UNIVERSIDADE FEDERAL DO PARÁ

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

Petiveria alliacea L.: ETNOBOTÂNICA, FITOQUÍMICA EFEITOS NEUROFARMACOLÓGICOS E COGNITIVOS

Diandra Araújo da Luz

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas, área de concentração: Fármacos e Medicamentos, do Instituto de Ciências da Saúde, da Universidade Federal do Pará, como requisito parcial para obtenção do título de Mestre em Ciências Farmacêuticas.

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FOLHA DE APROVAÇÃO

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Área de concentração: Fármacos e Medicamentos

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Prof(a). Dr(a).: _____

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Instituição: _____ Assinatura: _____

DEDICATÓRIA

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Habacuque 3, 17-18.

RESUMO

LUZ, D. A. da. ***Petiveria alliacea*** **L.: ETNOBOTÂNICA, FITOQUÍMICA, NEUROFARMACOLÓGICOS E COGNITIVOS.** 2016, 75 f, Dissertação (Mestrado) – Faculdade de Farmácia, Universidade Federal do Pará, Pará, 2016.

Petiveria alliacea L., é uma planta arbustiva, nativa de regiões tropicas, utilizada para tratar memória fraca e melhorar a aprendizagem. No presente estudo avaliou-se os efeitos do extrato hidroalcoólico das folhas de *P. alliacea* (EHFPa, 900 mg/Kg) sobre a memória e aprendizado de ratos adultos, submetidos aos testes comportamentais esquiva inibitória e labirinto aquático de Morris. Adicionalmente, foi feita uma cromatografia em camada delgada (CCD) para a detecção de compostos de enxofre, a fim de tentar correlacioná-los com as respostas investigadas. Frações diclorometano, obtidas a partir de extratos aquosos de *Alllium sativum* e *Allium cepa* serviram como padrões de detecção*. P. alliacea* apresenta também ações controversas sobre o sistema nervoso central (SNC). Por esta razão, foi feita uma revisão bibliográfica sobre a etnobotânica, fitoquímica e efeitos neurofarmacológicos desta planta em bases indexadas, livros, dissertações, teses e fontes científicas similares. De acordo com os resultados o EHFPa promoveu melhora da memória de curta e de longa duração, memória espacial e aprendizagem. Na CCD, o EHFPa produziu pontos de retenção semelhantes as amostras padrão, indicando que há compostos de enxofre no extrato, sendo possível que eles participem das respostas observadas. Quanto a revisão, *P. alliacea* é utilizada popularmente no tratamento da epilepsia, ansiedade, memória fraca, como sedativo, etc. Tais propriedades foram demonstradas experimentalmente e variam em função da dose, parte da planta e preparação utilizada. Estudos fitoquímicos detectaram diversos metabólitos na *P. alliacea*, dos quais os compostos de enxofre, flavonóides e derivados são as classes com maior número de compostos isolados.

Palavras-chave: *Petiveria alliacea* L., compostos de enxofre, memória, aprendizado, etnobotânica, fitoquímica e efeitos neurofarmacológicos.

ABSTRACT

LUZ, D. A. da. ***Petiveria alliacea* L.: ETHNOBOTANY, PHYTOCHEMISTRY AND NEUROPHARMACOLOGIAL AND COGNITIVE EFFECTS.** 2016, 75 f, Dissertação (Mestrado) – Faculdade de Farmácia, Universidade Federal do Pará, Pará, 2016.

Petiveria alliacea L. is a shrubby plant, native from tropic regions, used to treat poor memory and improve learning. In the present study, it was evaluated the effects of hydroalcoholic extract of the leaves of *P. alliacea* (PaLHE, 900 mg/kg) on learning and memory of adult rats, subjected to behavioral tests inhibitory avoidance and Morris Water Maze. In addition, it was performed a thin layer chromatography (TLC) to detect sulphur compound, to correlate them with the investigated responses. Dichloromethane fractions, obtained from aqueous extracts of *Alllium sativum* and *Allium Cepa* served as detection patterns. *P. alliacea* is also present controversial activities on central nervous system (CNS). For this reason, it was realized a bibliographic review of ethnobotanical, phytochemical and neuropharmacological activities of this plant in indexed databases, books, dissertations, thesis and similar scientific sources. According to the results, EHFPa improved short and long term memory, spatial memory and learning. In TLC, the EHFPa produced retention points similar to the standard samples, indicating that there are sulfur compounds in the extract, being possible that they contribute to observed responses. About the review, *P. alliacea* is popularly used to treat epilepsy, anxiety, poor memory, as a sedative, etc. Such properties have been demonstrated experimentally, varying depending on the dose, preparation, and part of plant used. Phytochemical studies detected several metabolites in *P. alliacea*, among which sulfur compounds, flavonoids and derivatives classes the most isolated compounds.

Keywords: *Petiveria alliacea* L., sulfur compounds, memory, learning, ethnobotany, phytochemistry and neuropharmacological effects.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

AMPA	Ácido α-amino-3-hidroxi-5-metil-4-isoxazol propiônico
BDZ	Benzodiazepínicos
DTS	Trissulfeto de Dibenzila
ERKs	Quinases reguladas por sinal extracelular
GABA	Ácido gama-aminobutírico
LTP	Potenciação de longa duração
MAPK	Proteína quinase ativadas por mitógeno
MCD	Memória de curta duração
MLD	Memória de longa duração
NMDA	N-metil-D-aspartato
SNC	Sistema nervoso central
TRP	Receptor de potencial transitório
TRPV1	Receptor de potencial transitório do tipo vanilóide 1

LISTA DE SÍMBOLOS E UNIDADES

g	Grama
µL	Microlitro
µg	Micrograma
mg	Miligrama
mL	Mililitro
Kg	Quilograma

SUMÁRIO

I INTRODUÇÃO

1. INTRODUÇÃO

1.1 Memorial

Plantas medicinais são fontes de substâncias dotadas de efeitos biológicos, cujo emprego como recurso terapêutico é uma prática antiga e disseminada. De acordo com a Organização Mundial de Saúde (OMS), durante a década de 1990, cerca de 65-80 % da população de nações em desenvolvimento tinham nas plantas medicinais a única forma de acesso a cuidados básicos de saúde (VEIGA JÚNIOR et al. 2005). Tal é sua relevância para a saúde pública, que desde 1978 a OMS reconhece preparações de espécies vegetais como opções terapêuticas válidas. Estima-se que cerca de 91% da população brasileira utilize preparações de plantas para fins medicinais (ETHUR, 2011; GIRALDI e HANAZAKI, 2010; OLIVEIRA et al. 2010).

Muitas vezes o conhecimento tradicional sobre o uso de determinadas espécies provê direcionamento às pesquisas com produtos naturais. Por esta razão, levantamentos etnobotânicos ou revisões bibliográficas que integrem dados (indicações, fitoquímica, atividades detectadas, etc.) de espécies utilizadas popularmente são de grande relevância para percepção do potencial farmacológico de cada uma delas. Este respaldo bibliográfico também é importante no sentido de prover suporte científico ou não a alegação popular, com base em resultados experimentais. Ademais, a realização de trabalhos que reúnam e sistematizem tais informações possibilitam a percepção de lacunas científicas que precisem ser esclarecidas, subsidiando a continuidade das pesquisas.

Uma das espécies que se inserem neste contexto é a *Petiveria alliacea* L. Esta planta tem sido popularmente utilizada por diversas finalidades, inclusive no tratamento de desordens do SNC. Há relatos etnofarmacológicos do uso de *P. alliacea* no combate a epilepsia, ansiedade, “memória fraca”, aprendizagem e como estimulante. Por esta razão, nosso grupo de pesquisa realizou um screening comportamental, a fim investigar a ocorrência de tais atividades, a partir da administração do extrato de partes totais de *P. alliacea*. Um dos efeitos observados a melhora na memória de longa duração (MLD), induzida pelo pré-tratamento com o extrato de planta total na dose de 900 mg/Kg. No entanto, neste estudo não observou-se atividade (melhora ou prejuízo) sobre a memória de curta duração (MCD) e aprendizagem (ANDRADE et al. 2012).

Diversos estudos indicam que a composição química de uma planta pode variar em função de diversos fatores, inclusive de acordo com a parte utilizada. Ao buscar na literatura, descobrimos também que suas folhas são utilizadas para melhora cognitiva, em associação ou não com outras plantas (MORS; RIZZINI; PEREIRA, 2000; RODRIGUES et al. 2008). Considerando estes fatos, nosso grupo decidiu investigar o efeito do extrato de folhas sobre o processo mnemônico.

Investigações a respeito de seus efeitos centrais demonstram que a *P. alliacea* exerce ações proeminentes no SNC. Logo, preparações desta espécie poderiam ser uma possível fonte para o tratamento de desordens como epilepsia, ansiedade, déficits de memória, etc. que são em sua maioria incapacitantes e necessitam de recursos terapêuticos para seu manejo clínico. Contudo, a fragmentação das pesquisas sobre suas atividades centrais dificulta a compreensão de seu potencial farmacológico. Diferentes partes da planta e doses do extrato utilizado parecem produzir respostas distintas ou mesmo controversas (BLAINSKI et al. 2010; CIFUENTES et al. 2001). Além disso, pouco se sabe a respeito de como a *P. alliacea* exerce os efeitos biológicos reportadas.

Por esta razão, além do trabalho experimental, foi realizado uma revisão crítica, que teve por objetivo confrontar os usos etnofarmacológicos, as atividades no SNC já demonstrados e os constituintes fitoquímicos já detectados na *P. alliacea*. Adicionalmente foram descritas as características botânicas, taxonômicas, distribuição geográfica dados toxicológicos da planta. Esta revisão teve por objetivo identificar as lacunas científicas a respeito desta espécie, bem como subsidiar pesquisas futuras que permitam uma melhor compreensão de seu potencial farmacológico.

1.2 *Petiveria alliacea* L.

A família Phytolaccaceae, compreende cerca de 17 gêneros e 120 espécies pantropicais amplamente distribuídas por todo o continente americano (DUARTE e LOPES, 2005). Os gêneros desta família que estão presentes no Brasil são: *Phytolacca, Microtea, Petiveria, Rivina* e *Seguieria*. No gênero *Petiveria* está inserido um dos mais importantes membros desta família, a *Petiveria alliacea* L. Esta planta possui como válidas as seguintes sinonímias: *P. foetida* Salisb, *P.alliacea* var. grandifolia Moq., *P.alliacea* var. octandra (L.) Moq., *P. foetida* Salisb., *P. hexandria*

Sessé & Moc., *P. ochroleuca* Moq., *P. octandra* L. e *P. paraguayensis* D. Parodi (TROPICOS.ORG, 2015).

Nativa de regiões tropicais, como a Amazônia, África Subsaariana, América do Sul e Central, *P. alliacea* desenvolve-se, preferencialmente, em locais subúmidos e sombreados (ROCHA; MARANHÃO; PREUSSLER, 2006). Dotada de leve aroma aliáceo, apresenta-se como um arbusto perene, que pode atingir altura de até 5-150 cm. Possui uma haste rígida e reta, ramificada com ramos compridos, eretos, delicados e ascendentes (ALMANZA, 2012; DUARTE e LOPEZ, 2005; RZEDOWSKI e DE RZEDOWSKI, 2000). A raiz fusiforme, de cor parda acinzentada clara e parda amarelada, é irregularmente ramificada e de comprimento variável, com cicatrizes verrucosas (GOMES, 2006).

Suas folhas são curto-pecioladas, alternas, estipuladas, membranosas, agudas no ápice e estreitas na base, livres e lisas. As flores são bissexuais, sésseis e pequenas, reunidas em inflorescência (espigas ou cachos) axilares e terminais espiciformes, podendo apresentar-se sob as cores branca, branco-rosa ou verde. Os frutos são do tipo aquênio, cilíndrico, achatado e dotado de espinhos, os quais funcionam como meio de disseminação, devido à possibilidade de se prenderem em animais, roupas, etc. (ANDRADE et al. 2012; DUARTE e LOPES, 2005; RZEDOWSKI e RZEDOWSKI, 2000; SOARES et al. 2013).

