December 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.3 • 15-27

ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Piper amalago* L. (PIPERACEAE) COLLECTED IN COASTAL ECUADOR

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Abstract

The essential oil of the leaves from *Piper amalago* L. (Piperaceae) obtained through hydrodistillation was analyzed by gas chromatography and gas chromatography-mass spectroscopy (GC/MS). The antibacterial and antifungal activities of the volatile oil was evaluated by the broth microdilution technique and was tested against *Gram* positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and *Gram* negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria, and the yeast *Candida albicans*. Altogether 38 compounds (90.16%) were identified in the sample and the major constituents found were β-phellandrene (20.42%), spathulenol (10.34%), bicyclogermacrene (8.50%) and α-pinene (7.29%). The essential oil howed a moderate antibacterial effect against *S. aureus* and *E. faecalis* (MIC 100-200 µg/mL). Additionally, *E. coli* was the *Gram* negative strain resulting be most susceptible (MIC 50-200 µg/mL), since the oil had little ability to inhibit the growth of *P. aeruginosa* and *K. pneumoniae* (MIC > 1000 µg/mL). A weak antifungal efect was showed against *C. albicans*. These results suggest that the *P. amalago* essential oil may be used as antimicrobial agent in biotechnological processes employed in food, pharmaceutical and other industries. According to the literature consulted, this is the first report on the antimicrobial activity of the essential oil of *P. amalago* from Ecuador.

Keywords: *Piperaceae, Piper amalago, antimicrobial activity, essential oil.*

Introduction

The acuteness in the prevalence of infectious diseases produced by antimicrobial resistant pathogens [1,2] together with the accelerated increase of antibiotic resistance genes into the environment in recent years [3-7], are a huge challenge for medical and environmental sciences worldwide.

Since ancient time a large portion of the world`s population, depending directly on traditional medicine for the cure of numerous infectious diseases [8]. In this context, is estimated that about 26% plants species are used to treat infections caused by bacteria, viruses, fungi and parasites out of 3118 medicinal plants reported in Ecuador [9]. The plants play an important role in providing an endless treasure of antimicrobial biomolecules [10-12].

Piper is the nomenclatural type of the family Piperaceae considered to be among the most antique families of global distribution flowering plants [13,14]. This genus is composed of about 1000 species [14] growing in tropical and subtropical regions of both hemispheres [15,16], where have commercial and economical importance since they are popularly used for centuries as dietary spices and remedies in folklore medicine [15,17,18]. In Ecuador, *Piper* is a representative genus of the family Piperaceae [19] being one of the most prominent for its numerous applications in rural daily life, with important reports of medicinal uses registered to cure several diseases [9].

Numerous scientific reviews on ethnomedical uses, phytochemistry and biological activities on species of the genus *Piper* have been published [20-26]. Phytochemical investigations of *Piper* species revealed the presence of a secondary metabolites set that belong to different chemical types: alkaloids, amides, aristolactams, arylpropanoids, aldehydes, ketones, volatile oils, chalcones, flavones, lignans, neolignans, long and short chain esters, phenylpropanoids, monoterpenes, sesquiterpenes, among others [15,27-35], that have been isolated from the seeds, leaves, and/or stem bark. Extracts and pure compounds obtained from many *Piper* plants have been examined through mainly *in vitro* studies and showed a wide variety of biological properties, many of which were based on their ethnomedical use [15,16,36-46].

Piper amalago L. has been extensively used in folk medicine as antipyretic [47], vermifuge [48], diuretic and against renal stones [49] as well as in several conditions, including stomachache [47,50], headache, chest pain [47], menstrual pain [51], toothache [52] for treatment of snakebites [53], skin inflammations such as rashes, sores and burns [47,54,55]. Furthermore, is used as a "blood tonic" [56], to prevent miscarriage and alleviate female disorders during pregnancy and postpartum [47,50].

Pharmacological researches points out that extracts, fractions and/or compounds isolated of *P. amalago* have different biological properties such as anti-inflammatory [57], healing [55], leishmanicidal [58,59], schistosomicidal [59] diuretic and antilithiasic [60], anxiolytic [61], antioxidant [62] antinociceptive and antihyperalgesic [63].

Essential oils are one of the major chemical constituents of *Piper* species [49,34], but their yield and composition can vary significantly depending on species and collection conditions [34,64]. A number of investigations has showed the volatile components of leaves from *P. amalago*, which monoterpenes and sesquiterpenes are predominant compounds [35, 49,65,67-71,]. Mezquita *et al*. [65] showed as the main compounds elemene (36.5%), E-caryophyllene (17.80%), bicyclogermacrene (16.4%) and germacrene D (10.9%), caryophyllene oxide (18.0 %) and α-pinene (9.30%). In a study developed by Potzernheim *et al*. [66] the occurrence of α-pinene (30.5%), camphene (8.9%) limonene (6.8%) and borneol (5.7%) were reported in the highest concentrations. In 2010, the main compounds determined in the essential oil of *P. amalago* by Morandim *et al*. [67] were bicyclogermacrene (27.91%), spathulenol (19.22%), germacrene D (9.94%), α-cadinol (7.6%) and γ-muurolene (7.27%), in the same year Ferraz *et al*. [68] revealed limonene (20.52%), α-pinene (5.23%), zingiberene (11.18%), δ-elemene (6.82%), and β-caryophyllene (4.69%) and da Carrara *et al*. [69] found β-copaen-4-α-ol (26%), 7-epi-α-eudesmol (21.84%), epi-α-cadinol (12.70%), and n-hexyl-benzoate (12.29%). da Silva Mota *et al*. [49],

