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Original Article

Chemical composition and biological activities of essential oil from flowers of *Psidium guajava* (Myrtaceae)

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(With 1 figure)

Abstract

Xylella fastidiosa is a plant-pathogenic bacterium that lives inside host xylem vessels, where it forms biofilm which is believed to be responsible for disrupting the passage of water and nutrients. *Pectobacterium carotovorum* is a Gram-negative plant-specific bacterium that causes not only soft rot in various plant hosts, but also blackleg in potato by plant cell wall degradation. Chagas disease, which is caused by *Trypanosoma cruzi*, has been commonly treated with nifurtimox and benzonidazole, two drugs that cause several side effects. As a result, the use of natural products for treating bacterial and neglected diseases has increased in recent years and plants have become a promising alternative to developing new medicines. Therefore, this study aimed to determine, for the first time, the chemical composition of essential oil from *Psidium guajava* flowers (PG-EO) and to evaluate its *in vitro* anti-*Xylella fastidiosa*, anti-*Pectobacterium carotovorum*, anti-*Trypanosoma cruzi* and cytotoxic activities. PG-EO was obtained by hydrodistillation in a Clevenger apparatus while its chemical composition was determined by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Major compounds identified in PG-EO were α-cadinol (37.8%), β-caryophyllene (12.2%), nerolidol (9.1%), α-selinene (8.8%), β-selinene (7.4%) and caryophyllene oxide (7.2%). Results showed that the PG-EO had strong trypanocidal activity against the trypomastigote forms of *Trypanosoma cruzi* (IC_{50} = 14.6 µg/mL), promising antibacterial activity against *X. fastidiosa* (MIC = 12.5 µg/mL) and *P. carotovorum* (MIC = 62.5 µg/mL), and moderate cytotoxicity against $LLCMK_2$ adherent epithelial cells in the concentration range (CC_{50} = 250.5 µg/mL). In short, the PG-EO can be considered a new source of bioactive compounds for the development of pesticides and trypanocide drugs.

Keywords: Neglected tropical diseases; bactericide efficacy; guava; α-cadinol; $LLCMK_2$.

Composição química e atividades biológicas do óleo essencial das flores de *Psidium guajava* (Myrtaceae)

Resumo

Xylella fastidiosa é uma bactéria patogênica que vive dentro dos vasos do xilema hospedeiro, onde forma um biofilme responsável por interromper a passagem de água e nutrientes. *Pectobacterium carotovorum* é uma bactéria Gram-negativa que causa não só podridão macia em várias plantas hospedeiras, mas também canela-preta na batata por degradação da parede celular da planta. A doença de Chagas, causada pelo *Trypanosoma cruzi*, é comumente tratada com nifurtimox e benzonidazol, duas drogas que causam vários efeitos colaterais. Como resultado, o uso de produtos naturais para o tratamento de doenças bacterianas e negligenciadas aumentou nos últimos anos e as plantas continuam sendo uma alternativa promissora para o desenvolvimento de novos medicamentos. Portanto, este estudo teve como objetivo determinar, pela primeira vez, a composição química do óleo essencial de flores de *Psidium guajava* (PG-EO) e avaliar suas propriedades anti-*Xylella fastidiosa*, anti-*Pectobacterium carotovorum*, anti-*Trypanosoma cruzi* e citotóxica *in vitro*. PG-EO foi obtido por hidrodestilação em um aparelho Clevenger, enquanto sua composição química foi determinada por cromatografia em fase gasosa com detecção por ionização por chama (CG-DIC) e por cromatografia em fase gasosa acoplada à espectrometria de massa (CG-EM). Os principais compostos identificados no PG-EO foram α-cadinol (37,8%), β-cariofileno (12,2%), nerolidol (9,1%), α-selineno (8,8%), β-selineno (7,4%) e óxido de cariofileno (7,2%). Os resultados mostraram que o PG-EO apresentou forte atividade tripanocida contra as formas tripomastigotas de *T. cruzi* (CI_{50} = 14,6 μg/mL), promissora atividade antibacteriana contra *X. fastidiosa* (MIC = 12,5 μg/mL) e *P. carotovorum* (MIC = 62,5 μg/mL) e citotoxicidade moderada contra células epiteliais aderentes ($LLCMK_2$) na faixa de concentração (CC_{50} = 250,5 μg/mL). Em suma, o PG-EO pode ser considerado uma nova fonte de compostos bioativos para o desenvolvimento de pesticidas e drogas tripanocidas.