Figura 01: *Petiveria alliacea* L.

FONTE: Duarte e Lopes (2005).

1.2.1 ETNOFARMACOLOGIA

Embora seja encontrada em outras regiões do mundo, *P. alliacea* é utilizada principalmente no continente americano. De acordo com o local de origem, esta planta recebe determinados nomes populares (GOMES et al., 2005; OLIVEIRA, 2012). Entre as diferentes denominações que recebe estão as seguintes: guiné, tipi, pênis-de-coelho, apacin, mucuracaá, anamú, zorrillo, amansa-senhor, erva-de-olho, caá, embayayendo e ouoembo (ANDRADE et al. 2012; DUARTE e LOPES, 2005; LIMA et al. 1991).

Quanto as possíveis propriedades medicinais, a alegação popular atribui à *P. alliacea* ação diurética, antirreumática, anti-helmíntica, antiespasmódica, antiemética, abortiva, analgésica, antipirética, antitumoral, hipoglicemiante, dentre outras (CAMARGO, 2007; GOMES et al. 2005, 2008; LOPES-MARTINS et al. 2002). Em rituais místicos, praticados por descendentes africanos e povos indígenas, *P. alliacea* é utilizada para combater “magia negra” ou “maus espíritos”, e/ou produzir visões e alucinações (TAYLOR, 1998).

Outro aspecto interessante é a indicação popular que esta planta recebe devido as possíveis ações que ela exerce no SNC. Por exemplo, durante o período escravagista, esta espécie era utilizada por seus efeitos sedativos e tóxicos. Há relatos de que as mulheres escravas recorriam a preparações de *P. alliacea* para seduzir seus “senhores” ou simplesmente para se protegerem do assédio por eles investido. Por esta razão, a denominação popular "amansa-senhor" foi dada à esta planta (CAMARGO, 2007; PECKOLT e PECKOLT, 1900). Atualmente, migrantes que vivem em regiões remanescentes da Mata Atlântica utilizam preparações de partes aéreas da de *P. alliacea* por inalação no tratamento de ansiedade (GARCIA; DOMINGUES; RODRIGUES, 2010).

Populações tradicionais do México e alguns lugares da América Latina recorrem à infusão de folhas para amenizar crises de epilepsia, “nervos” e paralisia (MARTINEZ, 1984; ZAMORA-MARTINS e POLA, 1992; TAYLOR, 1998). A infusão das folhas ou raízes é utilizada para tratar a memória fraca em diversas regiões do mundo (MORS; RIZZINI; PEREIRA, 2000). Remanescentes quilombolas utilizam

folhas de plantas consideradas “tônicos para o cérebro”, dentre as quais está a *P. alliacea* na produção de um cigarrete conhecido como “tira-capeta”. Esta preparação é utilizada para melhorar a capacidade de aprendizado de crianças e adolescentes, bem como em casos de esgotamento e distúrbios de sono (RODRIGUES et al. 2008).

1.2.2 FITOQUÍMICA E FARMACOLOGIA

A composição química de *P. alliacea* tem sido bastante estudada, o que levou a identificação de diversas substâncias, tendo algumas delas recebido proteção patentária (CUERVO, 2011). Ensaios fitoquímicos com diferentes extratos detectaram esteróis, triterpenos, saponinas, alcalóides, taninos, cumarinas, lipídeos, flavonóides e derivados (BANDONI et al. 1976; DE SOUSA et al. 1990; ROCHA e SILVA, 1969). Outras classes de metabólitos encontrados foram os polissulfetos, tiossulfinatos, dipeptídeos gutâmicos e derivados cisteína sulfóxido (BENEVIDES et al. 2001; KUBEC et al. 2001, 2002, 2003; KUBEC e MUSAH, 2005). Os polissulfetos são compostos de grande relevância farmacológica, especialmente o trissulfeto de dibenzila (DTS, do inglês “dibenzil trisulphide”, figura 02), e podem ser encontrados também em todas as partes da planta, bem como no óleo essencial dela extraído, ainda que em menor escala (AYEDOUN et al. 1998; BEZERRA, 2006; HERNÁNDEZ et al. 2014; NEVES et al. 2011; SILVA et al. 2014; WILLIAMS et al., 1997).

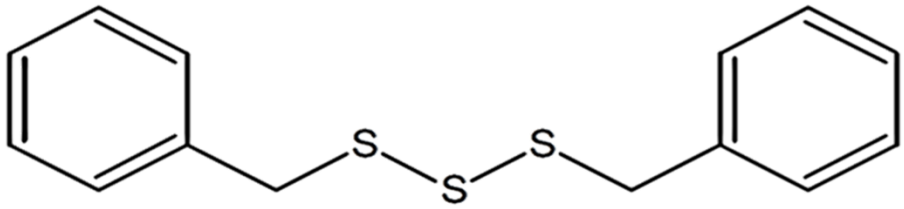

Figura 02: Tissulfeto de Dibenzila

Fonte: Adaptado de Pubchem Coumpound database.

Benevides et al. (2001) avaliaram o efeito antifúngico de extratos brutos e polissulfetos isolados das raízes de *P. alliacea*. De acordo com os resultados, os polissulfetos di-n-propil dissulfeto (0,1 µg/mL) e o DTS (1 µg/mL) apresentaram atividade antifúngica, sendo provável que estes dois compostos sejam responsáveis pela atividade que também foi observada no extrato bruto. Estudos *in vitro* demonstraram que o DTS estimula proteínas quinases ativadas por mitógenos (MAPK, do inglês *"Mitogen-activated protein kinases"*), as quais são enzimas regulam o processo de proliferação, diferenciação celular e resposta ao estresse (KONDON; NISHIDA, 2007). Ao que parece, esse seria um dos mecanismos responsáveis pela atividade antitumoral do DTS demonstrada em diversos estudos, frente a diferentes linhagens de células tumorais (WILLIAMS et al. (2007).

Adicionalmente, verificou-se que o DTS também estimula a atividade das ERKs 1 e 2 (do inglês *"Extracellular signal–regulated kinases"*), que estão envolvidas na expansão da memória de longa duração (MLD) e crescimento neuronal. Neste sentido, Andrade et al. (2012) descreveram uma melhora da memória de longa duração, promovida pelo extrato de partes totais de *P. alliacea* (900 mg/Kg) em ratos, porém estes efeitos não se estenderam a memória de curta duração e aprendizagem. Vale ressaltar fatores como, local de coleta, parte utilizada, etc., parecem influenciar na composição da planta, produzindo até mesmo atividades antagônicas. Por exemplo, ao avaliar o perfil neurofarmacológico de diferentes extratos de raízes e folhas de *P. alliacea*, foram obtidos efeitos controversos. As raízes produziram um ligeiro decréscimo da atividade motora espontânea em ratos, enquanto as folhas, hiperexcitabilidade (CIFUENTES et al. 2001).

A ocorrência de ações controversas também foi reportada nos estudos de Blainski et al. (2010). Neste trabalho constatou-se que o tratamento com extrato de planta total (300 e 900 mg/kg) apresentou efeito tipo ansiolítico, enquanto o de partes aéreas (300 mg/Kg), efeito tipo ansiogênico. Por outro lado, o extrato de raízes não apresentou atividade na avaliação deste paradigma, o que corrobora com os resultados obtidos por Gomes et al. (2008). Ainda de acordo com Blainski et al. (2010) foi detectado no extrato de partes aéreas uma quantidade de flavonoides superior as demais preparações estudadas (partes totais e raízes), sendo sugerido que estes compostos poderiam produzir os efeitos ansiolíticos observados.

Lopes-Martins et al. (2002) obtiveram os efeitos anti-inflamatório e analgésico mediante a administração do extrato etanólico das raízes de *P. alliacea* nas doses de 43,9 mg/Kg e 31,4 mg/Kg, respectivamente. Posteriormente, Gomes et al. (2005) avaliaram a ação de diferentes frações do extrato de raízes (100 e 200 mg/kg) da planta em modelos de nocicepção. Os resultados sugerem a ocorrência de antinocicepção tanto em nível periférico quanto central. Em outros trabalhos performados com extratos brutos de raízes e suas frações, também foi reportado a ocorrência de atividade anticonvulsivante (GOMES et al. 2008; LIMA et al. 1991).

1.3 Processos do SNC associados a *P. alliacea*

1.3.1 NOCICEPÇÃO E DOR

A percepção e interpretação de diferentes estímulos, ambientais ou endógenos, é uma função essencial do SNC, fundamental à sobrevivência e bem-estar. Particularmente, a detecção e discriminação de estímulos nocivos permite o desenvolvimento de comportamentos de defesa contra circunstâncias adversas, que muitas vezes podem ser fatais (BASBAUM et al. 2009). Em situações normais, a dor é decorrente da atividade de fibras aferentes primárias presentes nos tecidos periféricos, chamadas de nociceptores, que possuem como diferencial a capacidade de responder seletivamente a estímulos nóxicos (JULIUS e BASBAUM, 2001; SCHAIBLE, 2006), produzindo em resposta a sensação de dor.

A dor pode ainda ocorrer de forma transitória, sem obrigatoriamente haver dano tecidual e perdurar por dias ou poucas semanas, sendo consideradas como dor aguda (CARR e GOUDAS, 1999; LOEZER e MELZACK, 1999). Já aquelas que persistem durante a lesão ou após o reparo tecidual são classificadas como dor crônica (LOEZER e MELZACK, 1999). Embora seja difícil conceituá-la, pode considerada como um mecanismo fisiológico de proteção, funcionando como um sistema de alerta voltado à manutenção da integridade do organismo. Por outro lado, a dor (aguda ou crônica) pode alterar os padrões de sono, apetite e libido, irritabilidade, fadiga, e reduzir o desempenho em atividades familiares, profissionais e sociais (KRELING, 2006).

Os principais tipos de fibras nociceptivas são as mielinizadas de diâmetro médio (Aδ) e as não mielinizadas de pequeno diâmetro (fibras C). É proposto que o primeiro tipo medeia a dor aguda e localizada, ao passo que o segundo a dor difusa ou em “queimação”. As fibras e elas são chamadas de nociceptores polimodais, devido a sua capacidade em responder a diferentes estímulos. Quanto a sua localização, os corpos celulares dos nociceptores estão localizados nos gânglios da raiz dorsal e no gânglio trigeminal. Ambos possuem um ramo axonal periférico e outro central, onde o primeiro inerva o órgão-alvo e o segundo a medula espinhal.

Os nociceptores são ainda dotados de canais iônicos que respondem a estímulos térmicos, químicos e mecânicos. Estes canais participam como transdutores nas terminações nociceptivas, favorecendo a despolarização (canais de sódio), liberação de neurotransmissores nos terminais centrais ou periféricos para gerar dor e/ou inflamação neurogênica (canais de cálcio) ou hiperpolarizando o nociceptor (canais de potássio). Notadamente, os canais iônicos não seletivos para cátions, os quais pertencem a família do receptor de potencial transiente (TRP, do inglês *“Transient potential receptor”*) são os mais estudados.

O subtipo 1 destes receptores (TRPV1, do inglês *“Transient potential receptor vaniloid 1*) foi o primeiro a ser caracterizado e pode ser ativado por calor, baixos níveis de pH e substâncias exógenas, como por exemplo a capsaicina, uma substância presente na *Capsicum* sp. Estudos com a capsaicina demonstraram que ela inicialmente produz nocicepção, através da indução do aumento da condutância do canal e consequente liberação de mediadores pró-inflamatórios (SOUTHALL et al, 2003). No entanto, após exposições repetidas, provoca a dessensibilização dos receptores (SZALLASI; BLUMBERG, 1999; SZALLASI; DI MARZO, 2000; DRAY, 1992; GREEN, 1989; HAYES et al, 1984).