pointed out α-amorphene (25.7 %), p-cymene (9.4 %) and (E)-methyl geranate (7.8 %) as the major constituents. In 2016, on the one hand, Santos *et al.* [70] indicated the presence of β-phellandrene (13.64%), E-nerolidol (8.08%), α-muurulene (7.85%), δ-elemene (6.42%), germacrene D-4-ol (5.54%), β-cedrene (5.15%), and α-cadinol (4.96%) and on the other hand, Vas et al. in their study carried out with *P. amalago* from four different locations showed qualitative and quantitative differences. They found β-phellandrene (12.3-39.3%) in all oils, α-pinene (6.7-14.8%) and bicyclogermacrene (15.0-20.8%) in three oils, sabinene (6.3-8.2%) and spathulenol (5,6-9,1%) in two oils, and germacrene D (11.7%), myrcene (6.8%), γ-muurolene (5.9%) in one oil [71]. Finally, in a recent work, was revealed a total of 322 compounds with the predominance of bicyclogermacrene (12.95%), limonene (6.15%), α pinene (3.73%) and δ-cadinene (3.90%) [35].

Several pharmacological properties have been investigated in the volatile oil of leaves from *P. amalago* collected in Brazil, such as antifungal [69] antibacterial [70,71] and antilithiasic [35]. However, no scientific investigation has yet been undertaken to examine the essential oil chemical composition and antibacterial activity of *P. amalago* in the Ecuador. Taking into account the exploration of new alternatives for antimicrobial potentials based on essential oils, this study was conducted to evaluate the oil chemical constituents from leaves of *P. amalago* and their biological activity against six microbial species including *Gram* positive and *Gram* negative bacteria, as well as a yeast strain, which are representative for commonly involved microorganisms in human infections. According to our understanding, this is the first report on the composition and antimicrobial activity of essential oil from *P. amalago* from Ecuador.

Methods

Collection and identification of plant material:

Leaves of *P. amalago* were harvested in September 2015, at the area of Provincia Guayas, cantón Santa Elena, cordillera Chogón Colonche of the commune Olón, Ecuador at 375 m above sea level. The botanical identification was carried out by MSc. Xavier Cornejo and a voucher specimen was deposited in the GUAY Herbarium, Faculty of Natural Science, University of Guayaquil, Ecuador, under code number *Cornejo 2863*.

Extraction of the essential oil:

To obtain the essential oil from *P. amalago*, fresh botanical material (300 g) was cut into small pieces and submitted to hydrodistillation in a Clevenger-type apparatus for 4 h. The oil obtained was collected, dried over anhydrous sodium sulfate and stored in an amber glass vial at 4°C prior to testing and analysis. The calculated yield was 0.2% (v/w), based on fresh plant material.

Chemical composition of the essential oil:

The essential oil was analyzed by GC-MS using an Agilent gas chromatography (model 6890N series) coupled to a mass spectrometer-detector (model Agilent series 5973 inert). A non-polar column DB-5MS 5% phenyl-methylpolyxilosane glycol (Agilent 122-5532) 30 m x 0.25 mm, thickness 0.25 µm film was used. The source temperature was 200°C and the quadrupole temperature 200°C. Helium was used as a carrier gas at 0.7 mL/min. The ionization energy was 70 eV, and the scan range 30-350 amu at 3.9 scan/s. Essential oil was diluted (1:100) in dichloromethane. The injected volume was 1.0 µL. A automatic injector (series 7673) was used with a split ratio of 1:50. The initial oven temperature was programed at 50°C; this was then raised to 230°C at 3°C/min, and the final temperature maintained for 66 min. The injector and detector temperatures were 230°C and 250°C, respectively.

GC-FID analysis was performed on an Agilent chromatograph (model 6890N series) equipped with flame ionization detector. The same capillary column and analytical parameters as those used in the GC-MS measurement was also used in the GC-FID analysis.

The retention indices were determined based on the retention times of a mixture of the homologous series of n-alkanes, from C9 to C23, which were injected after the oils under the same conditions. The essential oil components were identified by comparison of their respective mass spectra with

reference spectra and their retention indices with those authentic compounds or data in the literature [72,73]. The quantitative data were obtained electronically from FID area percentage without the use of correction factor.

Microbial strains and preparation of cultures:

Six microorganisms tested for their susceptibility to the essential oil were either from the American Type Culture Collection (ATCC) provided by the Research Laboratory of the Faculty of Health Sciences of the National University of Chimborazo. The assay included *Gram* positive bacteria *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, *Gram* negative bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603 and the yeast *Candida albicans* ATCC 10231. The bacterial cultures were developed in nutrient broth (NB) except for *E. faecalis* that was grown in brain-heart infusion (BHI), and the yeast was cultured in sabouraud dextrose broth (SDB) at 37°C for 24 h in orbital agitation. All media were purchased from Oxoid.