Palavras-chave: Doenças tropicais negligenciadas; eficácia bactericida; goiaba; α-cadinol; $LLCMK_2$.

1. Introduction

Xylella fastidiosa, a bacterium found in xylem, is a pathogen that lives in several plant species, such as weeds and fruit trees of economic interest (Coletta-Filho et al., 2016). It is responsible for one of the most important plant diseases that has been reported over the last decades, i. e., a disease whose early symptoms are mostly silent, rather than apparent. An exception is citrus variegated chlorosis (CVC), a disease which is characterized by little yellow spots on leaves and severe fruit deterioration (Coletta-Filho et al., 2016). Fruits get more acid and smaller, since their maturation time is shorter; as a result, their commercial value decreases. In Brazil, the disease was first detected in 1987. In 2002, about a third of the country's orange groves had been infected by *X. fastidiosa*, while in 2016, about 43% of all produce had been overrun by the bacterium. It led to losses and posed negative impact on the economy. CVC may have arrived in Brazil from Argentina in the 1980's when the transportation of infected trees enabled the phytopathogen to quickly disseminate all over South America, where it is now endemic (Coletta-Filho et al., 2016).

Xylella fastidiosa is transmitted by vector insects, such as cicadas that belong to the subfamily Cicadellinae (Hemiptera: Cicadellidae). Synthetic insecticides have been used for controlling these vectors so as to decrease bacterial transmission and the number of infected plants (Bleve et al., 2018). However, resistance to conventional insecticides is the main cause of increase in the number of sick trees. Besides, bacterial infection has been observed even when good practices are applied to culture management. The literature has shown some strategies used against *X. fastidiosa*, such as the production of genetically-modified plants with either proteins or peptides that are capable of killing pathogens and the search for little molecules that may reach the xylem sap flow and inhibit its growth and movement (Bleve et al., 2018).

Soft rot caused by *Pectobacterium carotovorum* is considered a bacterial disease that affects economically important plants, such as lettuce and potato, not only in Brazil, but all over the world (Felix et al., 2014). In Pernambuco, a state in northeastern Brazil, lettuce has been successfully cultivated all along the year but soft rot has caused severe damage, mainly when temperature and humidity are high. *Pectobacterium carotovorum* has also been considered a highly aggressive bacterium which infects potato cultures in tropical and subtropical regions worldwide since 2004. Control of this type of disease has been hindered mainly by the wide range of host plants and the capacity that these bacteria have to survive in residues of cultures. The use of resistant cultivars is considered the most cost-effective and technically viable strategy, since pathogenic populations have resisted to pesticides (Felix et al., 2014; Czajkowski et al., 2015).

Pesticides have been widely used for controlling plant diseases around the world. Although the use of such products has positive short-term effect on producers, their long-term employment has many negative effects on society and the environment, such as soil and water pollution, deposition of agrochemical residues on food and the emergence of resistant pathogens, as mentioned before (Guimarães et al., 2015). In order to reduce negative effects of pesticides, natural products have been investigated so as to control phytopathogens. Therefore, there has been intense search for new antimicrobial agents from plants as the result of increasing resistance of pathogenic microorganisms to synthetic products (Guimarães et al., 2015). Essential oils are some of the natural products that exhibit a wide array of biological properties, such as insecticidal, antimicrobial, antioxidant and bioregulatory properties (Pandey et al., 2013). Regarding their application to agriculture, their antifungal activity against phytopathogens, such as *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides*, should be highlighted (Valadares et al., 2018; Sarkhosh et al., 2018). On the other hand, some essential oils have also exhibited satisfactory activity against several phytopathogenic bacteria, such as *Xanthomonas vesicatoria* and *Agrobacterium tumefaciens* (Vasinauskiene et al., 2006; Gormez et al., 2015).

Neglected tropical diseases are a group of 17 diseases considered to be chronic common infections among the poorest people from less developed countries. The World Health Organization recognizes the urgency of developing new tools and technologies to combat these diseases which are considered to be the world's greatest health problems (Hotez et al., 2016).

