Brazilian Green Propolis: Chemical Composition of Essential Oil and Their In Vitro Antioxidant, Antibacterial and Antiproliferative Activities

Ricardo Lanzellotti Quintino1
https://orcid.org/0000-0002-6199-3144

Ana Carolina Reis1
https://orcid.org/0000-0001-6858-2746

Cassia Cristina Fernandes2
https://orcid.org/0000-0003-2004-3166

Carlos Henrique Gomes Martins3
https://orcid.org/0000-0001-8634-6878

Ana Carla Colli4
https://orcid.org/0000-0001-8348-9013

Antônio Eduardo Miller Crotti4
https://orcid.org/0000-0002-1730-1729

Iara Silva Squarisi5
https://orcid.org/0000-0003-1962-3702

Arthur Barcelos Ribeiro5
https://orcid.org/0000-0002-4056-9571

Denise Crispim Tavares5
https://orcid.org/0000-0003-4646-5914

Mayker Lazaro Dantas Miranda6*
https://orcid.org/0000-0003-4689-572X

1Federal Institute of Education, Science and Technology of the South of Minas Gerais, Pouso Alegre Campus, Pouso Alegre, Minas Gerais, Brazil; 2Goiano Federal Institute, Rio Verde Campus, Rio Verde, Goiás, Brazil; Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil; 3University of São Paulo, Ribeirão Preto, São Paulo, Brazil; 4University of Franca, Franca, São Paulo, Brazil; 5Federal Institute of Education, Science and Technology of the Triângulo Mineiro, Uberlândia Centro Campus, Uberlândia, Minas Gerais, Brazil.

Received: 2019.07.09; Accepted: 2020.02.18.

*Correspondence: maykermiranda@iftm.edu.br; Tel.: +55-34-993024608 (M.L.D.M)

HIGHLIGHTS

- The chemical composition of propolis varies according to the region where it is produced.
- Production of green propolis in Minas Gerais state, Brazil, is related to the high amount of resin yielded by Baccharis dracunculifolia.
- Major compounds found in essential oil from Brazilian green propolis were carvacrol, acetophenone, spathulenol, (E)-nerolidol and β-caryophyllene.
- Brazilian green propolis showed high antibacterial, antioxidant and antiproliferative activities.

Abstract: Propolis is a resinous substance collected and processed by Apis mellifera from parts of plants, buds and exudates. In Minas Gerais (MG) state, Brazil, green propolis is produced from the collection of resinous substance found in shoot apices of Baccharis dracunculifolia. This paper aims to investigate the chemical composition and in vitro antioxidant, anti-Helicobacter pylori, antimycobacterial and antiproliferative activities of essential oil (EO) from Brazilian green propolis (BGP-EO). The oil showed high antibacterial activity against H. pylori (MIC = 6.25 µg/mL), Mycobacterium avium (MIC = 62.5 µg/mL) and M. tuberculosis (MIC = 64 µg/mL). Its antioxidant activity was evaluated in vitro by both DPPH (IC50 = 23.48 µg/mL) and ABTS (IC50 = 32.18 µg/mL) methods. The antiproliferative activity in normal (GM07492A, lung fibroblasts)
and tumor cell lines (MCF-7, HeLa and M059J) was analyzed by the XTT assay. BGP-EO showed inhibition of normal cell growth at 68.93 ± 2.56 µg/mL. Antiproliferative activity was observed against human tumor cell lines, whose IC₅₀ values were 56.17, 66.43 and 65.83 µg/mL for MCF-7, HeLa and M059J cells, respectively. Its major constituents, which were determined by GC-FID and GC-MS, were carvacrol (20.7 %), acetophenone (13.5 %), spathulenol (11.0 %), (E)-nerolidol (9.7 %) and β-caryophyllene (6.2 %). These results showed the effectiveness of BGP-EO as a natural product which has promising biological activities.

Keywords: free radicals; Helicobacter pylori; Mycobacterium tuberculosis; Mycobacterium avium; tumor cell lines; Baccharis dracunculifoli

INTRODUCTION

Propolis is a resinous material which is used by honeybees (Apis mellifera L.) in hive construction in order to seal exterior wall cracks and prevent insects, such as cicadas, butterflies, moths and beetles, from invading their hives. Propolis is also used inside beehives, since it protects bees against pathogenic microorganisms, such as bacteria, fungi and viruses [1].

Since bees produce it from substances secreted by different plant species, specificities of native vegetation are responsible for the variability found in its chemical composition worldwide [2]. Baccharis dracunculifolia, whose common names in Portuguese are ‘vassourinha’, ‘alecrim do campo’ and ‘alecrim de vassoura’, is native to Brazil and the biological precursor of green propolis. It belongs to the family Asteraceae and has been known for yielding phenolic compounds and essential oils (EO) that bestow high antiulcer, antibacterial and antifungal activities [3-5].