Determination of antimicrobial activity:

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the essential oil were determined against each microbial strain in triplicate, by means of the broth microdilution method in 96-well plates [74]. The concentrated solution of the oil was prepared in DMSO at 50 mg/mL, and dilutions were made in culture medium (NB or BHI in the case of bacteria and SBD for yeast) at twice the initial concentration required, from which serial dilutions were made descending down the microtiter plate columns. The concentrations ranged between 50 and 3200 µg/mL. After the preparation of an inoculum for each microorganism, 100 µL was added to each well where the initial microbial density was approximately 1-5 × 10^5 CFU/mL. Microbial Cell treated with DMSO, at the maximum concentration used in the samples, served as negative control to ensure that the solvent had no adverse effect on bacterial growth. The respective growth media of each microorganism were also included to ensure the support of microbial growth. After incubation for 24 h at 37°C in orbital shaking, the cell growth was monitored in a microplate reader (Biotek) at 550 nm and aliquots (100 µL) from all wells without visible growth were subcultured on nutritive, brain-heart infusion and sabouraud agar plates. After overnight incubation colony counts (viable cells) were performed in order to establish the MIC as the lowest concentration of the oil at which there was not growth and MBC or MFC as the lowest concentration of the oil that produced ≥ 99.9% killing of the initial inoculum.

Results

Chemical composition of the essential oil:

The quantitative and qualitative analysis of essential oil from *P. amalago* performed by GC and GG-MS is summarized in table 1. The results show that 38 compounds representing 90.16% of the total content were identified in the leaf volatile oil. In this investigation the chemical analysis of the leaf essential oil revealed the presence of the major constituent β-phellandrene (20.42%), followed by spathulenol (10.34%), bicyclogermacrene (8.50%), and α-pinene (7.29%).

Antimicrobial activity:

The MIC, MBC and MFC results of leaf essential oil from *P. amalago* against *Gram* positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and *Gram* negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria, and the yeast *Candida albicans* by using the microdilution technique are presented in Table 2. As demonstrated, the essential oil exhibited variable degrees of bacterial and fungal growth inhibition. The results indicate that the oil presented a moderate activity against *S. aureus* and *E. faecalis* for which the MIC ranged from 100 to 200 µg/mL with MBC of 400 µg/mL. The susceptibility of the *Gram* negative bacterial strains to oil compounds was different, a good inhibitory activity was observed against to *E. coli* since displayed a MIC from 50 to 100 µg/mL with MBC value of 200 µg/mL; the oil resulted inactive against *P. aeruginosa* and *K.*

pneumoniae (MIC > 1000 µg/mL). On the other hand, in the antifungal test, *P. amalago* oil presented weak activity against the yeast *C. albicans* which presented a MIC of 800 to 1600 µg/mL and a MFC of 1600 µg/mL. Some researchers consider that if the

Discussion

The monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes were the most abundant chemical fractions, in 33.34%, 27.37% and 27.31%, respectively. Previous study on chemical analysis of essential oil obtained from the leaves of this plant also reported the predominance of monoterpene hydrocarbons [35,66,68], sesquiterpene hydrocarbons [35,49,65,67-70,] and oxygenated sesquiterpenes [35,49,65,66,67,70].

On the basis of percentages of main compounds, was showed a difference between the chemical composition of *P. amalago* from the Ecuador and that reported in Brazilian species [35,49,65-71]. The percentage of β-phellandrene (20.42%) found was greater than that described for leaf oil from *P. amalago* (13.64%) collected in the Curitiba [70]. On the other hand, Perigo *et al*. [71] in the chemical analysis of the essential oil of *P. amalago* from four locations of Atlantic rainforest in Brazil reported higher percentages (39.3 and 33.1%) in two oils and lower percentages (15.9 and 12.3%) in the other oils.

Other major constituents identified in the present study as spathulenol, bicyclogermacrene and α-pinene have also been reported for *P. amalago* collected in different regions of Brazil. Spathulenol, have been found in lower quantity (10.34%) than the found in the leaf oil from this specie harvested at the Campus of the University of São Paulo (19.22%) [67]. Also, in the present investigation, bicyclogermacrene was found in a lower percentage (8.50%) to those reported for *P. amalago* collected in the state of Minas Gerais (16.4%) [65], at the Campus of the University of São Paulo (27.91%) [67] and at Dourados-MS (12.95%) [35]. Finally, α-pinene presents in this study in 7.29%, has been identified at higher percentages in oils of this plant from the locations: state of Minas Gerais (9.30%) [65], Federal District (30.5%) [66]; and at lower percentages at the Morro Reuter, Picada Café and Sao Francisco de Paula (5.23%) [68] and in Dourados-MS (3.73%) [35]. Different main compounds have been reported in essential oils of *P. amalago* collected in Paraná [69] and at Dourados-MS [49].

The variations in the yield and chemical composition of the essential oils are attributed to numerous factors like geographic variation, environmental and agronomic conditions, phenological stage of plants, genetic characteristics, harvest time, and the post collection processing and extraction techniques employed [34,64,76-80].