Uma vez estimuladas, as fibras aferentes primárias propagam a informação para o corno dorsal da medula espinal, a qual é dividida de acordo com propriedades anatômicas e eletrofisiológicas em cinco lâminas (BASBAUM E JESSELL, 2000). Os nociceptores Aδ e projetam-se às lâminas I e V, enquanto as fibras C projetam-se às lâminas I e II. As lâminas I e V quais possuem as principais vias que ascendem ao tálamo, cujos neurônios das regiões ventral e medial possuem axônios que se projetam para o córtex somatossorial. Apesar disto, não se pode afirmar que a dor resultaria unicamente da atividade integrada destas estruturas (APKARIAN et al.,

2005). Em vez disso, a dor resultaria da ativação tanto de regiões responsáveis por funções sensório-descriminativas (tálamo, córtex somatossensorial, etc.) quanto emocionais (giro do cíngulo anterior, córtex insular, amigdala, etc).

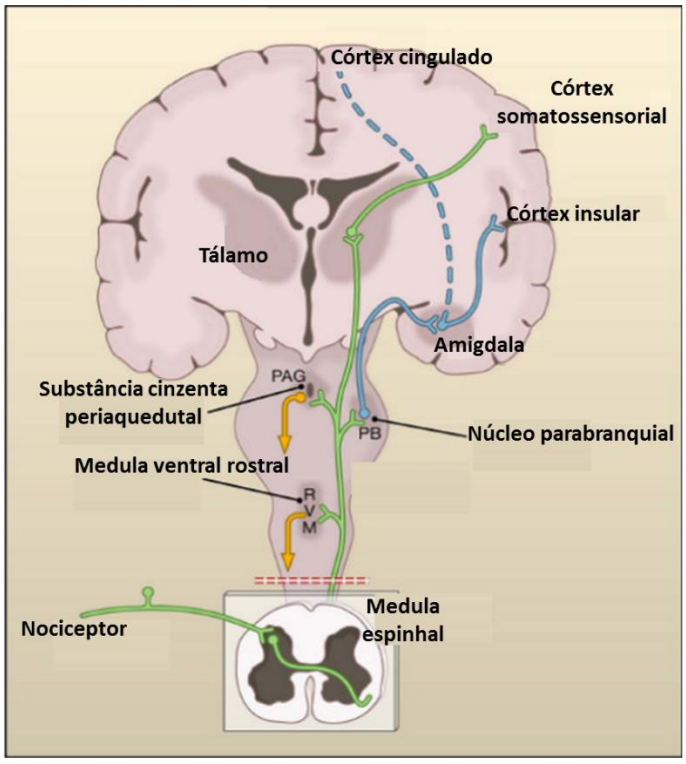

Figura 03: Caminho da dor

Fonte: Adaptado de BASBAUM et al. 2009

Ademais, após uma lesão ocorrem alterações no ambiente químico da fibra nervosa, provocada pelo acúmulo de fatores endógenos liberados nociceptores ativados ou células não-neuronais (macrófagos, mastócitos, etc.), residentes ou não da área afetada. Dentre as substâncias liberadas estão neurotransmissores, péptidos, eicosanoides, neurotrofinas, citocinas e quimiocinas, prótons, etc. Os nociceptores expressam receptores capazes de responder a cada um destes estímulos, o que leva a sua sensibilização a temperatura ou toque, resultando em uma manifestação dolorosa desproporcional a estimulação recebida (hiperalgesia) (BASBAUM et al. 2009).

1.3.2 ANSIEDADE

Em condições normais, a ativação do circuito do medo/ansiedade confere proteção contra perigos de ordem física e psicológica. O funcionamento adaptativo desta circuitaria restaura a homeostase e reduz o estresse. Por outro lado, sua desregulação pode resultar tanto no desenvolvimento de desordens físicas (hipertensão, resistência à insulina, etc.) quanto psicológicas, incluindo transtornos de ansiedade. Um dos tipos mais comuns de transtornos psiquiátricos, as desordens de ansiedade, possuem elevadas taxas de incidência e prevalência na população mundial.

Além do prejuízo social, o manejo terapêutico de pacientes que sofrem deste tipo de transtorno também representa um impacto financeiro considerável. Nos Estados Unidos estima-se que os valores envolvidos podem chegar a cerca de $42,3 bilhões de dólares por ano, sendo 50% desse valor voltado para custos não psiquiátricos (GARAKANI; MATHEW; CHARNEY, 2006; GREENBERG et al. 1999). Dentre as comorbidades relacionadas, destaca-se as desordens de humor e estima-se que cerca de 90% dos pacientes que sofrem com algum transtorno de ansiedade já tenham experimentado algum quadro depressivo, o que sugere uma correlação entre estas doenças (GARAKANI; MATHEW; CHARNEY, 2006; GORMAN, 1996).

Em termos neurobiológicos, estudos com animais revelaram que a amigdala, em conexão com uma complexa rede que inclui o córtex pré-frontal, tálamo e o hipocampo integram diversos aspectos da emocionalidade, incluindo a regulação de respostas adaptativas e patológicas ao medo (PHELPS; LEDOUX, 2005). A ativação do circuito medo/ansiedade pode ocorrer a partir de uma sensação dolorosa, ambientes abertos, presença de predadores, informações sensoriais do indivíduo, etc.

Inicialmente, o tálamo processa dados integrados referentes ao estimulo aversivo, a partir de informações previamente armazenadas (Figura 01). A seguir, esta informação é transmitida para a amigdala, uma estrutura que está localizada no lobo temporal medial, sendo composta por 13 núcleos, dos quais os núcleos basal, lateral e central constituem as vias de resposta ao medo (PARÉ; QUIRK; LE DOUX, 2004).

A informação pode chegar a amigdala por meio de dois circuitos paralelos, chamados de alça curta e alça longa (PERESE, 2012).

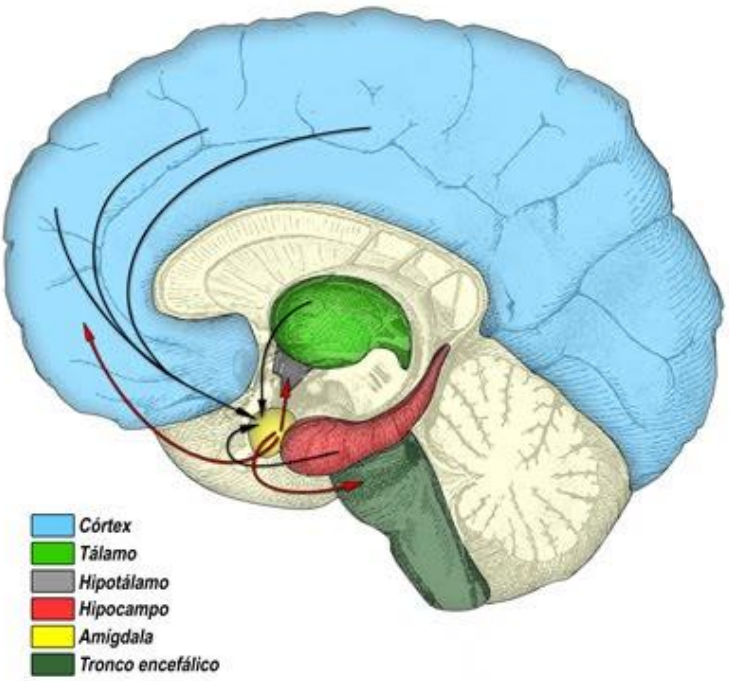

Figura 04: Regiões envolvidas na fisiopatologia do medo e ansiedade

Fonte: CAPDEVILA, 2013

Na alça curta a informação parte do tálamo é transmitido para a amigdala lateral e depois transferido ao núcleo central, enquanto na alça longa, as informações advêm do córtex somatossensorial, ínsula e córtex pré-frontal (GARAKANI; MATHEW; CHARNEY, 2006; GORMAN, 1996). A alça curta transmite informações sensoriais não processadas diretamente para a amigdala, enquanto a informação mais acurada é transmitida pela alça longa, que regula ainda a continuidade ou o cessar da resposta induzida por determinado estímulo. Assim, a resposta inicial a um agente estressor consiste na ativação da amigdala via alça curta, que transforma a informação recebida em respostas autonômicas, endócrinas e comportamentais de resposta ao medo (PERESE, 2012).

De forma simplificada, quando a informação chega ao núcleo central da amigdala é projetada para sítios efetores no tronco cerebral e hipotálamo. Por exemplo, as vias eferentes do núcleo central da amigdala que comunicam com o núcleo parabranquial produzem aumento da frequência respiratória. No núcleo lateral do hipotálamo e locus ceruleus, promove a liberação da adrenalina e norepinefrina, respectivamente, levando a aumento da pressão arterial, frequência cardíaca e resposta comportamental ao medo. Há ainda a estimulação do núcleo paraventricular o hipocampo, levando liberação aumentada de adrenocorticóides.

Este conjunto de sintomas compõem a clínica de diversas desordens de ansiedade, tais como a síndrome do pânico, na qual o indivíduo sofre ataques recorrentes e inesperados, de falta de ar, palpitações, dor no peito, sudorese, calafrios, náuseas, tremores, além de sintomas psicológicos, tais como medo de morrer ou de perder o controle, dormência e uma sensação de distanciamento ou irrealidade. Atualmente o tratamento de transtornos de ansiedade pode ser feito, de acordo com o diagnóstico, com benzodiazepínicos (BDZ), inibidores seletivos da receptação de noradrenalina e/ou serotonina (GARAKANI; MATHEW; CHARNEY, 2006). O grande inconveniente com estes fármacos são as reações adversas, que dificultam a adesão ao tratamento e, especialmente no caso dos BDZ, o potencial para produzir dependência química, o que ressalta a necessidade de opções eficazes e seguras para o manejo clínico destas desordens.

1.3.3 DEPRESSÃO

A depressão está entre os mais prevalentes tipos de doenças mentais, sendo associada a elevados índices de morbidade e mortalidade no mundo (BLAZER, 2000). O termo depressão refere-se tanto a um estado transitório experimentado pelos seres humanos em algum estágio da vida, quanto a uma síndrome que se diferencia pelo agravamento e duração das manifestações clinicas apresentadas, chamada depressão maior (FAVA; KENNETH; KENDLER, 2000).

Alterações de afeto, humor, apetite, sono, sentimento de culpa e inutilidade, e distúrbios na atividade locomotora, persistentes por mais de duas semanas estão entre os sintomas que compõem clínica da depressão maior e estão entre os critérios

para diagnosticá-la (NESTLER et al. 2002; PERITO e FORTUNATO, 2012). Outro fato importante é que a depressão pode ser decorrente de outras enfermidades, como ocorre na doença de Huntington, ou ser precipitada pelo uso de fármacos como a reserpina, um bloqueador de transportadores vesiculares de dopamina (FAVA; KENDLER, 2000). Inclusive, a partir desse tipo de descoberta e a observação de que fármacos como a imipramina, melhoravam o quadro de pacientes depressivos, surgiu a teoria mononaminérigica da depressão (MANJI; DREVETS; CHARNEY, 2001).

Esta teoria sugere que os sintomas depressivos seriam resultantes da depleção de monoaminas (principalmente serotonina, noradrenalina e dopamina), uma vez que estes neurotransmissores modulam diversas funções do SNC (Figura 05). Por exemplo, a serotonina e noradrenalina parecem regular o humor, estado de vigília e apetite, condições fisiológicas que, conforme citado acima estão alteradas em pacientes deprimidos (KANDEL; SCHWARTZ; JESSEL, 2000; KOBAYASHI, 2001). A dopamina, por sua vez, tem sido implicada principalmente por sua participação no sistema límbico, atuando diretamente as vias de recompensa cerebral, estando envolvida na fisiopatologia da anedonia, um comportamento característico de indivíduos depressivos (MANJI; DREVETS; CHARNEY, 2001; ann het ROT; MATHEW; CHARNEY, 2009).