Propolis is a natural product which has promising antioxidant potential since it acts as a body defense agent against free radicals that are found in all organisms. Its composition includes chemical constituents that protect organisms against chronic diseases caused by oxidative stress, such as cancer and metabolic disorders [6].

Some studies have not only shown that propolis exhibits satisfactory antibacterial activity against Helicobacter pylori, but have also highlighted its anti-inflammatory and anesthetic activities [7]. Besides, flavonoids, terpenoids, simple phenolics, pterocarpans, phenylethanoid derivatives, stilbenes and lignans are classes of compounds – found in propolis – which are responsible for its anti-Mycobacterium tuberculosis activity, a fact that justifies its use in folk medicine to treat tuberculosis, for instance [8].

Antiproliferative activity of propolis has also drawn researchers’ interest worldwide, since studies have proven that it may be applied as a nutritional supplement during cancer treatments. In addition, several reports have shown antiproliferative effects of propolis from different origins and their fractions in several cancer cell lines [9-10].

Taking into account the pharmacological potential of extracts and EOs extracted from samples of propolis found all over the world [11-12] and, mainly, from samples of EOs and compounds found in Brazilian green propolis [13-16], this study aimed at determining the chemical composition and in vitro antioxidant, anti-Helicobacter pylori, antimycobacterial and antiproliferative activities of EO from Brazilian green propolis (BGP-EO) found in São Lourenço, a city located in the south of Minas Gerais (MG) state, Brazil.

MATERIAL AND METHODS

EO extraction and GC-FID and GC–MS analyses

Fresh green propolis (500 g) produced by A. mellifera L. (B. dracunculifolia) in the Atlantic Forest in São Lourenço (22°06' 59" S and 45°03' 16" W), MG, Brazil, was purchased at Apiário Esperança (São Lourenço, MG, Brazil) on June 15th, 2017. EO from Brazilian green propolis (BGP-EO) was extracted by hydrodistillation with the use of a Clevenger-type apparatus for 3 h. Hydrodistillation was performed in quintuplicate. To this end, the material was divided into five 100 g samples and 500 mL distilled water was added to each sample. After manual collection of BGP-EO, traces of water which remained in the oil were removed with anhydrous sodium sulfate; filtration followed. BGP-EO was stored in an amber bottle and kept in a refrigerator at 4 °C until analysis. EO yield was calculated from green propolis and expressed as the average of quintuplicate analyses.

Gas chromatography (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 BGP-EO identification have been previously reported [17]. Identification
of volatile components of BGP-EO was based on their Kovats retention index 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 the Wiley 7, NIST 08 and FFNSC 1.2 spectral libraries while their fragmentation patterns were compared with literature data [18].

**Antioxidant assay**

Free radical scavenging activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH\(^*\)) and azino-bis (ethylbenzothiazoline-6-sulfonic acid) (ABTS\(^*\)) were determined by a spectrophotometric method [19], with modifications. In the DPPH assay, different concentrations of BGP-EO in methanol (10–100 \( \mu \)g/mL) were added to 2 mL 0.1 mM solution of DPPH that was previously prepared and incubated in the dark for 30 min. Absorbance was recorded at 517 nm by a UV spectrophotometer. In the ABTS\(^*\) solution was added to 20 \( \mu \)L BGP-EO, previously diluted in ethanol. Absorbance at 734 nm was measured 6 min after initial mixing. BHT was used as positive control. Assays were carried out in triplicate. Inhibition percentage was calculated as \((\%) = (A0 - A/A0) \times 100\), where \( A0 \) is the absorbance of the control and \( A \) is the absorbance of the samples. IC\(_{50}\) value was calculated as the concentration of sample required to scavenge 50% of free radicals by graphing the \( I \% \) versus EO concentration.

