SHORT COMMUNICATION

Hexane extracts from fruit of two varieties of Capsicum chinense Jacq.: their volatile constituents and antiacetylcholinesterase, antileishmanial and antiproliferative activities

Sandriny E. M. Toigo, Cassia C. Fernandes, Iara S. Squarisi, Arthur B. Ribeiro, Denise C. Tavares, Ana C. B. B. Candido, Lizandra G. Magalhães, Felipe F. Moreira, Antônio E. M. Crotti and Mayker L. D. Miranda

ABSTRACT

This article aims to investigate volatile constituents and antiacetylcholinesterase, antileishmanial and antiproliferative activities of hexane extracts from Capsicum chinense fruit (unripe bode pepper ‘HE-UB’ and ripe little beak pepper ‘HE-RB’). HE-UB and HE-RB were screened by the microplate assay method to determine their antiacetylcholinesterase activity. Both exhibited inhibitory potential, i.e., $IC_{50} = 41.5$ and $20.3 \, \mu g/mL$ respectively. HE-UB ($IC_{50} = 67.19 \, \mu g/mL$) and HE-RB ($IC_{50} = 38.16 \, \mu g/mL$) exhibited antileishmanial activity against promastigote forms of Leishmania (Leishmania) amazonensis. In addition, HE-UB and HE-RB demonstrated cytotoxic activity against different human tumor cell lines with $IC_{50}$ ranging from 325.40 to 425.0 $\mu g/mL$. Both GC-FID and GC-MS analyses revealed that the major component in both extracts was E-caryophyllene. In short, HE-RB was more satisfactory than HE-UB in all in vitro activities under evaluation. These findings may be used as initial data for further studies of Capsicum species.

CONTACT Mayker L. D. Miranda maykermiranda@iftm.edu.br

Supplemental data for this article can be accessed online at https://dx.doi.org/10.1080/14786419.2022.2057972.

© 2022 Informa UK Limited, trading as Taylor & Francis Group

ARTICLE HISTORY

Received 20 January 2022
Accepted 21 March 2022

KEYWORDS
non-polar extracts; E-caryophyllene; functional fruit; Alzheimer’s disease; antiparasitic activity; tumor cell lines
1. Introduction

The Alzheimer’s Disease (AD) is a neurodegenerative disorder which initially affects people’s memory and, later, their reasoning capacity and communication (Agatonovic-Kustrin et al. 2019). Since its treatment consists in trying to restore the cholinergic function, acetylcholinesterase (AChE) inhibitors have been widely used to modulate activities of enzymes that degrade acetylcholine (ACh) (Agatonovic-Kustrin et al. 2019). Pharmaceuticals that inhibit AChE are costly and lead to several adverse effects, a fact that makes it relevant to search for and develop alternative inhibitors, mainly the ones that derive from medicinal plants (Patel et al. 2018). Well-known researchers have also expressed concern for neglected diseases, such as leishmaniasis (Anversa et al. 2018). Leishmaniasis is caused by protozoa of the *Leishmania* genus and affects about 350 million people worldwide (Santana et al. 2014). Drugs used for treating this disease consist of pentavalent antimonials, which are highly toxic and cause adverse side effects. These issues also reinforce the need to find new therapeutic agents of natural origin that are safer and less aggressive to health (Santana et al. 2014). Another worrying scenario is the treatment of tumors and drugs used in chemotherapy. Researchers in the area of chemistry of natural products have worked hard to discover plants that are promising sources of metabolites with antiproliferative potential (Marrelli et al. 2020). In this respect, *Capsicum chinense* species and their varieties have been highlighted among plants that have biological potential (Figure S1). The literature has shown that their fruit are rich in biological activities, such as antifungal, antioxidant, antidiabetic, antitumor, antithrombotic and antimicrobial ones (Menichini et al. 2009; Sosa-Moguel et al. 2017; Buitimea-Cantúa et al. 2020). To the best of our knowledge, few studies have investigated antiproliferative, antileishmanial and antiacetylcholinesterase activities of Brazilian *Capsicum* species (Domenico et al. 2012). Therefore, this study aimed to evaluate (i) antiacetylcholinesterase, antileishmanial and antiproliferative activities of hexane extracts from fruit borne by two varieties of *Capsicum chinense* Jacq. (unripe bode pepper and ripe little beak pepper); and (ii) their volatile constituents identified by GC-FID and GC-MS.