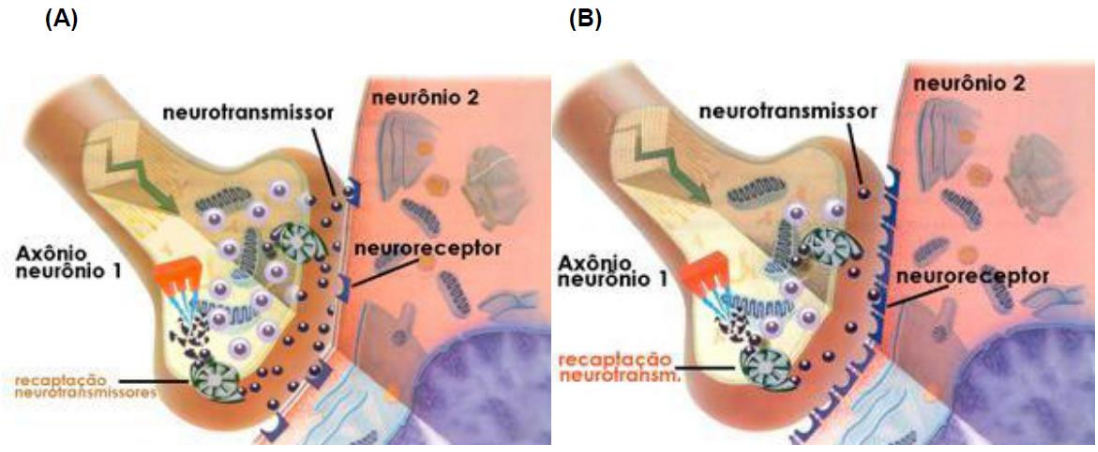

Figura 07: Hipótese monoaminérgica da depressão. Painel A: Liberação de neurotransmissores e expressão de receptores pós-sináticos em um indivíduo sadio. Painel B: Expressão aumentada de receptores pós-sinapticos e liberação de neurotransmissores em um paciente depressivo.

Fonte: BALLONE, 2007

Assim, o atual tratamento da depressão é realizado com fármacos que atuam melhorando a oferta destes neurotransmissores, embora outros sintomas chave como alterações no ciclo circadiano e cognição sejam relevantes tanto do ponto de vista clínico quanto para a compreensão das possíveis causas da doença (FAVA; KENDLER, 2000). Outras descobertas indicam o envolvimento do sistema glutamatérgico tanto na neurobiologia, pela indução de excitotoxicidade mediada por receptores N-metil-D-aspartato (NMDA), quanto no tratamento, através da liberação de glutamato para receptores não-NMDA (antagonismo competitivo) (DUTA; MCKIE; DEAKIN, 2015; HASHIMOTO, 2009.

Há ainda um crescente interesse por efeitos tróficos e antiapoptóticos de fármacos antidepressivos (DRZYZGA; MARCINOWSKA; OBUCHOWICZ, 2009). Tal fato se deve a estudos de neuroimagem estrutural e funcional que indicam haver redução do metabolismo e do volume de células em áreas como hipocampo, nos lobos frontais, gânglios da base e estruturas temporais do cérebro, envolvendo essencialmente conexões entre algumas destas regiões e o sistema límbico (KRISHNAN; NESTLER, 2008).

1.3.4 EPILEPSIA

A epilepsia compreende um grupo de distúrbios neurológicos, de ordem crônica, que possuem em comum o desenvolvimento recorrente e espontâneo de convulsões, na ausência de processos tóxicos-metabólicos ou febris (GASTAUT, 1973; ROGAWISKI e PORTER, 1990). Este tipo de transtorno pode ser causado por diversos processos patológicos, que ocorrem em regiões distintas do encéfalo. A epileptogênese decorre da transição de grupamentos de células normais para um estado de hiperexcitabilidade, o que as torna aptas para receber uma descarga anormal, que se converte nas convulsões (crises) observadas em pacientes epiléticos (SCHARFMAN, 2007). Essas descargas neuronais variam de acordo com o paciente, com a região de origem e local de propagação da descarga neural e, podem ser percebidas ou não pelo indivíduo (SANDER e HART, 1999).

Dentre as formas de classificar as crises epiléticas, a proposta mais aceita é a que as divide em dois grupos, considerando suas manifestações clínicas e eletroencefalográficas, proposta pela Liga Internacional Contra a Epilepsia (COMMISSION, 1989). O primeiro tipo são as crises parciais, na qual inicialmente ocorre a manifestação clínica, seguida por inconsciência, sendo as áreas corticais a origem da descarga neuronal, podendo generalizar-se posteriormente. O segundo grupo são as generalizadas, que se caracteriza pela perda súbita de consciência e com ambos os hemisférios sendo afetados simultaneamente (COMMISSION, 1989).

Embora existam diversos mecanismos capazes de produzir convulsões, o fato é que elas ocorrem quando o equilíbrio entre inibição e excitação do SNC é quebrado (SCHARFMAN, 2007). A excitação ou inibição de uma célula neuronal é resultante do balanço iônico, especialmente de sódio e potássio, presentes em maiores concentrações nos meios extra e intracelular, respectivamente (potencial de repouso de aproximadamente -60mV). Em condições normais, após a estimulação, um neurônio pré-sináptico libera um neurotransmissor, o qual estimula um neurônio pós-sináptico, propagando assim a informação recebida (MCCORMICK; HUGUENARD, 1994).

Dentre os controles que mantém a célula em estado de repouso ou que a trazem para ele estão os transportadores iônicos (bombas) presentes na membrana plasmática, principalmente a sódio/potássio ATPase. Inclusive, o bloqueio experimental desta bomba facilita o desenvolvimento de convulsões, o que sugere que alterações funcionais nesse transportador poderiam no mínimo ser um adjuvante na epileptogênese (GRISAR; GUILLAUME; DELGADO-ESCUE, 1992; TAVAILLEND; MASON; CUTTLE; ALGER, 2002).

Outra possível causa seria a mutação de canais de sódio, seja na redução em seu limiar de ativação ou na capacidade de inativação destes canais, levando a ativação excessiva destes. Neste sentido, estudos têm demonstrado mutações em canais de sódio voltagem dependente, inclusive em indivíduos com síndrome epilética generalizada, com convulsões febris (MEISLER; KEARNEY; OTTMAN; ESCAYG, 2001). Do ponto de vista clínico, diversos fármacos empregados como antiepiléticos atuam como inibidores de canais de sódio (carbamazepina, fenitoína, por exemplo), o que indica o envolvimento desses mecanismos na fisiopatologia da epilepsia.

No tocante a neurotransmissão, o ácido gama-aminobutírico (GABA, do inglês *"Gamma aminobutyric acid"*) e o glutamato têm sido implicados também na epilepsia, uma vez que são os principais transmissores inibitórios e excitatórios do SNC (SCHARFMAN, 2007). Exemplos de fármacos utilizados no tratamento da epilepsia e que atuam favorecendo a transmissão gabaérgica estão os barbitúricos e alguns benzodiazepínicos (Figura 06). Como esperado, a ação dos anticonvulsivantes na via glutamatérgica é inibitória, através do bloqueio de repceptores AMPA e NMDA, ou inibindo a liberação do glutamato na fenda sináptica.

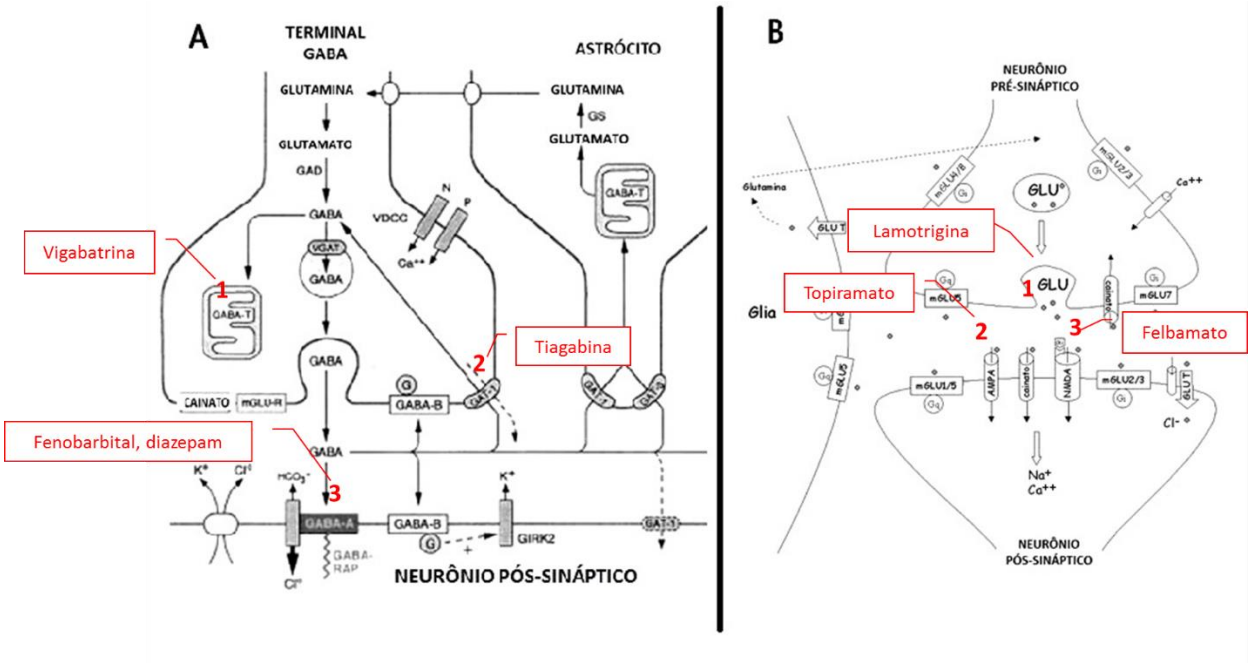

Figura 06: Alvos para drogas anticonvulsivantes. A: 1- Inibidores da GABA-transaminase; 2- Inibidores da receptação do GABA; 3- Agonistas gabaérgicos. B: 1- Inibidores da liberação de glutamato; 2- Antagonistas dos receptores AMPA; 3- Antagonistas dos receptores NMDA.

Fonte: Adaptado de Olsen 2002 e Carobrez, 2003.

Drogas antiepiléticas, em geral, podem potencializar alterações cognitivas, que na maioria das vezes são inerentes a doença. Além disso, muitas delas possuem uma janela terapêutica estreita, o que é bastante preocupante, especialmente em crianças

e idosos. Outro fato preocupante consiste na ocorrência de resistência ao tratamento com fármacos anticonvulsivantes, especialmente quando se trata de extremos de idade, já que não existem muitas opções seguras para estes grupos de pacientes (KWAN et al. 2011).

1.3.5 MEMÓRIA

O sistema nervoso possui diversas atribuições, mas sem dúvida, uma de suas mais intrigantes funções consiste na habilidade do cérebro em reter informações oriundas de experiências, bem como evocá-las. O aprendizado e a memória fazem parte deste complexo processo. A aquisição de uma nova informação, que pode expressar-se através de alterações comportamentais é denominada aprendizado. Por sua vez, o termo memória, refere-se à capacidade de adquirir, conservar e evocar a informações de aprendizados anteriores (IZQUIERDO et al. 2006). Portanto, a etapa de aquisição da informação é o que caracteriza o aprendizado, enquanto que a evocação é dita recuperação e lembrança.

O nível de consciência e estados de humor modulam a codificação, armazenamento e evocação da memória (Figura 07). Um dos exemplos de correlação entre memória e emocionalidade é a forma de aprendizado denominada esquiva inibitória, na qual animais submetidos a um estímulo aversivo passam a evitá-lo (CAMMAROTA et al. 2008). De fato, nem toda informação recebida é armazenada. Assim, uma das formas de classifica-las é quanto a sua duração em: memória de curta duração (MCD, duração de segundos, minutos ou poucas horas) e longa duração (MLD, duração por dias, meses ou anos). Há ainda a memória de trabalho, a qual possui estreita relação com a MCD, podendo ser vista como uma forma especializada, que integra funções executivas a partir da retenção temporária da informação durante a realização de alguma tarefa.