**Anti-Helicobacter pylori assay**

Minimum inhibitory concentration (MIC in \( \mu \)g/mL) of BGP-EO was calculated by the broth microdilution method on 96-well microplates. The following ATCC reference strain was used: *Helicobacter pylori* (ATCC 43526). Evaluation of the activity of EO with reference drugs was made by comparing bacterial growth on each plate of *H. pylori*. BGP-EO was dissolved in 5% dimethyl sulfoxide to reach final concentrations ranging between 0.195 and 400 \( \mu \)g/mL. The inoculum was adjusted at 625 nm in a spectrophotometer to produce a cell concentration equal to 5 \( \times \) \( 10^6 \) CFU/mL. Plates were incubated in a CO\(_2\) incubator at 37 °C for 3 days under microaerobic conditions. Tetracycline, at concentrations ranging from 0.115 to 59.0 \( \mu \)g/mL, was employed as the standard drug and incubated under the previously mentioned conditions. After incubation, 30 \( \mu \)L 0.01% aqueous resazurin solution was added to each well to evaluate microbial growth [20]. In addition, plates were incubated at 35 °C for 72 h under microaerophilic conditions and the MBC (Minimum Bactericidal Concentration) was defined as the lowest concentration of BGP-EO without any growth of microorganisms. MBC/MIC ratio was calculated to determine either bactericidal or bacteriostatic effects of BGP-EO under study.

**Antimycobacterial assay**

*Mycobacteria Mycobacterium tuberculosis* H37Rv (ATCC 27294) and *Mycobacterium avium* (ATCC 25291) were obtained from the American Type Collection (ATCC) and maintained at – 80 °C. Antimycobacterial activity of BGP-EO was evaluated by the MIC broth microdilution method conducted on microplates. Resazurin was employed to reveal mycobacterial growth by the Resazurin Microtiter Assay (REMA) method [21], with modifications. BGP-EO was diluted (two-fold) with Middlebrook 7H9 broth (DifcoTM, Detroit, MI, USA). The *Mycobacterium* inoculum was then added to BGP-EO solutions to obtain concentrations ranging from 50 to 2000 \( \mu \)g/mL. Afterwards, inoculated plates were incubated at 37 °C for 42 days (1st reading was carried out after 28 days while the 2nd one occurred after 42 days) and inhibition percentage was determined [22]. Isoniazid was used as positive control at concentrations (MIC =1.47 \( \mu \)g/mL) whereas Middlebrook 7H9 broth and the inoculum were used as solvent and negative control, respectively.

**Antiproliferative assay**

In this study, three different tumor cell lines were used: human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (HeLa) and human glioblastoma (M059J). A normal human cell line (lung fibroblasts, GM07492A) was included to evaluate possible selective activity of the natural product under investigation. Different cell lines were maintained as monolayers in plastic culture medium (HAM-F10 + DMEM, 1:1, Sigma-Aldrich) supplemented with 10% fetal bovine serum (Nutricell), antibiotics (0.01 mg/mL streptomycin and 0.005 mg/mL penicillin; Sigma-Aldrich) and 2.38 mg/mL Hepes (Sigma-Aldrich). Cells were incubated at 36.5 °C in humidified 5% CO\(_2\) atmosphere. Antiproliferative activity was measured by the in vitro Toxicology Colorimetric Assay Kit (XTT; Roche Diagnostics), in agreement with the manufacturer's instructions. In the experiments, cells (10^4 cells/well) were incubated on 96-well microplates. Each well was filled with 100 \( \mu \)L HAM-F10/DMEM medium which contained EO at concentrations ranging from 3.91 to 500
µg/mL. Negative (no treatment) solvent (0.02% DMSO, dimethylsulfoxide, Sigma-Aldrich) and positive (doxorubicin, DXR, Pharmacia Brasil Ltda.) controls were included. After incubation at 36.5°C for 24 h, the culture medium was removed. Cells were washed with 100 µL PBS (phosphate buffered saline) to remove treatments and exposed to 100 µL culture medium HAM-F10 without phenol red. Then, 25 µL XTT was added and cells were incubated at 36.5°C for 17 h. Sample absorbance was determined by a multi-plate reader (ELISA – Tecan – SW Magellan vs 5.03 STD 2P) at wavelength of 450 nm and reference length of 620 nm. Antiproliferative activity was evaluated with the use of IC_{50}, the concentration capable of inhibiting 50% of cell line growth as a response parameter, which was calculated by the GraphPad Prism program that plotted cell survival against concentrations of the natural product under investigation. One-way ANOVA was used for comparing means (p < 0.05). Experiments were performed in triplicate. The selectivity index was calculated by dividing the IC_{50} value of the BGP-EO obtained for GM07492A cells by the IC_{50} value obtained for the cancer cell line.

RESULTS AND DISCUSSION

Yield of EO extracted from green propolis, collected in Brazil, was 0.30 ± 0.15% (w/w). Major compounds found in BGP-EO were carvacrol (20.7%), acetophenone (13.5%), spathulenol (11.0%), (E)-nerolidol (9.7%) and β-caryophyllene (6.2%) (Table 1).