Chagas disease or American trypanosomiasis is caused by the flagellate protozoan *Trypanosoma cruzi*, which is transmitted to the human host, mainly by infected triatomines, commonly known as *barbeiro* (Delmondes and Stefani, 2018). This disease affects about 7-8 million Latin American people and the main mode of transmission to people is through feces of the infected vectors (Lavorato et al., 2015). There are other means of contamination such as contaminated blood transfusion, congenital transmission, organ transplantation, and even by ingestion of infected fruits, such as *açaí* (Lavorato et al., 2015; Passos et al., 2012).

In the treatment of Chagas disease, two nitroheterocyclic drugs are widely used: nifurtimox and benznidazole (Andrade et al., 2015). These drugs have several side effects, such as: anorexia, nausea, gastrointestinal disorders, allergic dermatopathy, polyneuritis, bone marrow depression, peripheral neuropathy among others (Oliveira et al., 2008).

Due to these complications caused by available drugs, the search for new chemotherapeutic agents that are effective and have low toxicity becomes increasingly relevant. In this sense, the wide biodiversity of bioactive compounds found in essential oils extracted from plants has increasingly aroused the interest of researchers worldwide (Ngahang Kamte et al., 2018). These essential oils can be obtained from different plant species and have various biological activities, such as: antibacterial, anticancer, anti-inflammatory, antimutagenic, antifungal, antioxidant and antiprotozoal (Raut and Karuppayil, 2014).

Psidium guajava L. belongs to the family Myrtaceae, which comprises about 80 genera and 3,000 species distributed in the tropics and subtropics, mainly in the Americas, Asia and Australia. The genus *Psidium* has around 150 species of bushes; *P. guajava* is the most well-known and most widely distributed one all over the world (Pereira et al., 2017). Guava is considered one of the most important cultures in horticulture in the world's tropical and subtropical regions due to its commercial and nutritional characteristics which result from its aggregated value and high content of vitamin C (Panneerselvam et al., 2012). This species was chosen to be used in this study because of its high yield of essential oils, a fact that has drawn the attention of researchers who aim at improving their use in different cases (Mendes et al., 2018).

Based on previously described facts and on several benefits shown by essential oils, this study aimed at determining, for the first time, the chemical composition and the *in vitro* antibacterial, trypanocidal and cytotoxic effects of essential oil extracted from *P. guajava* flowers (Figure 1) against *X. fastidiosa*, *P. carotovorum*, *T. cruzi* and LLCMK$_2$ adherent epithelial cells.

2. Material and Methods

2.1. Plant material

Flowers were collected at the Instituto Federal Goiano, Rio Verde, Goiás, Brazil (17°48′12.006″S and 50°54′19.083″W), at 5 pm on 21st March 2017. The plant material was identified and samples were deposited as voucher specimens in the herbarium at the State University of Montes Claros in Minas Gerais, Brazil (identification number 4481).

2.2. Essential oil extraction

Essential oil from *Psidium guajava* flowers (PG-EO) was extracted from fresh flowers by hydrodistillation for 3 h in a Clevenger-type apparatus. Hydrodistillation was performed in triplicate. To this end, the plant material was divided into three 100-g samples and 500 mL distilled water was added to each sample. After manual collection of the essential oil, traces of water which remained in the oil were removed with anhydrous sodium sulfate, followed by filtration. PG-EO was stored in an amber bottle and kept in a refrigerator at 4°C until analysis. Calculation of PG-EO yield was based on the weight of fresh flowers and expressed as the average of triplicate analyses.

2.3. Chemical analyses of essential oil (PG-EO)

Gas chromatography-flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS) analyses were performed by Shimadzu QP2010 Plus and GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) systems. GC-MS and GC-FID conditions and the identification of PG-EO have been previously reported (Lemes et al., 2018). Identification of volatile components of PG-EO (Table 1) was based on their retention indices on an Rtx-5MS capillary column under the same operating conditions used for GC relative to a homologous series of *n*-alkanes (C_8-C_{20}). Structures were computer-matched with Wiley 7, NIST 08 and FFNSC 1.2 spectral libraries and their fragmentation patterns were compared with data found in the literature (Adams, 2007).