The differences in the level of susceptibility that bacteria show to essential oil can be mainly due to their specific morphology (bacterial cell wall structure, capsule formation). Numerous reviews indicate that *Gram* positive bacteria are more susceptible to essential oils than *Gram* negative ones. The cell wall structure of *Gram* positive bacteria allows hydrophobic molecules to accumulate in the wall or pass to the interior of the cell to act as antimicrobials. The presence of the outer membrane composed of polysaccharides and lipopolysaccharides in the *Gram* negative bacteria may prevent that essential oils active compounds, generally hydrophobic, reach the cytoplasmic membrane, which makes them more resistant to their action [81-83]. There is no general rule with respect to sensitivity: the literature reports many conflicting studies showing that some *Gram* negative strains are more sensitive than some *Gram* positive ones to certain essential oils [71,84,85].

The biological activities reported of essential oils of *Piper* can be due to wide variety of active compounds belonging to the chemical classes of monoterpenes, sesquiterpenes and phenylpropanoids [86]. The good or moderate inhibitory activity of the oil on growth of some microorganisms, could probably be attributable to β-Phellandrene, spathulenol and/or bicyclogermacrene, which were characterized as dominant constituents in the present study. The magnitude of the essential oils biological effects could be dependent of synergistic functions between the major components, it has been observed that the effect in the combination of substances is greater than the sum of the individual effects [87], even, some studies have shown that

the action of the whole essential oil is higher than that of the major components used together, for what is possible that the activity of the these is modulated by other minor molecules [88].

Little has been reported on the antimicrobial activity of *P. amalago* essential oil and the studies have been carried out in agar diffusion tests on filter paper discs impregnated with volatile oil against microbial strains. Thus, the work of Santos *et al*. [70] on the essential oil obtained by hydrodistillation from the leaves of *P. amalago* grown in Curitiba, Brazil, exhibited bacterial activity against *S. aureus*, *P. aeruginosa*, *E. faecalis*, *E. coli* and *Salmonella typhymurium*. Similarly, researchers Perigo *et al*. [71] indicated that the essential oils of *P. amalago* from Adamantina and Mococa promoted significant levels of *E. coli* growth inhibition, moreover, *S. aureus* was susceptible to all oils, but those of Adamantina and Mococa locations in lower proportions. It is significant to highlight that β-phellandrene was identified as main compound (12.3 – 39.3%) in both works. On the other hand, in a study done by da Carrara *et al*. [69], the essential oil obtained of *P. amalago* from Paraná, Brazil, showed antifungal activity against nine *Candida* strains, but the chemical composition of this differ from the present work. The method used to test the antimicrobial activity of plants as well as the constitution and the number of volatile compounds presents in the oil of a plant species may significantly influence the observed levels of microbial growth inhibition, in addition to factors such as seasonality, variability the plant material, and others. Each compound may present a different mechanism to control the microorganisms, which involves a series of chemical reactions in the bacterial cell [82,89].

To the best of our knowledge this is the first report on the chemical composition of essential oil of *P. amalago* grown in the Ecuador.

Acknowledgments (optional)

We thank to Research Direction of the National University of Chimborazo by their support. We would also like to show our gratitude to the professionals of Research Laboratory of the Faculty of Health Sciences of the National University of Chimborazo who provided equipment and microbial strains from the ATCC.

References

1. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. The global threat of antimicrobial resistance: science for intervention. New microbes and new Infect. 2015; 6: 22–9.
2. Laxminarayan R, Boeckel T Van, Teillant A. The economic costs of withdrawing antimicrobial growth promoters from the Livestock sector. In: OECD Food, Agriculture and Fisheries. Paris: Organization for Economic Co-operation and Development; 2015. p. 1–41.
3. Wright GD. Antibiotic resistance in the environment: a link to the clinic?. Curr Opin Microbiol. 2010; 13(5): 589–94.
4. Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DGJ. Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front Microbiol. 2014; 5(648): 1–14.
5. Czekalski N, Gascon Diez E, Burgmann H. Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. ISME J. 2014; 8(7): 1381–90.
6. Yang Y, Xu C, Cao X, Lin H, Wang J. Antibiotic resistance genes in surface water of eutrophic urban lakes are related to heavy metals, antibiotics, lake morphology and anthropic impact. Ecotoxicology. 2017; 26(6): 831–40.
7. Yin Q, Yue D, Peng Y, Liu Y, Xiao L. Occurrence and distribution of antibiotic-resistant bacteria and transfer of resistance genes in Lake Taihu. Microbes Env. 2013; 28(4): 479–86.
8. Barnes J, Anderson LA, Phillipson D. Herbal Medicines. 3rd ed. Pharmaceutical Press-Royal Pharmaceutical Society; 2007. p.23-6.
9. de la Torre L, Alarcón D, Kvist L, Salazar J. Usos medicinales de las plantas. In: Torre, de la L, Navarrete H, Murie l M, Macía M, Balslev H, editors. Enciclopedia de las plantas útiles del Ecuador. Quito: Herbario QCA de la Escuela de