Previous reports of BGP-EO only showed terpenes β-caryophyllene (13.4%), (E)-nerolidol (17.1%) and selina-3,7(11)-diene (10.4%) as its major constituents [23]. Fernandes-Silva and coauthors studied EO from green propolis found in Viçosa, MG, Brazil, and reported the following major constituents: 3-prenylcinnamic acid allyl ester (26.3%), spathulenol (23.4%) and 7-phenyl-5-oxo-heptanol (13.3%) [14]. Taking into consideration that B. dracunculifolia, which is the botanical source of Brazilian green propolis, has been the most studied and exported type of propolis [24], it should be highlighted that, among the previously mentioned compounds, only spathulenol, β-caryophyllene and (E)-nerolidol were identified as major constituents of BGP-EO. Carvacrol, the most abundant chemical constituent in BGP-EO had also been previously isolated from B. dracunculifolia leaves [25].

Chemical constituents identified in BGP-EO may be directly related to the chemical composition of essential oils from B. dracunculifolia, since bees collect raw material from this plant species to yield this type of propolis. Mainly regarding this species of Asteraceae, EO from B. dracunculifolia collected in Pitangui, MG, exhibited high concentration of nerolidol (30.62%) [5]. On the other hand, when it was collected in Viçosa, MG [26], its EO was very similar to BGP-EO, even though most BGP-EO major constituents were found at lower concentrations.

Thus, the origin of green propolis produced by A. mellitfera in MG, Brazil, should be highlighted. It is a resinous substance collected in the shoot apices of B. dracunculifolia. It has a complex chemical composition, whose main components are EO, phenols, polyphenols, flavanones, chalcones and prenylates derived from p-coumaric acid [4]. It is also relevant to mention that bees collect regardless of gender (male or female) and phonological state (flowering or vegetative); the period of high resin collecting visits coincides with the harvest of green propolis in MG [4].
Table 1. Chemical composition of BGP-EO

| Compounds          | %RA | R\textsubscript{exp} | R\textsubscript{lit} |
|--------------------|-----|----------------------|---------------------|
| Benzaldehyde       | 0.3 | 969                  | 970                 |
| Limonene           | 0.4 | 1030                 | 1031                |
| **Acetophenone**   | 13.5| 1076                 | 1078                |
| Perillene          | 1.1 | 1097                 | 1099                |
| Linalool           | 0.9 | 1106                 | 1107                |
| Benzyl nitrile     | 0.8 | 1147                 | 1148                |
| 3-Ethyl-phenol     | 1.6 | 1171                 | 1171                |
| α-Terpineol        | 1.5 | 1188                 | 1189                |
| Decanal            | 0.3 | 1206                 | 1207                |
| Geraniol           | 0.2 | 1227                 | 1228                |
| Geranial           | 0.4 | 1272                 | 1270                |
| Thymol             | 3.0 | 1288                 | 1289                |
| **Carvacrol**      | 20.7| 1297                 | 1298                |
| Citronelly acetate | 0.6 | 1354                 | 1354                |
| α-Copaene          | 3.9 | 1370                 | 1372                |
| β-Elemene          | 1.9 | 1374                 | 1375                |
| α-Gurjunene        | 1.9 | 1408                 | 1410                |
| **β-Caryophyllene**| 6.2 | 1418                 | 1418                |
| Aromandendrene     | 5.2 | 1446                 | 1447                |
| Humulene           | 2.3 | 1455                 | 1455                |
| Alloaromadendrene  | 2.3 | 1460                 | 1460                |
| γ-Muurolene        | 0.2 | 1475                 | 1477                |
| Viridiflorene      | 0.9 | 1503                 | 1505                |
| γ-Cadinene         | 1.0 | 1512                 | 1513                |
| δ-Cadinene         | 1.8 | 1524                 | 1524                |
| Cubenene           | 1.2 | 1533                 | 1533                |
| Selina-3,7(11)-diene| 1.0| 1547                 | 1547                |
| **(E)-Nerolidol**  | 9.7 | 1562                 | 1564                |
| **Spathulenol**    | 11.0| 1578                 | 1578                |
| Viridiflorol       | 1.6 | 1592                 | 1593                |
| α-Cadinol          | 0.3 | 1651                 | 1652                |
| **Total**          |     | 97.7                 |                     |

R\textsubscript{exp}: Retention index relative to \(n\)-alkanes (C\textsubscript{8}-C\textsubscript{20}) on the Rtx-5MS (30 m X 0.25 mm; 0.250 µm) column; R\textsubscript{lit}: Retention index found in the literature [18]; %RA: relative area (peak area relative to the total peak area in the GC-FID chromatogram).