2.4. Pathogen preparation and identification

Both strains *X. fastidiosa* 9a5c and *P. carotovorum* Pca (424) used by this study were collected from CVC-affected Valencia sweet orange twigs in Macaubal (São Paulo, Brazil) and infected potatoes bought in Ipuíuna (Minas Gerais, Brazil), respectively. Strains were kept in the culture collection at the Laboratory of Research in Applied Microbiology (LaPeMA), University of Franca, São Paulo, Brazil, under cryopreservation at −80°C in periwinkle wilt (PW) broth with glycerol at 20% (v/v).

2.5. Minimum inhibitory concentrations and in vitro determination

Minimum inhibitory concentrations (MICs), i. e., the lowest compound concentrations that are able to inhibit microorganism growth, was determined in triplicate

Figure 1. Leaves and Flowers of *P. guajava* (Myrtaceae).

Table 1. Chemical composition of essential oil from *Psidium guajava* flowers (PG-EO).

Compounds	**RT_{exp}**	**RT_{lit}**	**%RA**
trans-β-Caryophyllene	1413	1414	12.2
α-Humulene	1442	1442	4.3
Nerolidol	1553	1554	9.1
β-Selinene	1475	1476	7.4
α-Selinene	1477	1478	8.8
Germacrene D	1479	1480	0.6
δ-Selinene	1494	1495	0.9
Caryophyllene oxide	1580	1581	7.2
Spathulenol	1583	1584	1.3
Globulol	1611	1611	3.0
Cubenol	1626	1628	2.9
Epi-α-Cadinol	1638	1638	4.5
α-Cadinol	1651	1652	37.8
Sesquiterpene hydrocarbons			34.2
Oxygenated sesquiterpenes			65.8
Total			100.0

RT: Retention time (minutes); **RI_{exp}:** Retention index relative to *n*-alkanes (C_8-C_{20}) on the Rtx-5MS (30 m X 0.25 mm; 0.250 µm) column; **RI_{lit}:** Retention index from the literature (Adams, 2007); **%RA:** relative area (peak area relative to the total peak area in the GC-FID chromatogram).

by using the microdilution broth method on a 96-well polystyrene tissue culture plate (TPP, Trasadingen, Switzerland). The methodology recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012) was followed. PG-EO samples (1 mg) were dissolved in 125 µL dimethylsulfoxide (DMSO; Merck, Darmstadt, HE, Germany) and diluted in PW broth. Then, samples were tested at concentrations ranging from 0.48 to 1,000 µg/mL. Inoculums were adjusted to produce cell concentrations of 1 × 10^6 CFU/mL, as advocated by the CLSI (2012). A growth control with no antibiotic and a sterility control with no inoculum were also included. DMSO at 5% was the maximum DMSO concentration (v/v) in the samples that allowed *X. fastidiosa* 9a5c and *P. carotovorum* Pca (424) to grow normally. Streptomycin (Sigma, St. Louis, MO, USA) was used as the reference antibiotic drug. The 96-well microplate was kept in biological oxygen demand (BOD, Cientec, Brazil) at 28°C for seven days. After incubation, 30 µL of a 0.02% aqueous resazurin (Sigma-Aldrich) solution was added to each well on the microplate. Resazurin is a dye that allows microbial growth to be observed. Blue and red represent absence and presence of microbial growth, respectively (Sarker et al., 2007). The microplate was incubated for additional 24 h so that observation and a descriptive analysis could be carried out.

2.6. *In vitro trypanocidal and cytotoxic activities*

To obtain the trypomastigotes of *T. cruzi*, $LLCMK_2$ adherent epithelial cells were cultured in RPMI medium supplemented with 2 x 10^{-6} mol/L L-glutamine, 10^{-5} mol/L $NaHCO_3$, 100 U/mL penicillin, 100 µg/mL streptomycin and 10% inactivated fetal bovine serum. The procedure was accomplished in culture bottles at 37 °C, under 5% ambient CO_2 and relative humidity of 95%. The trypomastigote forms were maintained in RPMI medium and the parasites were transferred to fresh medium every 48 h to furnish free parasite forms. The assay conducted after 24 h was based on the methodology of Rashed et al. (2016). Approximately 1 x 10^6 trypomastigotes was added to each well in a 96-well microtiter plate. Then, the essential oil was added at concentrations ranging from 12.5 to 200 µg/mL. After 24 h incubation, the biological activity of the samples was evaluated by the colorimetric MTT tetrazolium salt assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL). Readings were conducted by a microplate reader at 517 nm wavelength. Positive and negative controls were benznidazole (from 12.5 to 200 µg/mL) and 0.5% dimethyl sulfoxide (DMSO), respectively. Assays were performed in triplicate.