Em nível celular, as memórias são codificadas pelos neurônios, armazenadas e evocadas a partir destas redes neurais ou por outras vias. No caso da MCD, a formação mnêmica ocorre no hipocampo e no córtex entorrinal, sendo armazenada de forma provisória (no máximo 6 horas), podendo a informação ser ou não consolidada a uma forma duradoura. Assim, as modificações envolvidas no processamento da MCD são transitórias, não envolvendo alterações plásticas ou

síntese proteica, ocorrendo provavelmente pela modificação covalente de proteínas pré-existentes (CAREW, 1996). Quanto a MLD, sua formação necessita da expressão gênica e síntese de proteínas nas em um período de 3 a 6 horas em regiões como o hipocampo que pode armazenar ou transferir informações para serem armazenadas em outras regiões do SNC (Figura 07).

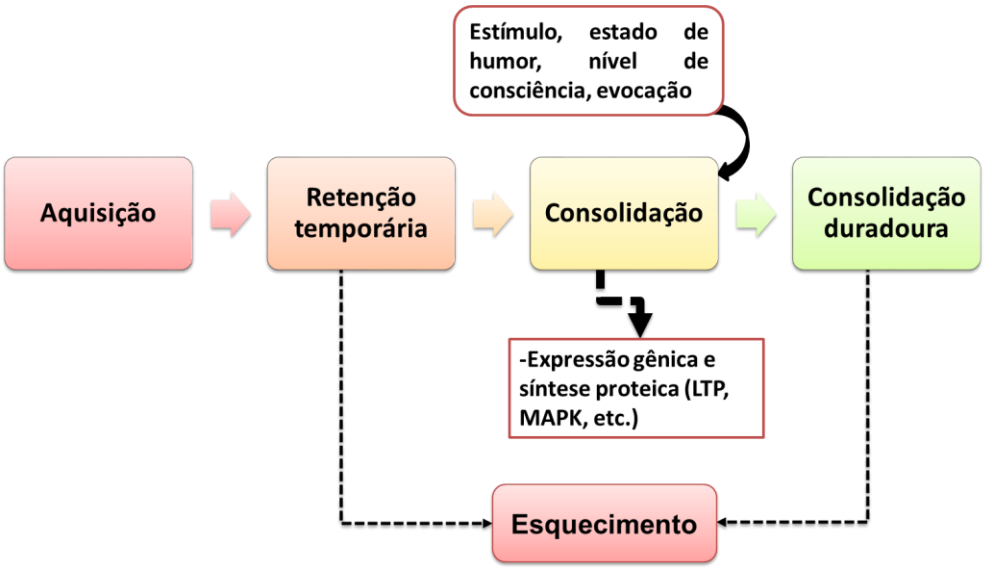

Figura 07: Etapas do processo cognitivo

Fonte: Adaptado de LENT, 2010

Um dos mecanismos que parece estar envolvido na formação da MLD é o LTP (do inglês "*long term potentiation*") descoberto por Bliss e Lomo (1973), durante a investigação da capacidade de armazenamento de informações do hipocampo. Neste estudo foi descoberto que em uma via hipocampal, durante um curto período, a atividade elétrica de alta frequência que aumentava a efetividade sináptica (LOMO, 2003). A LTP mais conhecida e estudada envolve a atividade glutamatérgica em regiões CA1 do hipocampo. De acordo com esses estudos, durante a LTP ocorre uma elevada liberação de glutamato, o qual se liga tanto aos receptores AMPA (do inglês *"α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid"*) quanto ao NMDA. No entanto, é a ligação ao primeiro que permite a ativação do receptor NMDA,

possibilitando o influxo de cálcio e ativando a via bioquímica do LTP (RUGGIERO et al. 2014).

Com o passar do tempo, a vivência de novas experiências e, portanto, a formação de novas memórias faz com que estas se sobreponham, sendo algumas informações perdidas ao longo do tempo (BERTÉ, 2002). Logo, o esquecimento, de certo modo, pode ser visto como um processo natural, cuja relevância pode depender de fatores intrínsecos ou ser agravado pela existência de algum quadro patológico. A doença de Alzheimer é uma das mais conhecidas desordens de memória, na qual inicialmente ocorre o comprometimento cortical e hipocampal e, consequentemente da MCD (MANN, 1996; NORFREY; PROVENZALE, 2004). Com o progresso da doença observa-se a redução da densidade celular em outras regiões do SNC, levando a prejuízos na MLD, execução de movimentos e alterações no humor e comportamento (KOROLEV, 2014).

Outros transtornos, tais como as doenças de Huntington e Parkinson tem a alteração mnemônica como sintoma subjacente (NIH, 2013). Apesar das diferentes origens, as desordens de memória possuem em comum a escassez de recursos terapêuticos para seu manejo clínico (SCHENCK, 2008). Além disso, os fármacos que já são utilizados na prática clínica, possuem efeitos indesejáveis ou mesmo não se mostram tão eficazes frente ao quadro patológico, principalmente no que se refere a capacidade de redução da morte celular observada no curso da doença (KOROLEV, 2014). Desta forma, é essencial a busca por novos insumos que possam tratar satisfatoriamente o paciente, melhorando sua qualidade de vida.

Considerando a relevância das desordens de memória, inicialmente, o objetivo deste estudo foi investigar a influência do tratamento com o extrato hidroalcoólico das folhas de *P. alliacea*, sobre os processos de aquisição e consolidação de memória, a fim de subsidiar as buscas por possíveis compostos biologicamente ativos. Adicionalmente, foi realizado um trabalho de revisão crítica acerca das características botânicas, taxonômicas, etnobotânica, efeitos no SNC, fitoquímica e dados toxicológicos de *P. alliacea*. Esta revisão teve por objetivo identificar as lacunas científicas a respeito desta planta, bem subsidiar pesquisas futuras que permitam seu melhor aproveitamento enquanto recurso terapêutico no tratamento de outros transtornos do SNC, tais como ansiedade, depressão, epilepsia, etc.

II OBJETIVOS

2. OBJETIVOS

2.1 Objetivo geral

Investigar a atividade do extrato hidroalcoólico das folhas de *P. alliacea* L. (Phytolaccaceae) sobre o processo mnemônico e de aprendizagem e fazer uma revisão bibliográfica a respeito da etnofarmacologia, fitoquímica e efeitos desta espécie sobre o SNC.

2.2 Objetivos específicos

a) Verificar a presença de compostos organossulfurados no extrato;
b) Avaliar seus efeitos sobre a aprendizagem;
c) Avaliar a atividade sobre a memória de curta e de longa duração;
d) Avaliar sua influência sobre a memória espacial;
e) Realizar um levantamento bibliográfico acerca dos usos aspectos botânicos, taxonômicos, usos etnofarmacológicos para o SNC, composição fitoquímica, efeitos neurofarmacológicos e toxicidade de *P. alliacea*.

III CAPÍTULO I

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Review

Ethnobotany, phytochemistry and neuropharmacological effects of *Petiveria alliacea* L. (Phytolaccaceae): A review

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Myricetin (PubChem CID 5281672)
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ABSTRACT

Ethnopharmacological relevance: *Petiveria alliacea* L. commonly grows in the tropical regions of the Americas such as the Amazon forest, Central America, Caribbean islands and Mexico, as well as specific regions of Africa. Popularly known by several different names including 'mucuracaá', 'guiné' and 'pipi', *P. alliacea* has been used in traditional medicine for the treatment of various central nervous system (CNS) disorders, such as anxiety, pain, memory deficits and seizures, as well as for its anaesthetic and sedative properties. Furthermore, the use of this species for religious ceremonies has been reported since the era of slavery in the Americas. Therefore, the present review aims to provide a critical and comprehensive overview of the ethnobotany, phytochemistry and pharmacological properties of *P. alliacea*, focusing on CNS pharmacological effects, in order to identify scientific lacunae and to open new perspectives for future research.

Materials and methods: A literature search was performed on *P. alliacea* using ethnobotanical textbooks, published articles in peer-reviewed journals, unpublished materials, government survey reports and scientific databases such as PubMed, Scopus, Web of Science, Science Direct and Google Scholar. The Plant List, International Plant Name Index and Kew Botanical Garden Plant name databases were used to validate the scientific names.

Results and discussion: Crude extracts, fractions and phytochemical constituents isolated from various parts of *P. alliacea* show a wide spectrum of neuropharmacological activities including anxiolytic, antidepressant, antinociceptive and anti-seizure, and as cognitive enhancers. Phytochemistry studies of *P. alliacea* indicate that this plant contains a diversity of biologically active compounds, with qualitative and quantitative variations of the major compounds depending on the region of collection and the harvest season, such as essential oil (Petiverina), saponinic glycosides, isoarborinol-triterpene, isoarborinol-acetate, isoarborinol-cinnamate, steroids, alkaloids, flavonoids and tannins. Root chemical analyses have revealed coumarins, benzyl-hydroxy-ethyl-trisulphide, benzaldehyde, benzoic acid, dibenzyl trisulphide, potassium nitrate, b-sitosterol, isoarborinol, isoarborinol-acetate, isoarborinol-cinnamate, polyphenols, trithiolaniacine, glucose and glycine.

Conclusions: Many traditional uses of *P. alliacea* have now been validated by modern pharmacology research. The available data reviewed here support the emergence of *P. alliacea* as a potential source for the treatment of different CNS disorders including anxiety, depression, pain, epilepsy and memory

Abbreviations: CNS, central nervous system; KNO_3, potassium nitrate; EAF, acetate fraction; FH, hexanic fraction; FHA, hydroalcoholic fraction; FHAppt, precipitated hydroalcoholic fraction; EPM, elevated plus maze; WP, whole plant; AP, aerial parts; R, roots; FST, forced swimming test; OFT, open field test; ETM, elevated T-maze; MWM, Morris water maze; SCE, sister chromatid exchanges; PaLHE, *P.* alliacea leaf hydroalcoholic extract; IC_{50}, half-maximal inhibitory concentration; OECD, Organisation of economic co-operation and development; DTS, dibenzyl trisulphide; TLC, thin-layer chromatography; MeOH, methanol; HCl, hydrochloric acid; NH_4OH, ammonium hydroxide; HPLC, high-performance liquid chromatography; MAPK, mitogen-activated protein kinase; RSK, ribosomal S6 kinase; NFTs, neurofibrillary tangles; BSA, bovine serum albumin; RBCs, red blood cells; PKC, protein kinase C; NO, nitric oxide; NFκB, nuclear factor kappa B; TNF-α, tumour necrosis factor alpha; IL-1β, interleukin 1β; COX-2, cyclooxygenase-2; CG/MS, gas chromatography coupled to mass spectrometry

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impairments. However, further studies are certainly required to improve the knowledge about the mechanisms of action, toxicity and efficacy of the plant as well as about its bioactive compounds before it can be approved in terms of its safety for therapeutic applications.

Contents

1. Introduction

The *Petiveria* belongs to the Phytolaccaceae, the most archaic family of the Caryophyllales, comprising about 17 genera and 120 pantropical species widely distributed throughout the American continent (Duarte and Lopes, 2005). Among the species of *Petiveria*, the most popular is *Petiveria alliacea* L. It is a perennial shrub with a rigid and straight stem, reaching a height of up to 5–150 cm (Almanza, 2012; Duarte and Lopes, 2005; Rzedowski and de Rzedowski, 2000). The alternating and elliptical leaves, small bisexual flowers (white, whitish-pink or green) and achene-type fruits are typical of this plant (Andrade et al., 2012; Duarte and Lopes, 2005; Rzedowski and de Rzedowski, 2000; Soares et al., 2013). This plant is native in tropical regions such as the Amazon rainforest, Central and South America, the Caribbean islands and sub-Saharan Africa (Rzedowski and de Rzedowski, 2000). The medicinal use of *P. alliacea* occurs in several regions of the world, mainly in American continent. In the folk medicine, it has curative and mystical purposes, which illustrates the importance to local tradition and culture.

