



## Essential oil from *Pterodon emarginatus* as a promising natural raw material for larvicidal nanoemulsions against a tropical disease vector



Anna E.M.F.M. Oliveira<sup>a,b</sup>, Desirane C. Bezerra<sup>a</sup>, Jonatas L. Duarte<sup>a,b</sup>, Rodrigo A.S. Cruz<sup>a,b</sup>, Raimundo N.P. Souto<sup>c</sup>, Ricardo M.A. Ferreira<sup>c</sup>, Jeane Nogueira<sup>d</sup>, Edemilson C. da Conceição<sup>e</sup>, Suzana Leitão<sup>f</sup>, Humberto R. Bizzo<sup>g</sup>, Paola E. Gama<sup>g</sup>, José C.T. Carvalho<sup>a,b</sup>, Caio P. Fernandes<sup>a,b,\*</sup>

<sup>a</sup> Laboratório de Nanobiotecnologia Fitofarmacêutica - Curso de Farmácia, Universidade Federal do Amapá, Campus Universitário Marco Zero do Equador, Rodovia Juscelino Kubitschek de Oliveira, KM – 02 Bairro Zéão, CEP: 68902-280, Macapá, AP, Brazil

<sup>b</sup> Laboratório de Pesquisa em Fármacos, Curso de Farmácia, Universidade Federal do Amapá, Campus Universitário Marco Zero do Equador, Rodovia Juscelino Kubitschek de Oliveira, KM – 02 Bairro Zéão, CEP: 68902-280 Macapá, AP, Brazil

<sup>c</sup> Laboratório de Artrópodes, Universidade Federal do Amapá, Curso de Ciências Biológicas, Universidade Federal do Amapá, Campus Universitário Marco Zero do Equador, Rodovia Juscelino Kubitschek de Oliveira, KM – 02 Bairro Zéão, CEP: 68902-280 Macapá, AP, Brazil

<sup>d</sup> Laboratório de Tecnologia de Produtos Naturais – LTPN, Departamento de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal Fluminense, UFF, Rua Mário Viana, 523, CEP: 24241-000, Santa Rosa, Niterói, RJ, Brazil

<sup>e</sup> Laboratório de Pesquisa - Desenvolvimento e Inovação em Bioprodutos, Universidade Federal de Goiás, Faculdade de Farmácia, Praça Universitária, 1166, Setor Leste Universitário, CEP: 74605220 Goiânia, GO, Brazil

<sup>f</sup> Faculdade de Farmácia - Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho – 373 - Sala A2-10 - Ilha do Fundão, CEP: 21.941-902 Rio de Janeiro, RJ, Brazil

<sup>g</sup> Embrapa Food Technology, Avenida das Américas – 29501, CEP: 23020-470 Rio de Janeiro, RJ, Brazil

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### ABSTRACT

The oleoresin obtained from fruits of *Pterodon emarginatus* was previously subjected to development of larvicidal nanoemulsions. To our knowledge, no efforts aiming at developing nanoemulsions with the essential oil from this species were carried out. This study describes the preparation and evaluation of the larvicidal activity of a novel oil in water nanoemulsion prepared with essential oil from fruits of *P. emarginatus* against *Aedes aegypti* larvae.  $\beta$ -caryophyllene is the major compound, corresponding to 25.8% of relative percentage of the essential oil. A series of nanoemulsions were obtained and better physical results were achieved using polysorbate 80, suggesting that a required Hydrophile-Lipophile balance value of this oil is around 15. Mean droplet size decreased from  $128.0 \pm 6.2$  nm to  $53.2 \pm 0.5$  nm, when surfactant to oil ratio increased from 1.0 to 2.0. The nanoemulsion prepared with *P. emarginatus* essential oil and polysorbate 80 (1:1) was able to induce mortality on early 4th instar larvae. This study allowed preparation of nanoemulsions with essential oil from fruits of *P. emarginatus* for the first time, which proved to be a potential larvicidal against *Aedes aegypti*. The utilization of a low cost and solvent-free method can be considered an advantage in terms of potential applications of this natural product in ecofriendly integrative practices of vector control. Moreover, the great potential of this plant species is highlighted by the fact that its fruits can be obtained by a sustainable management of biodiversity, using the concept of standing trees to protect and stimulate conservation of the species.