2. Results and discussion

Volatile constituents of hexane extracts from *C. chinense* fruit – unripe bode pepper (HE-UB) and ripe little beak pepper (HE-RB) – were identified by gas chromatography–flame ionisation detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS). Firstly, remarkable *E*-caryophyllene concentrations were found in both extracts, i.e., 49.4% in HE-RB and 21.6% in HE-UB (Table S1). Besides, HE-RB exhibited *E*-caryophyllene and *n*-octyl isobutyrate (6.1%) as its major constituents. However, HE-UB exhibited five other major constituents: hexyl isovalerate (8.5%), 4-methyleclocane (7.6%), isobutyl caprate (7.5%), octadecanal (5.2%) and hemimellitene (5.0%). *C. chinense* fruit of Cuban origin had high chemical variety of volatile constituents while their major constituents were hexyl isopentanoate, hexyl pentanoate, hexyl 2-methylbutanoate, 3,3-dimethylcyclohexanol, c-himachalene and germacrene D (Pino et al. 2011). A recent study reported that different types of esters are commonly found in
Capsicum species (C. chinense, C. frutescens, C. annuum, C. baccatum and C. pubescens) and mentioned that 3-methylbutanoyl moiety is apparently a characteristic of C. chinense (Murakami et al. 2019). The volatile composition found by the study reported by this short communication, which refers to C. chinense fruit grown in Goiás (GO) state, Brazil, is very similar to the one of fruit borne by two other C. chinense varieties found in Brasília, Brazil’s capital (Garruti et al. 2013). An important similarity is the remarkably high E-caryophyllene concentration (60.0%) identified in a variety of C. chinense known as seriema in Brazil (Garruti et al. 2013). Antiacetylcholinesterase activity of HE-UB and HE-RB was screened by the microplate assay method and both hexane extracts exhibited inhibitory potential, whose IC$_{50}$ values were 41.5 and 20.3 μg/mL, respectively (Table S2). The positive control physostigmine exhibited IC$_{50}$ = 1.3 μg/mL. Ogunruku et al. (2014) evaluated antiacetylcholinesterase activity of aqueous extract prepared with C. chinense ripe and unripe fruit and found that it also has inhibitory potential. Antiacetylcholinesterase activity of ethanolic extract from C. chinense ripe fruit was also evaluated and its IC$_{50}$ was 18.8 μg/mL (Vargas-Méndez et al. 2016), a little lower than the one of HE-RB. Thus, IC$_{50}$ values of HE-UB and HE-RB may be considered highly satisfactory. The literature has shown that extracts from medicinal plants, such as the ones from Blumea lacera (IC$_{50}$ = 150 μg/mL) and Cyclea barbata (IC$_{50}$ = 176 μg/mL), are strong inhibitors of acetylcholinesterase (Uddin et al. 2021). Regarding antileishmanial activity, HE-UB (IC$_{50}$ = 67.19 μg/mL) and HE-RB (IC$_{50}$ = 38.16 μg/mL) were moderately active and active, respectively, against promastigote forms of Leishmania amazonensis (Table S2). Researchers have stated that samples whose IC$_{50}$ values range between 10 and 50 μg/mL are active, while the ones whose IC$_{50}$ values range from 50 to 100 μg/mL are moderately active (Cabral et al. 2020). Furthermore, HE-UB and HE-RB had their cytotoxic potential against different human tumor cell lines evaluated. Extracts exhibited IC$_{50}$ ranging from 325.40 to 425.0 μg/mL after a 24-h treatment (Table S3). HE-RB showed IC$_{50}$ (373.80 μg/mL) in the U251 cell line significantly lower than in the non-tumor cell line (GM07492A, 447.30 μg/mL), thus, revealing a selective effect. It should be emphasised that both HE-UB and HE-RB exerted cytotoxic effect on human non-tumor cell line at concentrations that were higher (343.06 and 447.30 μg/mL, respectively) than those in which they revealed antileishmanial effect. Therefore, extracts exerted antileishmanial activity at non-cytotoxic concentrations. This study suggests that activities exhibited by both HE-UB and HE-RB may be related to their high E-caryophyllene concentrations since it is a promising constituent that has well-known antileishmanial, anticholinesterase and antitumor activities (Soares et al., 2013; Moreira et al., 2019; Salleh and Khamis 2020).