Regarding antioxidant activity, the results show that BGP-EO exhibited significant DPPH free radical activity, with IC\textsubscript{50} values of 23.48 ± 10.11 µg/mL. In the azino-bis (ethylbenzothiazoline-6-sulfonic acid) (ABTS\textsuperscript{+}) method, the value of IC\textsubscript{50} = 32.18 µg/mL was a little higher. The positive control was BHT (butylated hydroxytoluene), whose IC\textsubscript{50} = 19.66 ± 0.07 µg/mL. BHT was chosen to be the positive control because it inhibits both free radical formation and lipid peroxidation effectively [27]. The high antioxidant activity exhibited by BGP-EO may be explained by its major constituents, i.e., the ones that have already had their antioxidant activity reported by the literature: acetophenone [28], carvacrol [29], (E)-nerolidol [30], β-caryophyllene [31] and spathulenol [32]. Green propolis has been highlighted as a natural product that exhibits antioxidant activity, which has recently been evaluated by both DPPH e ABTS\textsuperscript{+} methods for the extract [33].
Antibacterial activity of BGP-EO was evaluated against the \textit{H. pylori} strain; its MIC was measured by the broth microdilution method. BGP-EO proved highly active against \textit{H. pylori} with MIC = 6.25 µg/mL. The positive control was tetracycline whose MIC = 1.0 µg/mL. BGP-EO has shown promising antibacterial activity against \textit{H. pylori} (MIC = 6.25 µg/mL and MBC = 12.5 µg/mL – MBC/MIC = 2). According to Malm and coauthors, MBC/MIC values of samples ≤ 4 suggested their bactericidal activity [34]. It should be highlighted that antibacterial agents are usually regarded as bactericidal if MBC/MIC is up to four-fold the MIC [35]. Anti-\textit{Helicobacter pylori} activity of green propolis extract has already been described by the literature [36]. The chemical and biological study of BGP-EO reported by this paper adds new and valuable information to the promising antibacterial activity of this type of propolis.

Antibacterial activity of BGP-EO was evaluated against the \textit{M. tuberculosis} and \textit{M. avium} strains; its MICs were measured by the broth microdilution method. BGP-EO proved active against \textit{M. tuberculosis} and \textit{M. avium} with MICs = 64 µg/mL and 62.5 µg/mL, respectively. The positive control was isoniazid whose MIC = 1.47 µg/mL. Some authors have considered that MICs ≤ 200 µg/mL of extracts and EO indicate good activity against \textit{M. tuberculosis} [37]. In addition, according to Holetz and coauthors natural products with MIC values lower than 100 µg/mL, between 100 and 500 µg/mL, from above 500 to 1000 µg/mL, and greater than 1000 µg/mL display good antibacterial activity, moderate antibacterial activity, weak antibacterial activity, and absence of antibacterial activity, respectively [38]. Interestingly, propolis has been used as an ingredient in traditional cures for tuberculosis. Previous in vitro studies have shown that extracts from propolis can inhibit the growth of \textit{M. tuberculosis} and synergise the effect of established antitubercular drugs, such as isoniazid, rifampicin and streptomycin [8]. The excellent antibacterial activity against \textit{M. tuberculosis}, \textit{M. avium} and \textit{H. pylori} may be related to the concentrations of its major constituents, whose bactericidal potential has already been proven. They are carvacrol [39], (E)-nerolidol [30], spathulenol [40], β-caryophyllene [41] and acetophenone [42].

Regarding antiproliferative activity, BGP-EO cytotoxicity was evaluated against the GM07492A normal cell line, whose IC\textsubscript{50} was 68.93 ± 2.56 µg/mL, and against MCF-7, HeLa and M059J tumor cell lines whose IC\textsubscript{50} values were 56.17 ± 8.41, 66.43 ± 0.40 and 65.83 ± 0.79 µg/mL, respectively (Table 2). The positive control was doxorubicin and IC\textsubscript{50} values are also shown in Table 2. The lowest IC\textsubscript{50} value was observed for MCF-7 cells, whose SI was 1.22. Even though the activity of EO against tumor cell lines has not been totally clarified yet, Gautam and coauthors stated that mechanisms underlying antiproliferative activity of EO and constituents may reach several ways of cell cycle regulation, which may often overlap [43]. They include the ones involved in apoptosis, cell growth interruption, antimetastatic and antiangiogenic activities, besides the one that leads to increase in the yield of reactive species, such as oxygen. Taking into account that EO are complex mixtures of several bioactive constituents, its synergic activity should also be considered, since synergism may be responsible for their large number of biological activities [44]. However, cytotoxicity of carvacrol, the major constituent of BGP-EO, has been observed against cancer cells [45]. Studies have shown that carvacrol induced cell death mediated apoptosis [46, 47].