### 1. Introduction

Dengue fever is a tropical neglected disease and more than 2.5 billion of people worldwide are in risk to be infected with dengue virus. Moreover, approximately 500,000 people require hospitalization, being most of them children, while 2.5% of cases result in death (WHO,

2014). In Brazil, a total of 170,103 probable cases of dengue were reported in the year 2016. Higher prevalence of virus serotype 1 (DENV1) has been observed in this country. Moreover, growing cases related to other diseases that can be transmitted by *Aedes aegypti*, including chikungunya fever and Zika, have been reported (Brasil, 2016). The main strategy to prevent diseases transmitted by *A. aegypti* is associated to

**Abbreviations:** DLS, dynamic light scattering; HLB, hydrophile/lipophile balance; PDI, polydispersity index; PG, particle growth; rHLB, required hydrophile lipophile balance; SOR, surfactant to oil ratio

\* Corresponding author at: Laboratório de Nanobiotecnologia Fitofarmacêutica, Curso de Farmácia, Universidade Federal do Amapá, Campus Universitário Marco Zero do Equador, Rodovia Juscelino Kubitschek de Oliveira, KM – 02 Bairro Zéão, CEP: 68902-280 Macapá, AP, Brazil

E-mail address: [caiofernandes@unifap.br](mailto:caiofernandes@unifap.br) (C.P. Fernandes).

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vector control (WHO, 2014). These methods involve elimination of water media in which mosquitoes oviposit and where larvae development occurs, application of larvicidal chemical agents, utilization of biological control and adulticidal agents (WHO, 2009). Several studies have demonstrated the larvicidal activity of plant-based natural products against *Aedes aegypti*, including essential oils (Rouis et al., 2013; Feitosa et al., 2009; Oliveira et al., 2013).

*Pterodon* Vogel is a genus that comprises some plants native to Brazil. In this country, it is widely distributed in several regions and phytogeographical domains, including the Amazon and the Brazilian savanna (cerrado) (Lima and de Lima, 2015). Species from this genus are commonly known as “sucupira” and “sucupira-branca”. They have fruits that are widely used in folk medicine, mainly due to their anti-inflammatory and analgesic properties (Hansen et al., 2010; Hoscheid et al., 2015). Most studies about this genus are associated to the species *P. emarginatus* Vogel, mainly using its fruits or seeds. These plant parts have an oleoresin that is highly viscous and considered a source of vouacapan diterpenes (Mahajan and Monteiro, 1973). Fruits or seeds of *P. emarginatus* are also used as source of an essential oil, which differs in method of extraction and chemical composition from the oleoresin. *P. emarginatus* essential oil has a wide range of biological activities, being able to ameliorate autoimmune encephalomyelitis (Alberti et al., 2014) and to inhibit contractions in rat isolated trachea (Evangelista et al., 2007). Moreover, it shows antinociceptive (Dutra et al., 2008), anti-ulcerogenic (Dutra et al., 2009a), anti-inflammatory (Dutra et al., 2009a; Velozo et al., 2013), antiproliferative (Dutra et al., 2012) effects, antimicrobial activity against *Staphylococcus aureus* ATCC25923 (Dutra et al., 2009b) and bactericidal activity against *Mycobacterium bovis*. *P. emarginatus* essential oil also presents a larvicidal activity against *Aedes aegypti* (Diptera, Culicidae) (Pimenta et al., 2006). However, intrinsic low water miscibility of these natural products is a technological challenge for development of viable and effective aqueous products.

Nanoemulsions are dispersed systems constituted by two immiscible liquids, usually stabilized by surfactants (Bruxel et al., 2012). They are metastable and have transparent or translucent aspect (McClements, 2011). Due to the small droplet size, nanoemulsions have enhanced kinetic stability against sedimentation and creaming, when compared to macroemulsions (Tadros et al., 2004; Maali et al., 2013). The term macroemulsion refers to conventional emulsions with micrometer-size droplets. On the other hand, microemulsions are thermodynamically stable systems and they are neither macroemulsions nor nanoemulsions. Despite some misconceptions that may occur in the literature, the term nanoemulsion or nano-emulsion is well-established to describe these non-equilibrium systems with kinetic stability (Solans and Solé, 2012) which can be reached even using less amounts of surfactant than those required for microemulsion preparation (Tadros et al., 2004). Two major groups of methods in terms of energy can be used in order to obtain nanoemulsions. Increasing interest has been observed for low energy methods, since they enable obtainment of nanoemulsions with fine droplets using low cost equipment. Spontaneous emulsification or phase inversion composition are the main mechanism of nanoemulsification on low energy methods (Solans and Solé, 2012).