3. Experimental

Supplementary material related to this article is available online, alongside Tables S1–S3 and Figure S1.

4. Conclusions

This study showed the chemical and biological potential of hexane extracts from ripe and unripe fruit borne by two varieties of pepper (Capsicum chinense Jacq.) grown in
Brazil: bode pepper and little beak pepper. Resulting hexane extracts exhibited the same chemical constituents but differed significantly in terms of their concentrations. The only similarity is that the sesquiterpene $E$-caryophyllene was identified as the major constituent in both HE-UB and HE-RB. Concerning biological activities under evaluation, both extracts exhibited promising IC$_{50}$ values but the ones of HE-RB were more satisfactory. In short, the study of extracts from medicinal plants is a key step to achieve quality research outcomes.

**Disclosure statement**

No potential conflict of interest is reported by the authors.

**Funding**

The authors would like to thank CAPES, FAPEG, CNPq and IFGOIANO - *Campus* Rio Verde for their financial support.

**ORCID**

Cassia C. Fernandes [http://orcid.org/0000-0003-2004-3166](http://orcid.org/0000-0003-2004-3166)
Iara S. Squarisi [http://orcid.org/0000-0003-1962-3702](http://orcid.org/0000-0003-1962-3702)
Arthur B. Ribeiro [http://orcid.org/0000-0002-4056-9571](http://orcid.org/0000-0002-4056-9571)
Denise C. Tavares [http://orcid.org/0000-0003-4646-5914](http://orcid.org/0000-0003-4646-5914)
Lizandra G. Magalhães [http://orcid.org/0000-0003-2959-6752](http://orcid.org/0000-0003-2959-6752)
Antônio E. M. Crotti [http://orcid.org/0000-0001-6047-4871](http://orcid.org/0000-0001-6047-4871)
Mayker L. D. Miranda [http://orcid.org/0000-0003-4689-572X](http://orcid.org/0000-0003-4689-572X)

**References**

Agatonovic-Kustrin S, Kustrin E, Morton DW. 2019. Essential oils and functional herbs for healthy aging. Neural Regen Res. 14(3):441–445.

Anversa L, Tiburcio MGS, Richini-Pereira VB, Ramirez LE. 2018. Human leishmaniasis in Brazil: a general review. Rev Assoc Med Bras (1992). 64(3):281–289.

Buitimea-Cantú GV, Velez-Haro JM, Buitimea-Cantú NE, Molina-Torres J, Rosas-Burgos EC. 2020. GC-EIMS analysis, antifungal and anti-aflatoxigenic activity of *Capsicum chinense* and *Piper nigrum* fruits and their bioactive compounds capsaicin and piperine upon *Aspergillus parasiticus*. Nat Prod Res. 34(10):1452–1455.

Cabral FD, Fernandes CC, Ribeiro AB, Squarisi IS, Tavares DC, Candido ACBB, Magalhães LG, Souza JM, Martins CHG, Miranda MLD. 2020. Bioactivities of essential oils from different parts of *Spiranthera odoratissima* (Rutaceae). Rodriguésia. 71:e00902019.

Domenico CI, Coutinho JP, Godoy HT, Melo AMT. 2012. Caracterização agronômica e pungência em pimento de cheiro. Hortic Bras. 30(3):466–472.