Ciencias Biológicas de la Pontificia Universidad Católica del Ecuador y Herbario AAU del Departamento de Ciencias Biológicas de la Universidad de Aarhus; 2008. p. 106–8.
10. Walsh C. Where will new antibiotics come from? Nat Rev Microbiol. 2003; 1(1): 65–70.
11. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J Ethnobiol Ethnomed. 2006; 2(14): 1–8.
12. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, et al. Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases: "reverse pharmacology" and "bedside to bench" approach. Curr Drug Targets. 2011; 12(11): 1595–653.
13. Burger W. Flora Costaricensis. Fieldiana Botany. Field Museum of Natural History; 1971.
14. Dyer L, Palmer A. *Piper*: A model genus for studies of phytochemistry, ecology, and evolution. New York: Kluwer Academic/Plenum; 2004. p. 117-34.
15. Parmar V, Jain S, Bisht K, Jain R, Taneja P, Jha A, et al. Phytochemistry of the genus Piper. Phytochemistry. 1997; 46(4): 597–673.
16. Trindade FTT, Stabeli RG, Facundo VA, Cardoso CT, Silva MA da, Gil LHS, et al. Evaluation of larvicidal activity of the methanolic extracts of *Piper alatabaccum* branches and *P. tuberculatum* leaves and compounds isolated against *Anopheles darlingi*. Rev Bras Farmacogn. 2012; 22(5): 979–84.
17. Simpson B, Ogorzaly M. Economic botany: plants in our world. 2nd ed. New York: McGraw-Hill; 1995. 742 p.
18. Chauret DC, Bernard CB, Arnason JT, Durst T, Krishnamurty HG, Sanchez-Vindas P, *et al*. Insecticidal neolignans from *Piper decurrens*. J Nat Prod. 1996; 59(2): 152–5.
19. Jørgensen P, León-Yánez S. Catalogue of the vascular plants of Ecuador. Monogr. Syst. Bot. Missouri Bot. Gard.; 1999. p. 785–805
20. Scott I, Jensen H, Philogene B, Arnason J. A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. Phytochem Rev. 2008; 7(1): 65–75.
21. Roersch C. *Piper umbellatum* L.: a comparative cross-cultural analysis of its medicinal uses and an ethnopharmacological evaluation. J Ethnopharmacol. 2010; 131(3): 522–37.
22. Perez-Gutierrez RM, Neira-Gonzalez AM, Hoyo-Vadillo C. Alkaloids from *Piper*: A review of its phytochemistry and pharmacology. Mini-Reviews Med Chem. 2013; 13(2): 163–93.
23. Monzote L, Scull R, Cos P, Setzer WN. Essential Oil from *Piper aduncum*: Chemical analysis, antimicrobial assessment, and literature review. Medicines. 2017; 4(3): 49.
24. Mgbeahuruike E, Yrjönen T, Vuorela H, Holm Y. Bioactive compounds from medicinal plants: Focus on *Piper* species. South African J Bot. 2017; 112: 54–69.
25. Durant-Archibold A, Santana A, Gupta M. Ethnomedical uses and pharmacological activities of most prevalent species of genus *Piper* in Panama: A review. J Ethnopharmacol. 2018; 217: 63–82.
26. Salehi B, Zakaria Z, Gyawali R, Ibrahim S, Rajkovic J, Shinwari ZK, *et al*. *Piper* species: A comprehensive review on their and applications. Molecules. 2019; 24(7): 1–118.
27. Achenbach H, Groß J, Portecop J. Ishwarol, the main sesquiterpene in *Piper amalago*. Planta Med. 1984; 50(6): 528–9.
28. Sengupta S, Ray A. The chemistry of *Piper* species: a review. Fitoterapia. 1987; 58(3): 147–66.
29. Lago JHG, Ramos CS, Casanova DCC, Morandim A de A, Bergamo DCB, Cavalheiro AJ, *et al*. Benzoic acid derivatives from *Piper* species and their fungitoxic activity against *Cladosporium cladosporioides* and *C. sphaerospermum*. J Nat Prod. 2004; 67(11): 1783–8.
30. Scott IM, Puniani E, Jensen H, Livesey JF, Poveda L, Sánchez-Vindas P, *et al*. Analysis of Piperaceae germplasm by HPLC and LCMS: A method for isolating and identifying unsaturated amides

