin can be isolated from *P. cubeba* or semi-synthesized by oxidation of cubebin. This substance has demonstrated high analgesic activity and anti-inflammatory, antimutagenic, chemopreventive, anticancer activities, in the treatment of Chagas disease and antibacterial activity against oral pathogens. The hinokinin certainly deserves attention and has a great therapeutic potential. Considering the patents on the use of cubebin and conduction of non and clinical trials, undoubtedly, in a few years it is possible to have a new drug arising from natural products studies to be used as new medicinal agent.

Conflict of Interests

The authors have not declared any conflict of interests.

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