**Table 2.** Concentration inhibiting 50\% growth (IC\textsubscript{50}) and selectivity index (SI) of BGP-EO against different cell lines

| Cell line   | Treatment (µg/mL) | BGP-EO | DXR | BGP-EO | DXR |
|-------------|------------------|--------|-----|--------|-----|
|             | IC\textsubscript{50} | SI     | IC\textsubscript{50} | SI     |
| GM07492A    | 68.93 ± 2.56     | 0.50 ± 0.20 |
| MCF-7       | 56.17 ± 8.41     | 1.22   | 62.10 ± 2.00 |
| HeLa        | 66.43 ± 0.40     | 1.03   | 5.30 ± 1.30 |
| M059J       | 65.83 ± 0.79     | 1.04   | 16.20 ± 2.50 |

Doxorubicin (DXR) was used as positive control. GM07492A, human lung fibroblasts; MCF-7, human breast adenocarcinoma; HeLa, human cervical adenocarcinoma; M059J, human glioblastoma. The selectivity index is the ratio between the IC\textsubscript{50} value of BGP-EO obtained for GM07492A cells and the value found for the tumor cell line. Values are mean ± SD, n = 3.
CONCLUSION

Since several classifications of Brazilian propolis, such as yellow, brown, red and green ones, exhibit important biological activities, they have drawn researchers’ interest worldwide. Results of this study showed the promising activities of green propolis collected in São Lourenço, a city located in the south of MG, Brazil. Thus, there was evidence that BGP-EO may constitute an alternative source of compounds with promising activities, such as antioxidant, anti-Helicobacter pylori, antimycobacterial and antiproliferative ones. In sum, these data are valuable, since they show the potential application of BGP-EO to the development of new drugs. The literature has shown that compounds that had previously been identified in EO from Baccharis dracunculifolia and green propolis can guarantee the authenticity of the plant material, green propolis resin and their products. Therefore, these studies corroborate the use of this plant to produce green propolis by honeybees. However, further in vivo studies are needed to ensure and elucidate the mechanism of action of this natural product.

Funding: The authors would like to thank the IFSULDEMINAS – Campus Pouso Alegre for its financial support. Conflicts of Interest: The authors declare no conflict of interest. Funders had no role in the design of the study; in the collection, analyses and interpretation of data; in the writing of the manuscript, nor in the decision to publish the results.

REFERENCES

1. Sahinler N, Kaftanoglu O. Natural product propolis: chemical composition. Nat. Prod. Res. 2005; 19(2): 183-188.
2. Popova M, Reyes M, Conte YL, Bankova V. Propolis chemical composition and honeybee resistance against Varroa destructor. Nat. Prod. Res. 2014 Feb;28(11):788-794.
3. Búfalo MC, Candeias JMG, Sousa JPB, Bastos JK, Sforcin JM. In vitro cytotoxic activity of Baccharis dracunculifolia and propolis against Hep-2 cells. Nat. Prod. Res. 2010 Nov; 24(18):1710-1718.
4. Bastos EMAF, Santana RA, Calaça-Costa AGF, Thiago PS. Interaction between Apis mellifera L. and Baccharis dracunculifolia DC, that favours green propolis production in Minas Gerais. Braz. J. Biol. 2011 Aug;71(3):727-734.
5. Alves KF, Caetano FH, Garcia IJP, Santos HL, Silva DB, Siqueira JM, Tanaka AS, Alves SN. Baccharis dracunculifolia (Asteraceae) essential oil toxicity to Culex quinquefasciatus (Culicidae). Environ. Sci. Pollut. Res. 2018 Nov;25(31): 31718-31726.
6. Calegari MA, Prasniewski AC, Silva C, Sado SY, Maia FMC, Tonial LMS, Oldoni TLC. Propolis from Southwest of Parana produced by selected bees: influence of seasonality and food supplementation on antioxidant activity and phenolic profile. An. Acad. Bras. Ciênc. 2017 Feb;89(1):45-55.
7. Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, Mitov I, Krastev Z. Activity of Bulgarian propolis against 94 Helicobacter pylori strains in vitro by agar-well diffusion, agar dilution and disc methods. J Med Microbiol. 2005 Apr;54(5):481-483.
8. Ali MT, Blicharska N, Shilpi JA, Seidel V. Investigation of the anti-TB potential of selected propolis constituents using a molecular docking approach. Nature 2018 Aug;8(1):12238.
9. Pratsinis H, Kletsas D, Mellieu E, Chinou I. Antiproliferative activity of Greek propolis. J. Med. Food 2010 Apr;13(2): 286-290.
10. Borges KS, Brassesco MS, Scrideli CA, Soares AEE, Tone LG. Antiproliferative effects of Tubi-bee propolis in glioblastoma cell lines. Genet. Mol. Biol. 2011 Feb;34(2):310-314.
11. Ramanauksiene K, Inkeniene AM. Propolis oil extract: quality analysis and evaluation of its antimicrobial activity. Nat. Prod. Res. 2011 Feb;25(15):1463-1468.
12. Bankova V, Popova M, Trusheva B. Propolis volatile compounds: chemical diversity and biological activity: a review. Chem. Cent. J. 2014 May;8(2):28.
13. Tavares LC, Lemos TLG, Arriaga AMC, Santiago GMP, Braz-Filho R. Estudo quimico de uma amostra de própolis verde de Passa Quatro, Minas Gerais, Brasil. Quim. Nova 2010 Oct;33(10):2051-2054.
14. Fernandes-Silva CCF, Lima CA, Negri G, Salatino MLF, Salatino A, Mayworm MAS. Composition of the volatile fraction of a sample of Brazilian green propolis and its phytotoxic activity. J. Sci. Food Agric. 2015 Nov;95(15):3091-3095.
15. Oliveira PF, Lima IMS, Munari CC, Bastos JK, Filho AAS, Tavares DC. Comparative evaluation of antiproliferative effects of Brazilian green propolis, its main source Baccharis dracunculifolia, and their major constituents artepelin C and baccharin. Planta Med. 2014 Apr;80(6):490-492.
16. Figueiredo FJB, Souza MVD, Nascimento EA, Lima LRP. Physicochemical characterization and flavonoid contents of artesanial Brazilian green propolis. Int. J. Pharm. Sci. 2015 Feb;7(3):64-68.
17. Santos LS, Alves CCF, Estevam EBB, Martins CHG, Silva TS, Esperandim VR, Miranda MLD. Chemical composition, in vitro trypanocidal and antibacterial activities of the essential oil from the dried leaves of *Eugenia dysenterica* DC from Brazil. J. Essent. Oil Bear. Pl. 2019 Jun;22(2):347-355.

18. Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, Illinois, USA: Allured Publishing Corporation, 2007.

19. Justus B, Almeida VP, Gonçalves MM, Assunção DPSF, Borsato DM, Arana AFM, Maia BHLNS, Paula JFP, Budel JM, Farago PV. Chemical composition and biological activities of the essential oil and anatomical markers of *Lavandula dentata* L. cultivated in Brazil. Braz. Arch. Biol. Technol. 2018 Nov;61:e18180111.

20. Sarkar SD, Nahar L, Kumarsamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007 Aug;42(4):321-324.

21. Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 2002 Aug;46(8):2720-2722.

22. Gupta R, Thakur B, Singh P, Sharma VD, Katoh VM, Chauhan SVS. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. Indian J. Med. Res. 2010 Mar;131(1):809-813.

23. Albuquerque IL, Alves LA, Lemos TLG, Dorneles CA, Morais MO. Constituents of the essential oil of Brazilian green propolis from Brazil. J. Essent. Oil Res. 2008 Aug;20(5):414-415.

24. Santos RF, Isobe MTC, Laila JG, Haber LL, Marques MOM, Ming LC. Composição química e produtividade dos principais componentes do óleo essencial de *Baccharis dracunculifolia* DC. em função da adubação orgânica. Rev. Bras. Pl. Med. 2012 Mar;14(spe):224-234.

25. Fukuda M, Ohkoshi E, Makino M, Fujimoto Y. Studies on the constituents of the leaves of *Baccharis dracunculifolia* (Asteraceae) and their cytotoxic activity. Chem. Pharm. Bull. 2006 Oct;54(10):1465-1468.

26. Lage TCA, Montanari RM, Fernandes SA, Monteiro CMO, Senra TOS, Zeringota V, Matos RS, Daemon E. Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). Exp. Parasitol. 2015 Jan;148(1):24-29.

27. Bajpai VK, Baek KH, Kang SC. Antioxidant and free radical scavenging activities of taxoquinone, a diterpenoid isolated from *Metasequoia glyptostroboides*. S. Afr. J. Bot. 2017 May;111(1):93-98.

28. Emami S, Esmaili Z, Dehghan, G, Bahmani M, Hashemi SM, Mirzaei H, Shokrzadeh M, Moradi SE. Acetophenone benzoylhydrazones as antioxidant agent: Synthesis, in vitro evaluation and structure-activity relationship studies. Food Chem. 2018 Dec;268(1):292-299.