$LLCMK_2$ adherent epithelial cells were grown in RPMI 1640 medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin and 5% inactivated fetal calf serum. They were kept at 37°C in 5% CO_2. A cell suspension was seeded at a concentration of 1 x 10^6 cells/mL in a 96-well microplate with RPMI 1640 medium. Thereafter, cells were treated with essential oil at different concentrations (6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL). Plates were incubated at 37°C for 24 h and the biological activity was evaluated by the MTT colorimetric method [MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] in a microplate reader at 540 nm. RPMI 1640 medium was the positive control whereas DMSO and RPMI 1640 media were the negative ones. All experiments were performed in triplicate. The percentage of cell viability was determined by the following formula: % cell viability = 1 - [(Y-N)/(N-P)] x 100, where Y = absorbance of wells containing cells and essential oil at different concentrations; N = negative control; and P = positive control (Rashed et al., 2016).

3. Results and Discussion

Thirteen volatile compounds were identified in PG-EO, while its yield was 1.0%. Major compounds of PG-EO were α-cadinol (37.8%), β-caryophyllene (12.2%), nerolidol (9.1%), α-selinene (8.8%), β-selinene (7.4%) and caryophyllene oxide (7.2%) (Table 1). They were determined by GC-FID and GC–MS.

It is important to mention that chemical constituents which predominate in PG-EO had already been previously identified at different concentrations in the essential oil from leaves of this species found in Brazil (Mendes et al., 2018; Souza et al., 2017). When the chemical composition of PG-EO is compared with the one of oils extracted from the same species in other regions around the world, some similarities can be found. In China, major constituents were α-cubebene, caryophyllene, aromadendrene, α-cadinene and calamenene in essential oil from *P. guajava* leaves (Xu et al., 2017). Oils extracted from Tunisian *P. guajava* stems and leaves exhibited the following major constituents: α-humulene, germacrene D, valerenol, viridiflorol and β-caryophyllene (Khadhri et al., 2014). In India, major components of oil from waste leaves of *P. guajava* were cineole, caryophyllene, copaene, azulene and eucalyptol (Kamran et al., 2012). Major components of this essential oil in Nepal were (*E*)-nerolidol and (*E*)-caryophyllene (*P. guajava* leaves) while limonene and β-caryophyllene (*P. guajava* leaves) were identified in Nigeria (Satyal et al., 2015; Ogunwande et al., 2003). In Cuba, major constituents identified in essential oil from *P. guajava* leaves were the following terpenes: β-caryophyllene, (*E*)-nerolidol and selin-11-en-4α-ol (Pino et al., 2001). Even though this study is the first report of the chemical composition of PG-EO (flowers), it may be noticed that the chemical composition found by this study is similar to the chemical composition which has already been described in the literature for other species belonging to the same genus and Myrtaceae family (Stefanello et al., 2011).

In the literature, differences in chemical compositions of essential oils from this botanical species may be due to the method of extraction, region of origin of the plant, climate, soil composition, plant organ, age, seasonality and circadian cycle. These factors affect the quality and quantity of the composition of essential oils, besides biotic and abiotic agents. It is known that chemotypical variation guides the use of plants, since chemical characterization and chemotype identification enable more refined chemical and agricultural analyses to be carried out (Souza et al., 2017; Bouyahya et al., 2019).