from *Piper* spp extracts. J Agric Food Chem. 2005; 53(6): 1907–13.
31. Felipe D, Filho B, Nakamura C, Franco S, Cortez D. Analysis of neolignans compounds of *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck by HPLC. J Pharm Biomed Anal. 2006; 41(4): 1371–5.
32. Silva MLA, Coímbra HS, Pereira AC, Almeida VA, Lima TC, Costa ES, et al. Evaluation of *Piper cubeba* extract, (-)-cubebin and its semi-synthetic derivatives against oral pathogens. Phytother Res. 2007; 21(5): 420–2.
33. Vadlapudi V, Kaladhar D. Phytochemical evaluation and molecular characterization of some important medicinal plants. Asian Pacific J Trop Dis. 2012; 2: S26–32.
34. Xiang CP, Han JX, Li XC, Li YH, Zhang Y, Chen L, *et al*. Chemical composition and acetylcholinesterase inhibitory activity of essential oils from *Piper* species. J Agric Food Chem. 2017; 65(18): 3702–10.
35. dos Santos AL, Novaes A da S, Polidoro A dos S, de Barros ME, Mota JS, Lima DBM, *et al*. Chemical characterization of *Piper amalago* (Piperaceae) essential oil by comprehensive two-dimensional gas chromatography coupled with rapid-scanning quadrupole mass spectrometry (GC×GC/qMS) and their antilithiasic activity and acute toxicity. Phytochem Anal. 2018; 29(5): 432–45.
36. Alécio AC, Bolzani V da S, Young MCM, Kato MJ, Furlan M. Antifungal amide from leaves of *Piper hispidum*. J Nat Prod. 1998; 61(5): 637–9.
37. Navickiene HMD, Alécio AC, Kato MJ, Bolzani V da S, Young MCM, Cavalheiro AJ, *et al*. Antifungal amides from *Piper hispidum* and *Piper tuberculatum*. Phytochemistry. 2000; 55(6): 621–6.
38. Felipe CB, Filho-Jucélio TS, de Oliveira-Souza LE, Silveira JA, de Andrade Uchoa DE, Silveira ER, *et al*. Piplartine, an amide alkaloid from *Piper tuberculatum*, presents anxiolytic and antidepressant effects in mice. Phytomedicine. 2007; 14(9): 605–12.
39. Nascimento JCD, Paula VFD, David JM, David JP. Occurrence, biological activities and 13C NMR data of amides from *Piper* (Piperaceae). Quim Nova. 2012; 35(11): 2288–311.
40. Bodiwala H, Chadha G, Singh R, Dey C, Sharma S, Bhutani K, *et al*. Antileishmanial amides and lignans from *Piper cubeta* and *Piper retrofractum*. J Nat Med. 2007; 61(4): 418–21.
41. Tang G-H, Chen D-M, Qiu B-Y, Sheng L, Wang Y-H, Hu G-W, *et al*. Cytotoxic amide alkaloids from *Piper boehmeriaefolium*. J Nat Prod. 2011; 74(1): 45–9.
42. Ferreres F, Oliveira A, Valentão P, Andrade P. *Piper betle* leaves: Profiling phenolic compounds by HPLC/DAD-ESI/MSn and anti-cholinesterase activity. Phytochem Anal. 2014; 25(5):453–60.
43. Niu M, Xu X, Shen Y, Yao Y, Qiao J, Zhu F, *et al*. Piperlongumine is a novel nuclear export inhibitor with potent anticancer activity. Chem Biol Interact. 2015; 237: 66–72.
44. Pereira Bezerra D, Pessoa C, De Moraes MO, Rocha Silveira E, Sousa Lima MA, Martins Elmiro FJ, *et al*. Antiproliferative effects of two amides, piperine and piplartine, from *Piper* species. Zeitschrift für Naturforsch C. 2005; 60(7–8): 539–43.
45. Bezerra DP, de Castro FO, Alves APNN, Pessoa C, de Moraes MO, Silveira ER, *et al*. In vitro and in vivo antitumor effect of 5-FU combined with piplartine and piperine. J Appl Toxicol. 2008; 28(2): 156–63.
46. Bokesch HR, Gardella RS, Rabe DC, Bottaro DP, Linehan WM, McMahon JB, *et al*. A new hypoxia inducible factor-2 inhibitory pyrrolinone alkaloid from roots and stems of *Piper sarmentosum*. Chem Pharm Bull. 2011; 59(9): 1178–9.
47. Domínguez XA, Alcorn JB. Screening of medicinal plants used by Huastec Mayans of northeastern Mexico. J Ethnopharmacol. 1985; 13(2): 139–56.
48. Carrara V, Vieira SCH, de Paula RG, Rodrigues V, Magalhães LG, Cortez DAG, *et al*. In vitro schistosomicidal effects of aqueous and dichloromethane fractions from leaves and stems of *Piper* species and the isolation of an active amide from *P. amalago* L. (Piperaceae). J Helminthol. 2014; 88(3): 321–6.