Garruti DS, Pinto NOF, Alves VCC, Penha MFA, Tobaruela EC, Araújo IMS. 2013. Volatile profile and sensory quality of new varieties of *Capsicum chinense* pepper. Ciênc Tecnol Aliment. 33:102–108.

Marrelli M, Pisani F, Amodeo V, Duez P, Conforti F. 2020. *Echinophora tenuifolia* L. branches phytochemical profile and antiproliferative activity on human cancer cell lines. Nat Prod Res. 34(18):2664–2667.
Menichini F, Tundis R, Bonesi M, Loizzo MR, Conforti F, Statti G, Cindio B, Houghton PJ, Menichini F. 2009. The influence of fruit ripening on the phytochemical content and biological activity of Capsicum chinense Jacq. Cv habanero. Food Chem. 114(2):553–560.

Moreira RRD, Santos AG, Carvalho FA, Perego CH, Crevelin EJ, Crotti AEM, Cogo J, Cardoso MLC, Nakamura CV. 2019. Antileishmanial activity of Melampodium divaricatum and Casearia sylvestris essential oils on Leishmania amazonensis. Rev Inst Med Trop São Paulo. 61:e33.

Murakami Y, Iwabuchi H, Ohba Y, Fukami H. 2019. Analysis of volatile compounds from chili peppers and characterization of habanero (Capsicum chinense) volatiles. J Oleo Sci. 68(12):1251–1260.

Ogunruku OO, Oboh G, Ademosun AO. 2014. Water extractable phytochemical from peppers (Capsicum spp.) inhibit acetylcholinesterase and butyrylcholinesterase activities and prooxidants induced lipid peroxidation in rat brain in vitro. Int J Food Sci. 2014:1–7.

Patel SS, Raghuwanshi R, Masood M, Acharya A, Jain SK. 2018. Medicinal plants with acetylcholinesterase inhibitory activity. Rev Neurosci. 29(5):491–529.

Pino J, Fuentes V, Barrios O. 2011. Volatile constituents of cachucha peppers (Capsicum chinense Jacq.) grown in Cuba. Food Chem. 125(3):860–864.

Salleh WMNHW, Khamis S. 2020. Chemical composition and anticholinesterase inhibitory activity of Pavetta graciliflora Wall. Ex. Ridl. Essent Oil. Z Naturf. 75:467–471.

Santana LCLR, Carneiro SMP, Caland-Neto LB, Arcanjo DDR, Moita-Neto JM, Citó AMGL, Carvalho FAA. 2014. Brazilian brown propolis elicits antileishmanial effect against promastigote and amastigote forms of Leishmania amazonensis. Nat Prod Res. 28(5):340–343.

Soares DC, Portella NA, Ramos MFS, Siani AC, Saraiva EM. 2013. Trans-beta-caryophyllene: an effective antileishmanial compound found in commercial copaiba oil (Copaifera spp.). Evid Based Complement Alternat Med. 761323.

Sosa-Moguel O, Pino JA, Ayora-Talavera G, Sauri-Duch E, Cuevas-Glory L. 2017. Biological activities of volatile extracts from two varieties of habanero pepper (Capsicum chinense Jacq.). Int J Food Proper. 20(sup3):S3042–S3051.

Uddin MJ, Russo D, Rahman M, Uddin SB, Halim MA, Zidorn C, Milella L. 2021. Anticholinesterase activity of eight medicinal plant species: in vitro and in silico studies in the search for therapeutic agents against Alzheimer’s disease. Evid Based Compl Alternat Med. 2021:9995614.

Vargas-Méndez LY, Rosado-Solano DN, Sanabria-Flórez PL, Puerto-Galvis CE, Kouznetsov V. 2016. In vitro antioxidant and anticholinesterase activities and in vivo toxicological assessment (Zebrafish embryo model) of ethanolic extracts of Capsicum chinense Jacq. J Med Plants Res. 10:59–66.