Oil in water nanoemulsions have been considered very promising for delivery of poor water-soluble larvicidal substances in aqueous media, such as essential oils and other natural products. Thus, it is in the spotlight of research for integrative practices of tropical diseases vector control (Ghosh et al., 2014; Rodrigues et al., 2014; Sugumar et al., 2014). The oleoresin obtained from seeds of *P. emarginatus* was successfully used for the preparation of a potential larvicidal nanoemulsion against *A. aegypti* (Oliveira et al., 2016) and *Culex quinquefasciatus*, the main vector of the tropical neglected disease called lymphatic filariasis or elephantiasis (Oliveira et al., 2017a).

However, to our knowledge, no study was carried out in order to generate an oil in water nanoemulsion with the essential oil from this plant, which also has great bioactive potential and has limited water-solubility. Thus, as part of our ongoing studies with nanobiotechnology

of plant-based larvicidal agents, the aim of the present study was to obtain a novel oil in water nanoemulsion prepared with essential oil from fruits of *P. emarginatus* against *Aedes aegypti* larvae.

## 2. Material and methods

### 2.1. Chemicals

The non-ionic surfactants (sorbitan monooleate – HLB 4.3 and polysorbate 80 – HLB 15) were obtained from Praid (SP, Brasil).

### 2.2. Essential oil extraction

*Pterodon emarginatus* fruits (760 g) were crushed with distilled water. Then, the plant material was placed in a 5 L bottom flask and submitted to hydrodistillation during 3 h using a Clevenger-type apparatus. In the end, the essential oil was collected and stored at 4 °C for further utilization and chemical analysis.

### 2.3. Gas-chromatography analysis

Characterization of the essential oil from fruits of *P. emarginatus* was performed by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 N mass spectrometer. Separation was accomplished with a HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm phase thickness). The essential oil was dissolved in dichloromethane at a ratio of 1:1000 and a volume of 1 µL was injected. Operating conditions were as follows: split ratio 1:20; Injector temperature 250 °C; carrier gas: helium, 1.0 mL/min, constant flow; column temperature, 60 °C (no hold), 3 °C per min to 240 °C. Mass spectra were acquired in electron ionization mode at 70 eV using a scan range of 40–450 *m/z* and a sampling rate of 3.15 scan/s. The ion source temperature was 230 °C, mass analyzer 150 °C and transfer line 260 °C.

The essential oil relative composition was obtained using gas chromatography coupled with flame ionization detection (GC-FID). Analyses were performed using an Agilent 7890 A gas chromatograph and separation was accomplished with a HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm phase thickness). The detector (FID) was operated at 280 °C. The injection procedure and conditions were the same as described above, except the carrier gas, which was hydrogen, at 1.5 mL/min constant flow.

#### 2.3.1. Compounds identification

Linear retention index was calculated by injection of a series of *n*-alkanes (C7–C26) (Van den Dool and Kratz, 1963) using the same column and condition as described above for the GC analyses. The identification of peaks was performed by comparison of mass spectra with an electronic library database (Wiley, 1999) and comparing their calculated linear retention index with the literature data (Adams, 2007).

### 2.4. Preparation of the nanoemulsions

Nanoemulsions were prepared by a low energy titration method (Ostertag et al., 2012). The final mass was 50g and the nanoemulsions contained 2500 µg/mL of *P. emarginatus* essential oil. The essential oil and surfactants (sorbitan monooleate/polysorbate 80) were pooled together and stirred for 30 min. Then, water was added drop wise and the system was stirred for additional 60 min under 800 rpm.

#### 2.4.1. Factors of influence on nanoemulsion formation

Surfactant mixtures with different hydrophile lipophile balance (HLB) values were achieved by mixing sorbitan monooleate (HLB = 4.3) and polysorbate 80 (HLB 15) at different blends according to the following equation:

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