For example, in Brazil, this plant has been used in religious ceremonies in Brazil at least since the slavery era. Slaves used *P. alliacea* for its toxic and sedative effects. Thus, the plant is also popularly known as 'Remedy to tame the Master', which refers to its sedative property and potential to alter the mind and brain function (Bastide, 1971; Caminhoá, 1884; Camargo, 2007; Gomes et al., 2005, 2008; Peckolt and Peckolt, 1900; Ramos, 1988; Rodrigues et al., 2003; Santos Filho, 1947).

According to indigenous medicine, the root, powder and leaf of *P. alliacea* have been associated with several therapeutic properties, such as diuretic, antispasmodic, emmenagogic, analgesic, anti-inflammatory, antileukaemic, antirheumatic, antihelminthic, antimicrobial and depurative properties (Duarte and Lopes, 2005; Lima et al., 1991). In addition, different preparations of *P. alliacea* are utilized for its activities on the central nervous system (CNS) such as *anti*convulsant, anxiolytic, mnemonic, anaesthetic and sedative (Gomes et al., 2005; Lima et al., 1991).

Over the last two decades, different research groups have validated many traditional uses of *P. alliacea* through the use of laboratory animals and a range of neurobehavioural paradigms and pharmacological approaches. Moreover, phytochemical research has expanded the knowledge about the metabolites present in the plant (i.e. sulphur derivatives, flavonoids, alkaloids and many others), revealing their potential to interact with biological systems, including many targets in the CNS (Benevides et al., 2001; De Sousa et al., 1990; Monache and Suarez, 1992; Williams et al., 2007). However, the *P. alliacea* mechanisms of action remain mostly unknown as well as the compounds involved in such activities.

On the other hand, despite its beneficial pharmacological properties, *P. alliacea* is also known to exert toxic effects on the CNS (Lima et al., 1991). Remarkably, deaths after one year of chronic exposure to this plant have been reported (Peckolt and Peckolt, 1900). Therefore, the toxicological profile of this species has been addressed in recent studies.

The purpose of this review is to provide comprehensive information on the botany, traditional uses, phytochemistry, neuropharmacology and toxicological research of *P. alliacea* in order to explore its therapeutic potential focused on neuropharmacological properties, highlight the lacunae in the current knowledge and evaluate future research opportunities. The available information on *P. alliacea* was collected via electronic search (using PubMed, Scopus, Web of Science, Science Direct, Google Scholar) and a library search for articles published in peer-reviewed journals, unpublished materials, theses and ethnobotanical textbooks. The Plant List (www.theplantlist.org), International Plant Name Index and Kew Botanical Garden Plant name databases were used to validate the scientific names. This review thus may provide the

scientific basis for future research work on the central effects of *P. alliacea*. Besides, this data compilation highlights the security in traditional medicine, religious rituals and ceremonies.

2. Ethnobotany

2.1. Taxonomy and botanical aspects

The taxonomic rating of *P. alliacea* shows some diversions, probably because earlier studies were performed using more archaic analytical techniques. Including, the plant has some scientific, registered and valid synonyms of *P. alliacea* include: *Petiveria foetida* Salisb., *P. alliacea* var. grandifolia Moq., *P. alliacea* var. octandra (L.) Moq., *P. foetida* Salisb., *P. hexandria* Sessé& Moc., *P. ochroleuca* Moq., *P. octandra* L. and *P. paraguayensis* D. Parodi (Tropicos.org, 2015). It is probably that the diversions on taxonomy occurs because earlier studies were performed using more archaic analytical techniques. Consequently, the accurate morphology of some structures (the seed, for example) could not be identified and a genus was included in the wrong family (Neves and Bauermann, 2006). For instance, this species was classified as a member of the Rivinoideae subfamily (Nowicke, 1968; Rohwer, 1993). Other authors classified *P. alliacea* as a member of the Petiveriaceae, which is a subgroup of the Phytolaccaceae (Brown and Varadarajan, 1985; Culham, 2007; Judd et al., 2002). The species of the Petiveriaceae have four tepals, a minimum of four stamens, a gynoecium with one carpel and a drupe or achene with indehiscent-type fruit, with a slightly lenticular seed and embryonic distinctions (Almanza, 2012; APG III, 2003; Brown and Varadarajan, 1985; Culham, 2007; Cronquist, 1981; Engler and Prantl, 1894; Judd et al., 2002; Neves and Bauermann, 2006).

On the other hand, according to the majority of the botanical studies on *P. alliacea*, the plant belongs to the order of Caryophyllales, also known as Centrospermae, and a member of Phytolaccaceae, which is a more accurate taxonomic classification (Almanza, 2012; APG III, 2009; Cronquist, 1981). The Phytolaccaceae comprises 17 genera, and 70–125 pantropical species have been reported. The members of this family are better adapted to shaded and subhumid places (Almanza, 2012; Duarte and Lopes, 2005; Gomes, 2006; Ke et al., 2003; Marchioretto, 1989; Soares et al., 2013; Steinmann, 2010; Stevens, 2010).

Robert Brown first described the Phytolaccaceae. It is primarily composed of perennial herbs, but shrubs, trees and vines have also been reported. Members of this family have a straight stem as well as alternating, petiolate leaves in the majority of cases. The flowers are actinomorphic in shape, are more frequently hermaphroditic and are organized into inflorescences, which may be of auxiliary,

Table 1
Botanical characteristics of *Petiveria alliacea* L.Adapted from Almanza (2012).

***Petiveria alliacea* L.**			
Representatives and structures	**Description**	**Dimension**	**Reference**
Representatives	Perennial herbaceous or shrubby plant.	5 cm to 1.5 m	Almanza, 2012; Duarte and Lopes, 2005; Rzedowski and de Rzedowski, 2000.
Stem	It is a straight and rigid structure. It is commonly contains a slender branch with longitudinal stripes.	Variable length	Almanza, 2012; Andrade, 2011; Joly, 1979; Gomes, 2006; Rzedowski and de Rzedowski, 2000.
Roots	Fusiform roots with irregular branches and fine longitudinal stripes. The external surface presents a light greyish-brown or yellowish-brown colour.	Variable length	Gomes, 2006.
Leaves	It is an acuminate leaf with oblong or elliptical anatomy. The leaves are alternately distributed and membranous with herbaceous consistency, short petiole of pinnate camptodromous (brachidodromous) venation type. The length of the petioles and stipules vary between 0.6 and 1 cm and 2 mm, respectively.	5–10 cm in length;2–6 cm wide	Almanza, 2012; Di Stasi and Hiruma-Lima, 2002; Duarte and Lopes, 2005; Gomes, 2006; Rocha et al., 2006; Rzedowski and de Rzedowski, 2000; Soares et al., 2013.
Peduncle	Simple free peduncle, characterized by its narrow shape and flexibility.	0.5–2.5 cm in length	Rzedowski and de Rzedowski, 2000.
Flowers	Flowers white, sessile and bisexual, with actinomorphic symmetry, whitish-pink or green to pale brown in colour. It has three main longitudinal ribs, oblong linear petals and a tetramerous perigonium. The flowers are composed of spikes or inflorescences.	4–6 mm long and about 1 MM wide (the petals)	Almanza, 2012; Andrade, 2011; Di Stasi and Hiruma-Lima, 2002; Gomes, 2008; Rzedowski and de Rzedowski, 2000; Soares et al., 2013; Udulutsch et al., 2007.
Inflorescence	The inflorescences are racemose and present two possible localizations, terminal or axial.	10–15 cm in length	Di Stasi and Hiruma-Lima, 2002; Gomes, 2008.
Filaments	There are fine filaments uneven in length, more or less persistent.	3–5 mm in length	Rzedowski and de Rzedowski, 2000.
Androecium	The androecium presents four to eight irregular stamens shorter than tepals. The androecium is localized in a fleshy structure and contains free filiform fillets.	–	Soares et al., 2013; Udulutsch et al., 2007
Gynoecium	The gynoecium is a unicarpellate, subulate, deflexed and laterally flattened organ. This structure is tomentose, and the stigma is sessile and penicillate.	–	Di Stasi and Hiruma-Lima et al., 2002; Soares et al., 2013; Udulutsch et al., 2007
Fruit	Cylindrical achene-type fruit, with longitudinal stripes and similar seed dimensions. It is flattened and rounded and linear, with adherent pericarp and membranous forehead situated close to the rachis. It may be wrapped by rigid petals.	6–8 mm in length;1–2 mm in width	Di Stasi and Hiruma-Lima, 2002; Rzedowski and de Rzedowski, 2000; Soares et al., 2013; Udulutsch et al., 2007
Anthers	Frequently, the anthers are prematurely obsolete, sagittal or cylindrical, localized near the oblong, long linear slit at both ends.	1.5–2 mm in length	Soares et al., 2013; Udulutsch et al., 2007
Ovary	The ovary is cylindrical or flattened.	–	Rzedowski and de Rzedowski, 2000.
Stigma	It is sessile or pedicle, localized laterally.	–	Rzedowski and de Rzedowski, 2000; Soares et al., 2013; Udulutsch et al., 2007
Pollen	Pollen grains are of the stephanopontoperculate type. It is tiny, circular and radially symmetrical with a high concentration of sexine in the pores.	24–27.1 μm	Bath and Barbosa, 1972; Neves and Bauermann, 2006

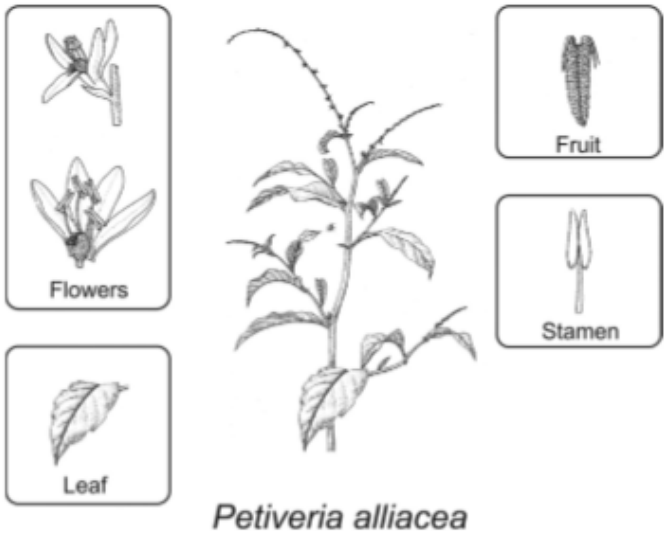

Fig. 1. Principal anatomic structures of *Petiveria alliacea* L. Adapted from Almanza (2012).

terminal, racemose, cymose, panicle or spike types. A minimum of three stamens constitute the androecium, which are not distributed ordinately but are associated with the tepals and inserted in a hypogynous disc. The ovaries are often superolateral, and the indehiscent fruits can be fleshy or dry with one unique wilt seed per locule (Almanza, 2012; Rzedowski and de Rzedowski, 2000; Shang et al., 2003; Steinmann, 2010; Stevens, 2010). The plants of this family show variable pollen morphology, with birds and wind spreading the seeds, and insects promoting pollination (Lorenzi, 1992; Neves and Bauermann, 2006).

The complete botanical description of *P. alliacea* is presented in Table 1. The *Petiveria* represents a group of herbaceous or shrub plants and perennial herbs. It is characterized by an erect, branched and cylindrical main structure or stem. The chemical components of the plant produce a strong odour, usually associated with garlic (Almanza, 2012; Rzedowski and de Rzedowski, 2000). This feature justifies its species name *alliacea* (Alonso, 1998). This genus is characterized by alternating leaves. As depicted in Fig. 1, the leaves also exhibit small stipules, and are petiolate, membranous and glabrous (Almanza, 2012; Rzedowski and de Rzedowski, 2000).

The flowers are small, actinomorphic, hermaphroditic, and white, green or pink in colour. The inflorescences are composed of long curls, which may be axillary or terminal. The curls are composed of 8–30 flowers with bracts and bracteoles as well. The perianth consists of four free petals, which are intimately associated with the fruit. The androecium is filiform in nature, with the number of stamen filaments ranging from four to nine. The anthers are fixed, and the fruit is an achene with two lobules and four to six spines on the dorsal area (3–5 mm in length) (Almanza, 2012; Rzedowski and de Rzedowski, 2000).