Minimum inhibitory concentrations values determined for PG-EO against *X. fastidiosa* and *P. carotovorum* were 12.5 µg/mL and 62.5 µg/mL, respectively. Streptomycin was the positive control and its MIC value was 0.0368 µg/mL. Regarding the antibacterial activity of natural products, Holetz et al. (2002) showed that samples with good, moderate and weak antibacterial activity and inactivity have MIC values below 100 µg/mL, from 100 to 500 µg/mL, from 500 to 1000 µg/mL and above 1000 µg/mL, respectively. Results showed that PG-EO exhibited high *in vitro* antibacterial activity against *X. fastidiosa* (MIC = 12.5 µg/mL) and *P. carotovorum* (MIC = 62.5 µg/mL) and may have promising activity against phytopathogenic bacteria. It should be highlighted that PG-EO exhibited more promising *in vitro* anti-*Xyllela fastidiosa* activity than the ones of seventeen essential oils whose MIC values had already been reported by the literature (Santiago et al., 2018). In addition, essential oil from *P. guajava* leaves has already been described as a natural product which has potential activity against *Sclerotinia sclerotiorum*, a phytopathogen that also causes damage to agriculture (Silva et al., 2018).

The excellent antibacterial activity exhibited by PG-EO against both phytopathogenic bacteria under investigation may be justified by the high concentrations of its major constituents, since they have already had their antibacterial potential described by the literature. For instance, α-cadinol, β-caryophyllene, α-selinene, β-selinene and caryophyllene oxide have already been identified as major constituents of essential oil from *Teucrium yemense, whose satisfactory antibacterial activity has been shown by the* disc diffusion test and the broth microdilution test (Ali et al., 2017). The third major constituent found in PG-EO – nerolidol – may have significantly contributed to good results of anti-*Xylella fastidiosa* and anti-*Pectobacterium carotovorum* activities, since this sesquiterpene has relevant antibacterial activity and has been considered a promising chemical or drug candidate in the field of agriculture by the specialized literature (Chan et al., 2016). According to Bajpai et al. (2011), certain essential oils act in many ways on various types of disease complex and may be applied onto the important crop plants in the same way as other agricultural chemicals. These oils can be used as a leading factor in a wide range of activities against many plant pathogenic bacteria, where these pathogens have developed resistance against the specific bactericide (Bajpai et al., 2011).

It should be emphasized that, in addition to the classes (sesquiterpene hydrocarbons and oxygenated sesquiterpenes) to which the constituents belong, other factors, such as isomerism and synergism among components, must be taken into account when antibacterial activity is evaluated (Costa et al., 2017). In short, the results in this study showed that PG-EO had a strong antibacterial activity against *X. fastidiosa* and *P. carotovorum.* It could be attributed also to its constituents of sabinene, α-pinene, β-pinene, limonene and β-caryophyllene (Table 1), which appear to make the cell membrane permeably and disintegrate the outer membrane of Gram-negative bacteria (Zhang et al., 2017).

In relation to the trypanocidal activity investigated, the essential oil from flowers of *P. guajava* has been shown to be active against trypomastigote forms of *Trypanosoma cruzi*. There was reduction in the viability of trypomastigote cells with increased concentration of essential oil. Thus, the essential oil exhibited satisfactory trypanocidal activity with IC_{50} = 14.6 µg/mL compared to positive control using benznidazole (positive control) with IC_{50} = 9.8 µg/mL (Table 2).

Table 2. Trypanocidal activity *in vitro* of the essential oil from flowers of *P. guajava* (Myrtaceae).

	% of lysis ± S.D./concentration (μg/mL)							
	6.25	**12.5**	**25**	**50**	**100**	**200**	**400**	**IC_{50} (μg/mL)**
PG-EO	17.6±4.2	58.9±4.0	57.5±0.4	79.1±4.9	96.9±0.8	99.9±0.8	99.5±0.4	14.6

PG-EO: essential oil from *P. guajava* flowers; **S.D.** Standard deviation; Positive control: benznidazole (IC_{50} = 9.8 μg/mL).

Table 3. Cytotoxic activity of the essential oil from flowers of *P. guajava* (Myrtaceae)

	% of lysis ± S.D./concentration (μg/mL)							
	6.25	**12.5**	**25**	**50**	**100**	**200**	**400**	**CC_{50} (μg/mL)**
PG-EO	100±0	100±0	100±0	86.3±2.7	62.2±0.9	35.9±1.1	15.2±4.3	250.5

PG-EO: essential oil from *P. guajava* flowers; **S.D.** Standard deviation.

A current study with the essential oil of *Eugenia dysenterica* dried leaves reports that essential oil samples with IC_{50} <10 μg/mL had trypanocidal activity considered to be highly active, active (IC_{50} > 10 < 50 μg/mL), moderately active (IC_{50} > 50 < 100 μg/mL) and inactive (IC_{50} > 100 μg/mL) against trypomastigote forms of *T. cruzi* (Santos et al., 2019).