49. da Silva Mota J, de Souza DS, Boone CV, Lima Cardoso CA, Bastos Caramão E. Identification of the volatile compounds of leaf, flower, root and stem oils of *Piper amalago* (Piperaceae). J Essent Oil Bear PL. 2013; 16(1): 11–6.
50. Zamora-Martinez MC, de Pascual Pola CN. Medicinal plants used in some rural populations of Oaxaca, Puebla and Veracruz, Mexico. J Ethnopharmacol. 1992; 35(3): 229–57.
51. Coe FG. Rama midwifery in eastern Nicaragua. J Ethnopharmacol. 2008; 117(1): 136–57.
52. Ribeiro RV, Costa Bieski IG, Balogun SO, de Oliveira Martins DT. Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. J Ethnopharmacol. 2017; 205(9): 69–102.
53. Arnason T, Uck F, Lambert J, Hebda R. Maya medicinal plants of San Jose Succotz, Belize. J Ethnopharmacol. 1980; 2(4): 345–64.
54. Alves EO, Mota JH, Soares TS, Vieira M do C, da Silva CB. Etnobotanical survey and medicinal plants characterization in forest fragments in Dourados-MS. Ciênc agrotec, Lavras. 2008; 32(2): 651–8.
55. Coelho RM, Brand G, Ricardo LL, Bonfim NM, de Brito LP, de Macedo CS, *et al*. Atividade cicatrizante e isolamento de amidas pirrolidínicas do extrato metanólico em *Piper amalago*. In: 32a Reunião Anual da Sociedade Brasileira de Química. Brazil: Sociedade Brasileira de Quımica (SBQ); 2009. p. 1.
56. Morton JF. Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan. Thomas CC, editor. Springfield; 1981. 1420 p.
57. Sosa S, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G, *et al*. Screening of the topical anti-inflammatory activity of some Central American plants. J Ethnopharmacol. 2002; 81(2): 211–5.
58. da Silva Carrara V, Serra LZ, Cardozo-Filho L, Cunha Júnior EF, Torres-Santos EC, Garcia Cortez DA. HPLC analysis of supercritical carbon dioxide and compressed propane extracts from *Piper amalago* L. with antileishmanial activity. Molecules. 2011; 17(1): 15–33.
59. da Silva Carrara V, Cunha-Júnior EF, Torres-Santos EC, Gonçalves Corrêa A, Monteiro JL, Demarchi IG, *et al*. Antileishmanial activity of amides from *Piper amalago* and synthetic analogs. Rev Bras Farmacogn. 2013; 23(3): 447–54.
60. Novaes A, Mota J, Barison A, Veber C, Negrão F, Kassuya C, *et al*. Diuretic and antilithiasic activities of ethanolic extract from *Piper amalago* (Piperaceae). Phytomedicine. 2013; 21(4): 523–8.
61. Mullally M, Cayer C, Muhammad A, Walshe-Roussel B, Ahmed F, Sanchez-Vindas PE, *et al*. Anxiolytic activity and active principles of *Piper amalago* (Piperaceae), a medicinal plant used by the Q'eqchi' Maya to treat susto, a culture-bound illness. J Ethnopharmacol. 2016; 185: 147–54.
62. Rovani G, dos Santos V, Obdulio Gomes M, Budel J, Campos R. Investigação fitoquímica e antioxidante de partes vegetativas aéreas de *Piper amalago* L. Cad Esc Saúde. 2013; 2(10): 164–77.
63. da Silva Arrigo J, Balen E, Júnior UL, da Silva Mota J, Iwamoto RD, Barison A, *et al*. Anti-nociceptive, anti-hyperalgesic and anti-arthritic activity of amides and extract obtained from *Piper amalago* in rodents. J Ethnopharmacol. 2016; 179: 101–9.
64. Srivastava A, Lall R, Sinha A, Gupta RC. Essential oils. In: Gupta R, Srivastava A, Lall R, editors. Nutraceuticals in veterinary medicine. Springer; 2009. p. 157–71.
65. Mesquita JMO, Cavaleiro C, Cunha AP, Lombardi JA, Oliveira AB. Estudo comparativo dos óleos voláteis de algumas espécies de Piperaceae. Rev Bras Farmacogn. 2005; 15(1): 6–12.
66. Potzernheim M, Bizzo HR, Agostini-Costa TS, Vieira RF, Carvalho-Cilva M, Gracindo L. Chemical characterization of seven *Piper* species (Piperaceae) from Federal District, Brazil, based on volatile oil constituents. Rev bras plantas med. 2006; 8: 10–2.

67. Morandim A, Pin A, Pietro N, Oliveira H, Mendes Giannini MJ, Alecio A, *et al*. Composition and antifungal activity against *Candida albicans*, *Candida parapsilosis*, *Candida krusei* and *Cryptococcus neoformans* of essential oils from leaves of *Piper* and *Peperomia* species. J Med Plants. 2010; 4(17): 1810–4.
68. Ferraz A, Balbino J, Zini C, Ribeiro V, Bordignon S, Poser G. Acaricidal activity and chemical composition of the essential oil from three *Piper* species. Parasitol Res. 2010; 107(1): 243–8.
69. da Carrara VS, Souza A, Filho B, Nakamura C, Paulo L, Young M, *et al*. Chemical composition and antifungal activity of the essential oil from *Piper amalago*. Lat Am J Pharm. 2010; 29(8): 1459–62.
70. Santos V, Lima C, Campos R, Ribeiro C, Marques F, Budel J, *et al*. Chemical composition and antimicrobial activity of volatile oils of *Piper amalago* L. Lat Am J Pharm. 2016; 35(8): 1883–9.
71. Perigo CV, Torres RB, Bernacci LC, Guimarães EF, Haber LL, Facanali R, *et al*. The chemical composition and antibacterial activity of eleven *Piper* species from distinct rainforest areas in Southeastern Brazil. Ind Crops Prod. 2016; 94: 528–39.
72. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4.1 ed. Stream C, editor, IL, USA: Allured Publishing Corporation, Carol Stream, 2007.
73. Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J Chromatogr A. 1990; 503: 1–24.
74. Moujir LM, Seca AML, Araujo L, Silva AMS, Barreto MC. A new natural spiro heterocyclic compound and the cytotoxic activity of the secondary metabolites from *Juniperus brevifolia* leaves. Fitoterapia. 2011; 82(2): 225–9.
75. Holetz FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, Dias Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz. 2002; 97(7):1027–31.
76. Asekun O, Grierson DS, Afolayan A. Effects of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. subsp. *Capensis*. Food Chem. 2007; 101(3): 995–8.
77. Sefidkon F, Abbasi K, Jamzad Z, Ahmadi S. The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Satureja rechingeri* Jamzad. Food Chem. 2007; 100(3): 1054–8.
78. Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 2008; 108(3): 986–95.
79. Anwar F, Hussain AI, Sherazi STH, Bhanger MI. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (*Foeniculum vulgare* Mill.) fruit at different stages of maturity. J Herbs Spices Med Plants. 2009; 15(2): 187–202.
80. Djerrad Z, Kadik L, Djouahri A. Chemical variability and antioxidant activities among *Pinus halepensis* Mill. essential oils provenances, depending on geographic variation and environmental conditions. Ind Crops Prod. 2015 ;74: 440–9.
81. Paduch R, Kandefer-Szerszeń M, Trytek M, Fiedurek J. Terpenes: substances useful in human healthcare. Arch Immunol Ther Exp. 2007; 55(5): 315–27.
82. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013; 6(12): 1451–74.
83. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils? chemical characterization and investigation of some biological activities: A critical review. Medicines. 2016; 3(4): 25.
84. Hudson J, Kuo M, Vimalanathan S. The antimicrobial properties of cedar leaf (*Thuja plicata*) oil; a safe and efficient decontamination agent for buildings. Int J Environ Res Public Health. 2011; 8(12): 4477–87.