In this genus, the seed and embryo are straight and membranous in the superior region. The ovary is unicarpelar and unilocular in nature, and is localized on the apocarps (top region) presenting a single egg. The stigma may be sessile or subsessile, and is connected by a lateral gap (Almanza, 2012; Rzedowski and de Rzedowski, 2000). The South American samples were found to be of stephanopontoperculate pollen morphology. This pollen morphology could only be characterized through electron microscopy analysis (Neves and Bauermann, 2006). Thus, earlier studies on the pollen morphology of this genus, which used only optic microscopy, reported a pantoporate morphology (Barth and Barbosa, 1972; Bortenschlager, 1973; Erdtman, 1952).

In *P. alliacea* samples from the Amazon, flowering was the highest in the months of September and November, for an average of 20–21 days. The lowest flowering average was recorded in February and July, for nine and seven days, respectively. Fruiting was not observed in March, June, July and November. The highest fruiting average was 21 days in the months of April and May. The lowest fruiting average was six days in the month of December (Assis et al., 2013).

For the samples collected from the southern region of Brazil, flowering was the highest from the months of November to March (Neves and Bauermann, 2006). Flowering and fruiting were also reported from December until April (Hatschbach and Guimarães, 1973). The different periods of flowering and fruiting reported in various studies can be primarily attributed to the different climate conditions of the regions from which the samples were collected. These variations may influence other plant traits, such as phytochemical composition (see the following sections).

2.2. Distribution and traditional uses

Different theories have been proposed for the original geographic distribution of *P. alliacea*. This species was first described in Jamaica in the 18th century (Linnaeus, 1753), which supports the theory of slaves bringing *P. alliacea* L. from Africa to Brazil in South America (Gomes, 2008; Kubec and Musah, 2001).

According to studies conducted by Germosén-Robineau (1995), this plant is native to Central America (Rocha et al., 2006), whereas Di Stasi and Hiruma-Lima (2002) indicated that South America was the first area where this plant occurred. Moreover, recent studies have noted a higher incidence of this species in a wild and subspontaneous state throughout South America (Andrade, 2011; Marchioretto, 2010; Soares et al., 2013).

Based on these data, *P. alliacea* is found to be common in tropical regions of the Americas such as the Amazon forest, Central America, Caribbean islands and Mexico, as well as specific regions of Africa. This species is endemic to these regions (Almanza, 2012; Gupta, 1995; García-González et al., 2006; Rzedowski and de Rzedowski, 2000). It adapts to humid, warm and shaded environments (Marchioretto, 1989; Soares et al., 2013). In addition, as summarized in Table 2, *P. alliacea* is known by various popular names according to their geographic localization.

Due to its diverse medicinal uses, this plant was exported to other continents. Nowadays, *P. alliacea* is available in North America (southern USA and Mexico), Central America (El Salvador, Guatemala, Panama, Nicaragua and Honduras), the Caribbean (Cuba, Haiti, Jamaica and Martinique), South America (Brazil, Argentina, Colombia, Peru, Venezuela and Paraguay), Africa and India (Almanza, 2012; Rzedowski and de Rzedowski, 2000) (see Fig. 2).

P. alliacea was employed ethnomedically primarily in the American continent, but nowadays it is widely used in traditional medicine in different regions of the world (Almanza, 2012; Camargo, 2007). In general, the consumption of 9 g of the dried plant with 600 ml of boiled water is recommended three times daily (Ferraz et al., 1991a,b). Other traditional preparations include a decoction or infusion prepared with 30 g of dried *anamu* whole herb in a litre of water. For this treatment, dosages from a quarter to half a cup must be consumed three times daily or used topically (Taylor, 2005). Additional uses of *P. alliacea* in folk medicine are presented in detail in Tables 3 and 4.

Ethnobotanical studies have shown that leaf infusions or decoctions from *P. alliacea* were used in sacred rituals in Nicaragua, Panamá, Guatemala and Brazil (Camargo, 2007; Coe and Anderson, 1996b; Girón et al., 1991; Joly et al., 1987; Leitão et al., 2009; Schardong and Cervi, 2000; Souza and Neto, 2010). Reports describe *P. alliacea* associated with other plants for diverse purposes. For example, the healers and indigenous communities of the

Table 2
Popular names of *Petiveria alliacea* L. According to geographic localization.

Popular name	Geographic localization	References
Ajillo	Colombia	Gupta, 1995.
Amansa-senhor (Tame-master)	Brazil: Amazonas and Bahia	Braga, 1992; Camargo, 2007; Gupta, 1995; Di Stasi and Hiruma-Lima, 2002; Gomes et al., 2005; Ximenes, 2008.
Amanu	Cuba	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Anamú	Colombia; Cuba; Panama; Peru; Dominican republic; Venezuela; USA	Braga, 1992; Gomes, 2006; Gupta, 1995; Rodríguez et al., 2004.
Apacin	Brazil: amazonas, Pará and Roraima; Guatemala	Braga, 1992; Gomes, 2006; Gupta, 1995; Rodríguez et al., 2004.
Apasote de zorro	Guatemala	Rodríguez et al., 2004.
Apurito	Colombia	Braga, 1992; Gomes, 2006; Gupta, 1995.
Ave	Haiti	Rodríguez et al. (2004).
Caá	Brazil: Amazonas	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Chambira	Peru	Braga, 1992; Gomes, 2006; Gupta, 1995.
Da-hua-ta	Colombia: Mikuna	Braga, 1992; Gomes, 2006; Gupta, 1995.
Erva de alho	Brazil: Amazonas, Pará and Roraima	Braga, 1992; Gomes, 2006; Gupta, 1995.
Erva-guiné	Brazil	Hoehne, 1939; Pio Correa, 1969.
Erva-pipi	Brazil: Pernambuco and São Paulo	Di Stasi and Hiruma-Lima, 2002.
Erva-de-tipi	Brazil	Lima et al., 1991.
Gambá-tipi	Brazil: Mato Grosso	DI Stasi and Hiruma-Lima, 2002.
Gorarema	Brazil: Amazonas	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Gorazema	Brazil: Amazonas	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Guiné	Brazil: Pernambuco and São Paulo	Braga, 1992; Gupta, 1995; Hoehne, 1939; Lima et al., 1991; Pio Correa, 1969; Ximenes, 2008.
Guinea hen	Colombia: San Andrés and Providencia	Rodríguez et al., 2004.
Guinea hen weed	Jamaica, Panamá and USA	Braga, 1992; Gupta, 1995; Rodríguez et al., 2004; Ximenes, 2008; Yukes and Balick, 2010.
Herbe aux poules	France	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Hierba del Zorrillo	Mexico	Fletes-Arjona et al., 2013.
Ipacina	Honduras and Nicaragua	Rodríguez et al., 2004.
Ipicina	Nicaragua	Braga, 1992; Gupta, 1995; Ximenes, 2008; Yukes and Balick, 2010.
Iratacaca	Brazil: Amazonas	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Koujourouck	Dominican Republic	Rodríguez et al., 2004.
Lanceilla	Colombia	Braga, 1992; Gomes, 2006; Gupta, 1995.
Macur	Brazil: Amazonas	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Mapurita	Colombia	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Mapurite	Venezuela	Braga, 1992; Gupta, 1995; Rodríguez et al., 2004; Ximenes, 2008.
Micura	Peru	Braga, 1992; Gomes, 2006; Gupta, 1995.
Mucura	Colombia, Peru	Braga, 1992; Gomes, 2006; Gupta, 1995; Rodríguez et al., 2004.
Mucuracaá or Mucura-caá	Amazon Region (Brazil): Amazonas, Pará and Roraima	Braga, 1992; DI Stasi and Hiruma-Lima, 2002; Gomes, 2006; Gupta, 1995; Hoehne, 1939; Pio Correa, 1969; Rocha, 2004; Ximenes, 2008.
Mapuro	Colombia	Braga, 1992; Gomes, 2006; Gupta, 1995.
Ocoembro	Brazil: Rio de Janeiro	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Ojúùsàjú	Africa	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Paraacaca	Brazil: Rio de Janeiro	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Paracoca	Brazil: Rio de Janeiro	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Patscang ay (skunk leaf)	Mexico: Isthmus of Tehuantepe (Zoque-Popoluca)	Leonti et al., 2003.
Pats ujts (Skunk herb)	Mexico: Isthmus of Tehuantepe (lowland Mixe)	Leonti et al., 2003.
Pipi	Brazil: Rio de Janeiro; Colombia; Venezuela	Braga, 1992; Gomes, 2006; Gupta, 1995; Di Stasi and Hiruma-Lima, 2002; Hoehne, 1939; Lima et al., 1991; Pio Correa, 1969; Ximenes, 2008.
Pipí	Argentina	Rodríguez et al. (2004).
Puante	France	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Raiz-de-conconha	Brazil: Pernambuco and São Paulo	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Raiz-de-guiné	Brazil: Pernambuco and São Paulo	Braga, 1992; Di Stasi and Hiruma-Lima, 2002; Gupta, 1995; Ximenes, 2008.
Raiz de pipi	Colombia	Braga, 1992; Gomes, 2006; Gupta, 1995.
Timbó	Brazil: Amazonas	Braga, 1992; Gupta, 1995; Ximenes, 2008.
Tipi	Brazil: Amazonas, Ceará and Bahia	Bezerra, 2006; Braga, 1992; DI Stasi and Hiruma-Lima, 2002; Gomes, 2006; Gupta, 1995; Hoehne, 1939; Lima et al., 1991; Pio Correa, 1969; Rodríguez et al., 2004; Ximenes, 2008.
Tipi verdadeiro	Brazil: Ceará and Bahia	Braga, 1992; Di Stasi and Hiruma-Lima, 2002; Gomes, 2006; Gupta, 1995.
Zorrillo	Colombia; Mexico	Gupta, 1995; Rodríguez et al., 2004.

Amazon forest used *P. alliacea* in herbal baths to protect against witchcraft. These ceremonies were a means of driving away 'bad spirits' and producing visions or hallucinations (Taylor, 1998; Maciel and Neto, 2006).

In Populaca culture (a Macro-Mayan ethnic group from Mexico), *P. alliacea* was used to ward off black magic (Leonti et al., 2003). Moreover, *P. alliacea*, combined with many other additives, was used to prepare the ritual drink of ayahuasca (Camargo, 2007; Hoehne, 1939). This preparation is utilized to treat illnesses of a *magical origin* or that are intractable by medicine. The ayahuasca drink is employed in religious ceremonies for inducing visions and increasing spiritual abilities (Rivier and Lindgren, 1972). Reports of the plant's hallucinogenic effects are indicative of its ability to act on the CNS. Furthermore, voodoo practitioners in Haiti used *P. alliacea* as a 'zombie poison' to induce a prolonged psychotic state, with subjects falling into a deathlike stupor (Albuquerque et al., 2012).

The Ticuna, an indigenous people of the Amazon, used roots of *P. alliacea* along with other plants to prepare curare, applied as poison to their arrows. This poison induces neuromuscular blockade, lethargy and death by asphyxia. The effects of these different preparations may not be exclusively related to *P. alliacea*,

Fig. 2. Geographical distribution of *Petiveria alliacea* L.

Table 3
Indications and forms of traditional use of *Petiveria alliacea* L.Source: Adapted from Gomes (2006).

Indication	Forms of use
Buccal anti-inflammatory and analgesic	Tea of roots and leaves: 10 g in 1 L of water, four times a day.
Cancer	Decoction of leaves: 40 g in 1 L of water, three times a day.
	Plant juice (juice): 25–30 fresh leaves (green), filtered with 1 L of cold water, pure. This filtered juice should be consumed three times a day (morning, afternoon and night).
Cystitis	Decoction of leaves or roots: 30 g/L.
Headache	Compress of macerated leaves.
Rheumatic pains, neuralgia, polyneuritis (beriberi), paralysis	Tincture to friction: 350 g from roots in 40% alcohol.
Stimulant, diaphoretic and diuretic	Decoction from leaves or roots: 30 g in 1 L of water to be taken in tablespoons during the day.
Paralysis	Baths: 500 g of roots in each bath.

because the association of plants possibly produces synergistic or antagonistic effects (Rodrigues and Carlini, 2004; Rodrigues et al., 2008). The leaves and roots of this plant were used as stimulants in various regions across Brazil (Negri and Rodrigues, 2010). Trinidadian hunters bathed their dogs with a preparation of *P. alliacea* roots for increased alertness, as well as dabbing it on the dogs' noses to improve their scent-tracking ability (Lans et al., 2001; Muñoz et al., 2000).