The antiparasitic activity presented by the essential oil of *P. guajava* flowers can be attributed to the synergism between the constituents present in the essential oil analyzed (Bakkali et al., 2008). In addition, among the constituents present in the oil, there are those that already have recognized trypanocidal activity reported in the literature, such as terpenes: α-cadinol (37.8%), β-caryophyllene (12.2%), nerolidol (9.1%), α-selinene (8.8%), β-selinene (7.4%), and caryophyllene oxide (7.2%) (Table 1) previously identified in the essential oils of the species *Annona vepretorum*, *A. squamosa*, *Cymbopognon giganteus*, *C. nardus*, *C. citratus*, *C. schoenantus*, *Hagenia abyssinica*, *Leonotis ocymifolia*, *Moringa stenopetala,* oils that also had significant trypanocidal effect (Meira et al., 2014; Kpoviessi et al., 2014; Nibret and Wink, 2010).

The various biological activities of essential oils, including against trypanosomatids, are mainly due to their terpenic composition and to the aforementioned synergism among their constituents (Borges et al., 2012). The terpenes are responsible for the hydrophobic nature of the essential oils, allowing their diffusion through the cell membrane of the parasite thus affecting the metabolic pathways and intracellular organelles (Raut and Karuppayil, 2014).

Cytotoxicity was assessed against $LLCMK_2$ cells because the cell medium is the same as the parasites are cultured. The execution of this assay is justified since the efficacy of the sample against the parasites is proved without lysing the healthy cells at the same time as the parasites are lysed. Cultures of $LLCMK_2$ adherent epithelial cells were treated with the essential oil at the concentrations of 6.25, 12.5, 25.0, 50.0, 100, 200 and 400 μg/mL for 24 h. The results showed that the essential oil from flowers of *P. guajava* presented moderate toxicity at the concentration evaluated, presenting CC_{50} = 250.5 μg/mL (Table 3) compared to the benznidazole positive control (CC_{50} = 147.3 μg/mL) and with data already reported in the literature (Carneiro et al., 2017).

It is important to evaluate the cytotoxicity of a given sample because it makes it possible to elucidate the biological mechanism that generates the cytotoxic effect and the mechanism of action of different compounds during their interaction with tissues (51). Toxicity levels are reported in the literature as highly toxic CC_{50} < 10 μg/mL, toxic (10 < CC_{50} < 100 μg/mL), moderately toxic (100 < CC_{50} < 1000 μg/mL), and nontoxic (CC_{50} > 1000 μg/mL) (Andrade et al., 2018; Lima et al., 2012; Camacho et al., 2003). The moderate cytotoxicity exhibited by the essential oil of *P. guajava* flowers against the $LLCMK_2$ adherent epithelial cells is an indicator that this essential oil can be well tolerated by the biological system, however, further studies are still necessary to evaluate its toxicity *in vivo*.

4. Conclusion

In summary, results demonstrated that PG-EO exhibited a mixture of sesquiterpenes in its chemical composition. Its major constituents were α-cadinol, β-caryophyllene, nerolidol, α-selinene, β-selinene and caryophyllene oxide, since they exhibited the highest concentrations. The high concentration of α-cadinol (37.8%) in PG-EO investigated by this study is the prospect of a new source of the secondary metabolite as a raw material in the synthesis of a new bactericide. In addition, results of this study show that there is good prospect of using these essential oil experimentally to control phytopathogens in both greenhouse and field conditions. On the other hand, PG-EO can also be considered the source of an important secondary metabolite which may be applied to agriculture, i. e., nerolidol. From an environmentally sustainable perspective, PG-EO may become an alternative to the use of synthetic pesticides in agriculture and may act as a natural insecticide which is capable of protecting cultures of economic interest. The PG-EO also showed satisfactory trypanocidal activity against *Trypanosoma cruzi* trypomastigote forms and exhibited moderate cytotoxicity against $LLCMK_2$ adherent epithelial cells. In sum, results provide support for further studies of PG-EO which aim at isolating bioactive compounds and investigating its *in vivo* antibacterial, cytotoxic and trypanocidal properties.

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