85. Maida I, Lo Nostro A, Pesavento G, Barnabei M, Calonico C, Perrin E, *et al*. Exploring the anti-burkholderia cepacia complex activity of essential oils: A preliminary analysis. Evid Based Complement Alternat Med. 2014; 2014: 10.
86. Takeara R, Gonçalves R, Ayres V, Guimarães A. Biological properties of essential oils from the *Piper* species of Brazil: A review. In: Aromatic and Medicinal Plants—Back to Nature. El-Shemy. 2017. p. 81–93.
87. Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–an overview. Complement Med Res. 2009; 16(2): 79–90.
88. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol. 2004; 94(3): 223–53.
89. Baser HC, Buchbauer G. Handbook of essential oils: Science, technology, and applications. 2nd ed. Londres: CRC Press; 2015. 1112 p.

Table 1. Chemical composition of *Piper amalago* L. essential oil.

N°	Compounds[a]	RT	% Area	KI[b]	KI[c]
1	α-Pinene	6.03	7.29	932	933
3	Sabinene	7.43	0.31	969	976
4	β-Pinene	7.57	0.62	980	980
5	β-Myrcene	8.17	2.25	991	1007
6	α- Phellandrene	8.75	0.3	1002	1002
7	delta-3-Carene	8.81	0.38	1008	1003
8	p-Cymene	9.56	1.77	1020	1021
9	β-Phellandrene	9.86	20.42	1025	1027
10	Linalool L	13.02	0.70	1095	1101
11	Cryptone	16.77	1.44	1183	1184
12	α-Cubebene	23.72	0.24	1345	1340
13	α-Copaene	24.88	0.21	1374	1367
14	β–Bourbonene	25.19	0.19	1387	1374
15	β-Cubebene	25.45	0.22	1387	1380
16	β- Elemene	25.56	0.66	1389	1383
17	trans-Caryophyllene	26.66	1.73	1417	1409
18	Beta-copaene	27.12	0.90	1432	1420
19	Aromadendrene	27.42	0.26	1439	1427
20	β-Humulene	28.13	0.19	1452	1445
21	α-Amorphene	29.04	0.40	1483	1467
22	Germacrene-D	29.21	0.83	1484	1471
23	Bicyclogermacrene	29.86	8.50	1500	1487
24	Alfa-muurolene	30.04	1.34	1500	1491
25	γ-cadinene	30.56	1.15	1513	1504
26	delta-cadinene	30.75	5.60	1523	1509
27	alfa-Cadinene	30.85	3.96	1533	1512
28	Cis-Calamenene	30.95	0.99	1528	1514
29	*Trans*- Nerolidol	32.77	0.45	1564	1561
30	Spathulenol	33.13	10.34	1577	1570
31	Veridiflorol	33.37	0.82	1592	1576
32	Guaiol	34.25	0.23	1598	1585
33	1,10-di-epi-cubenol	34.75	1.33	1619	1612
34	1-epi-cubenol	34.97	1.32	1628	1618
35	α-epi-Cadinol	35.58	1.98	1638	1635
36	Epi-alfa-Muurolol	35,67	2.30	1640	1637
37	Cubenol	35.79	3.05	1646	1641
38	t-Muurolol	36.13	5.49	1646	1650
	Total identified (%)		90.16		
	Monoterpenes		33.34		
	Oxygenated monoterpenes		0.7		
	Sesquiterpenes		27.37		
	Oxygenated sesquiterpenes		27.31		
	Oxygenated Cyclohexene		1.44		

[a]Compounds listed in sequence of elution from a DB-5 MS column; RT: retention time; [b]KI: Kovats retention indices calculated; [c]KI: Kovats retention indices from literature [72,73]

Table 2. Antimicrobial activity of *Piper amalago* L. essential oil.

Microorganism	Essential oil (µg/mL)	
	MIC	MBC/ MFC
Gram positive bacteria		
***S. aureus* ATCC 6538P**	100-200	400
***E. faecalis* ATCC 29212**	100-200	400
Gram negative bacteria		
***E. coli* ATCC 25922**	50-100	200
***P. aeruginosa* ATCC 27853**	1600-3200	3200
***K. pneumoniae* ATCC700603**	1600-3200	3200
Yeast		
***Candida albicans* ATCC10231**	800-1600	1600

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration; ATCC: American type culture collection