29. Ramos M, Beltrán A, Peltzer M, Valente AJM, Garrigós MC. Release and antioxidant activity of carvacrol and thymol from polymethylene active packaging films. LWT-Food Sci. Technol. 2014 Oct;58(2):470-477.

30. Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH. Nerolidol: a sesquietherpene alcohol with multi-faceted pharmacological and biological activities. Molecules 2016 Apr;21(5):529.

31. Calleja MA, Veites JM, Montero-Meterdez T, Torres MI, Faus MJ, Gil A, Suárez A. The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Br. J. Nutr. 2013 Feb;109(3):394-401.

32. Nascimento KF, Moreira FMF, Santos JA, Kassuya CAL, Croda JHR, Cardoso CAL, Vieira MC, Ruiz ALTG, Foglio MA, Carvalho JE, Formaggio AN. Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of *Psidium guineense* Sw. and spathulanol. J. Ethnopharmacol. 2018 Jan;210(1):351-358.

33. Augusto-Obara TR, Oliveira J, Gloria EM, Spoto MHF, Godoy K, Vieira TMFS, Scheuermann E. Benefits of superfine grinding method on antioxidant and antifungal characteristic of Brazilian green propolis extract. Sci. Agric. 2019 May;76(5):398-404.

34. Malm A, Glowiński-Lipa A, Korona-Glowniak I, Baj T. Anti-*Helicobacter pylori* activity *in vitro* of chamomile flowers, coneflower herbs, peppermint leaves and thyme herbs – a preliminary report. Curr. Issues Pharm. Med. Sci. 2015 May;28(1):30-32.

35. French GL. Bactericidal agents in the treatment of MRSA infections - the potential role of daptomycin. J. Antimicrob. Chemother. 2006 Oct;58(6):1107-1117.

36. Coelho LGV, Bastos EMAF, Resende CC, Silva CMP, Sanches BSF, Castro FJ, Moretzsohn LD, Vieira WLS, Trindade OR. Brazilian green propolis on *Helicobacter pylori* infection. A pilot clinical study. Helicobacter 2007 Sep;12:572-574.
37. Mota APP, Dantas JCP, Frota CC. Antimicrobial activity of essential oils from *Lippia alba*, *Lippia sidoides*, *Cymbopogon citrates*, *Plectranthus amboinicus*, and *Cinnamomum zeylanicum* against *Mycobacterium tuberculosis*. Ciênc. Rural 2018 Jun;48(06):e20170697.

38. Holetz FB, Pessini LG, Sanches RN, Cortez DAG, Nakamura VC, Day Son BP. Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases. Mem. Inst. Oswaldo Cruz 2002 Oct;97(7):1027-1031.

39. García-Salinas S, Elizondo-Castillo H, Arruebo M, Mendoza G, Irusta S. Evaluation of the antimicrobial activity and cytotoxicity of different components of natural origin present in essential oils. Molecules 2018 Jun;23(6):1399.

40. Vidal CS, Oliveira-Tintino CDM, Tintino SR, Galvao HFB, Costa JGM, Coutinho HDM, Menezes IRA. Chemical composition, antibacterial and modulatory action of the essential oil of *Croton rhamnifoliioides* leaves Pax and Hoffman. Biosci. J. 2016 Dec;32(6):1632-1643.

41. Dahham SS, Tabana YM, Iqbal MA, Ahamed MBK, Ezzat MO, Majid ASA, Majid AMSA. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules 2015 Jun;20(7):11808-11829.

42. Sivakumar PM, Sheshayan G, Doble M. Experimental and QSAR of acetophenones as antibacterial agents. Chem. Biol. Drug Des. 2008 Oct;72(4):303-313.

43. Gautam N, Mantha AK, Mittal S. Essential oils and their constituents as anticancer agents: a mechanistic view. BioMed Res Int. 2014 Jun;1(1):154106.

44. Bhalla Y, Gupta VK, Jaitak V. Anticancer activity of essential oils: a review. J. Sci. Food Agric. 2013 Dec;93(15):3643-3653.

45. Slamova D, Horvathova E. Cytotoxic, anti-carcinogenic and antioxidant properties of the most frequent plant volatiles. Neoplasma 2013 Apr;60(1):343-354.

46. Koparal AT, Zeytinoglu M. Effects of carvacrol on a human non-small cell lung cancer (NSCLC) cell line A549. Cytotechnology 2003 Nov;43(1):149-154

47. Liang WZ, LU. Carvacrol-induced [Ca2+]i rise and apoptosis in human glioblastoma cells. Life Sci. 2012 Mar;90(15):703-711.

© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC) license (https://creativecommons.org/licenses/by-nc/4.0/).