In contrast, *P. alliacea* is also consumed in Latin America for its sedative effects in the form of infusions and aqueous preparations of roots at high temperature (Germano et al., 1993). In the Dominican Republic, the root infusion is orally consumed to alleviate anxiety (Ososki et al., 2002; Mañon Rossi, 1983). The island communities of Brazil mash the leaves in alcohol and use this preparation to treat convulsions in children (Branch and Silva, 1983). In addition, migrants living in the remnants of the South-East Atlantic Forest (Brazil) treat cases of anxiety by the inhalation of the aerial parts (APs) of the plant (Garcia et al., 2010). Traditional populations of Mexico and some areas of Latin America use a leaf infusion to alleviate epilepsy crises, anxiety and paralysis (Martinez, 1984; Zamora-Martinez and Pola, 1992; Taylor, 1998). Indeed, weak infusion of the leaves or roots have been used in several parts of the world to boost memory (Mors et al., 2000).

During the era of slavery, female slaves used preparations of *P. alliacea* to seduce their masters or to protect themselves from being harassed by their employers. Thus, the plant was popularly known as *amansa-senhor* ('Tame-Sir' or 'Tame-Master' in English) (Camargo, 2007; Schardong and Cervi, 2000; Souza and Neto, 2010). Moreover, the surviving members of the quilombola communities have reported the use of this plant for its mind-altering effects (Rodrigues and Carlini, 2004, 2006). Quilombola communities prepare a cigarette known as *tira-capeta* ('removing-the-devil' in English). The *tira-capeta* is recommended for improving the learning abilities of adolescents and children, in cases of nervous breakdown due to overwork and for relieving sleep disturbances. Nine plants belonging to the *tonics for the brain* category are used in the preparation of this cigarette, including *P. alliacea* (Rodrigues et al., 2008). It is also indicated to reduce *cannabis* use and other non-CNS uses (i.e. sinusitis, cold, etc.) (Negri and Rodrigues, 2010).

Prolonged use of *P. alliacea* has been known to cause insanity. For instance, the acute consumption of high doses of this plant induces insomnia, hyperarousal and hallucinations, whereas chronic exposure leads to paradoxical symptoms including seizures, weakness and mental retardation. There are reports of deaths within one year of chronic use of *P. alliacea* (Peckolt and Peckolt, 1900).

3. Phytochemistry

Many compounds have been isolated from *P. alliacea*, and some of them are patent protected (Ferrer, 2007; Taylor, 1998). The main chemical components include sulphur compounds, flavonoids,

Table 4
Traditional uses of *Petiveria alliacea* L. worldwide.

Plant part	Location	Administration	Medicinal use	References
Aerial parts[1]	Bolivia	Oral	Colds	[1]Desmarchelier et al., 1997; [2]Barrett, 1994; [3]Desmarchelier et al., 1996a; [4]Coe and Anderson, 1996a; [5]Desmarchelier et al., 1996b.
Entire plant[2]	Nicaragua	Not stated		
Leaf[3]	Argentina	Infusion oral		
Leaf[4]	Nicaragua	Decoction oral		
Leaf[5]	Peru	Leaves oral		
Stem and root[1]	Guatemala	Powder inhalation	Sinusitis	[1]Girón et al., 1991.
Root[1]	Paraguay	Decoction oral		
Entire plant[1]	Nicaragua	Not stated	Other respiratory tract disorders	[1]Coe and Anderson, 1996a; [2]Perez and Anesini, 1994; [3]Ruffa et al., 2002; [4]Leonti et al., 2003.
Leaf[2]	Argentina	Decoction oral		
Leaves and stem [3]	Argentina	Not stated		
Not stated [4]	Mexico	Not stated		
Aerial parts[1]	Colombia	Infusion external	Snakebite	[1]Otero et al., 2000; [2]Barrett, 1994.
Entire plant[2]	Nicaragua	Plant external		
Aerial parts[1]	Colombia	Oral	Childbirth	[1]García-Barriga, 1974; [2]Cosminsky, 1982; [3]Hodge and Taylor, 1957.
Entire plant[2]	Mexico	Infusion oral		
Entire plant[3]	Dominican Republic	Oral		
Aerial parts[1]	Paraguay	External	Insecticide	[1]Schmeda-Hirschmann and Rojas de Arias, 1992; [2]Medeiros et al., 2013; [3]Schmeda-Hirschmann and Rojas de Arias, 1990.
Not stated[2]	Brazil	Not stated		
Leaf[3]	Brazil	Leaves not stated		
Entire plant[1]	Brazil	Oral	Abortive	[1]Dragendorff, 1898; [2]Milliken, 1997; [3]Schmeda-Hirschmann and Rojas de Arias, 1990; [4]Lores and Pujol, 1990; [5]Mihalik, 1978; [6]Roig and Mesa, 1945; [7]Amadeo, 1888; [8]Burlage, 1968.
Roots[2,3]	Brazil	Infusion or decoction		
Entire plant[4]	Cuba	Decoction oral		
Entire plant[5]	Guyana	Decoction oral		
Entire plant[6]	Mexico	Oral		
Root[7,8]	Puerto Rico and USA	Oral		
Entire plant[1]	Brazil	Oral	Diuretic	[1]Dragendorff, 1898; [2]Milliken, 1997; [3]Schmeda-Hirschmann and Rojas de Arias, 1990; [4]Silva, 2002; [5]Medeiros et al., 2013; [6]Bandoni et al., 1976.
Roots[2,3]	Brazil	Infusion or decoction		
Not stated[4]	Brazil	Not stated		
Leaf[5]	Brazil	Tea		
Not stated[6]	Argentina	Oral		
Leaf[4]	Argentina	Decoction oral	Urinary tract infections	[1]Perez and Anesini, 1994.
Aerial parts[1]	Brazil	Oral	Toothache or caries	[1]Van Den Berg, 1984; [2]Medeiros et al., 2013; [3]Silva, 2002; [4]Barrett, 1994; [5]Filipov, 1994; [6]Martínez, 2010; [7]Weniger et al., 1986.
Not stated[2]	Brazil	Not stated		
Root or leaves[3]	Brazil	Mash roots and apply on tooth		
Entire plant[4]	Nicaragua	Oral		
Roots[5]	Argentina	Root periodontal or smoke inhalation		
Entire plant[6]	Argentina	Infusion or decoction		
Leaf[7]	Haiti	Macerated leaves		
Entire plant[1]	Brazil	Infusion or decoction oral	Headache	[1]Elisabetsky and Castilhos, 1990; [2]Oliveira et al., 2010; [3]Schmeda-Hirschmann and Rojas de Arias, 1990; [4]Branch and Silva (1983); [5] Comerford, 1996; [6]Weniger et al., 1986; [7]Filipov, 1994; [8]Oakes and Morris, 1958.
Leaf[2]	Brazil	Pomade and tincture		
Leaf[3]	Brazil	Poultice and wash		
Leaf and root[4]	Brazil	Tea		
Leaf[5]	Guatemala	Leaves external		
Leaf[6]	Haiti	Leaves inhalation		
Root[7]	Argentina	Smoke inhalation		
Root[8]	Virgin Islands	Oral		
Entire plant[1] and leaf[2]	Nicaragua	Decoction oral OR infusion external	Analgesic (other types of pains)	[1]Coe and Anderson, 1996a; [2]Coe and Anderson, 1996b; [3]Girón et al., 1991; [4]Oliveira et al., 2010; [5]Garcia et al., 2010; [6]Ruffa et al., 2002.
	Paraguay	Decoction external		
Root[3]	Brazil	Decoction		
Leaf[4]		Pomade and tincture		
Leaf[5]	Brazil			
Leaves and stem[6]	Argentina	Not stated		
Entire plant[1]	Cuba	Decoction	Diabetes	[1]Lores and Pujol, 1990.
Entire plant[1]	Cuba	Decoction oral	Anti-inflammatory	[1]Lores and Pujol, 1990; [2]Germano et al. (1993); [3]Medeiros et al., 2013; [4]Quílez et al., 2004; [5]Ruffa et al., 2002.
Root[2]	Brazil	Infusion oral		
Not stated[3]	Brazil	Not stated		
Not stated[4]	Dominican Republic	Not stated		
Leaf and stem[5]	Argentina	Not stated		
Entire plant[1]	Mexico	Plant external	Pimples	[1]Zamora-Martínez and Pola, 1992; [2]Caceres et al., 1987.
Leaf[2]	Guatemala	Infusion external		
Entire plant[1]	Mexico	Oral	Emmenagogue	[1]Roig and Mesa, 1945; [2]Bandoni et al., 1976; [3]Moreno, 1975; [4] Gonzalez and Silva, 1987; [5]Heckel, 1897; [6]Amadeo, 1888; [7]Burlage, 1968.
Not stated[2]	Argentina	Oral		
Not stated[3]	Paraguay	Not stated		
Not stated[4]	Venezuela	Oral		
Root[5,6,7]	French Guiana, Puerto Rico and USA	Oral		
Leaf[1]	Guatemala	Decoction external	Fever	[1]Comerford, 1996; [2]Loustalot and Pagan, 1949; [3]Milliken, 1997; [4] David and Pasa, 2013; [5]Girón et al., 1991; [6]Bandoni et al., 1976.
Leaf and Stem[2]	Puerto Rico	Oral		
Root[3]	Brazil	Infusion oral or tea		
Leaf[4]	Brazil	Decoction oral		
Root[5]	Paraguay	Oral		

Table 4 (*continued*)

Plant part	Location	Administration	Medicinal use	References
Not stated[6]	Argentina	Oral		
Leaf[1]	Guatemala	Infusion external	Skin diseases	[1]Caceres et al., 1987; [2]Girón et al., 1991; [3]Martinez, 1984; [4]Medeiros et al., 2013; [5]Yukes and Balick, 2010.
Root[2]	Paraguay	Decoction external		
Leaf[3]	Mexico	Leaves external		
Not stated[4]	Brazil	Not stated		
Leaf[5]	Dominican republic	External (topically)		
Leaf and root[1]	Puerto Rico	Oral	Cholera	[1]Amadeo, 1888.
Entire plant[1]	Guatemala	Oral	Diarrhoea	[1]Logan, 1973; [2]Perez and Anesini, 1994.
Leaf[2]	Argentina	Decoction oral		
Entire plant[1]	Guatemala	Oral	Digestive disorders	[1]Logan, 1973; [2]Girón et al., 1991; [3]Comerford, 1996; [4]Branch and Silva, 1983.
Root[2]	Paraguay	Decoction oral		
Leaf[3]	Guatemala	Decoction oral		
Leaf and root[4]	Brazil	Tea		
Leaf[1]	Guatemala	Oral	Ringworm	[1]Caceres et al., 1990; [2]Germano et al., 1993.
Root[2]	Brazil	Infusion oral		
Not stated[1]	Brazil	Not stated	Malaria	[1]Moreno, 1975; [2]Milliken, 1997; [3]Carabalo et al., 2004; [4]Vigneron et al., 2005; [5]Ruffa et al., 2002.
Root[2]	Brazil	Infusion oral		
Entire plant[3]	Brazil	Decoction		
Leaf[4]	French Guiana	Not stated		
Leaf and stem[5]	Argentina	Not stated		
Entire plant[1]	Nicaragua	Not stated	Heart diseases	[1]Barrett, 1994; [2]Silva et al., 2009.
Leaf[2]	Brazil			
Leaf[1]	Guatemala	Leaves oral	Blood disorders	[1]Villar et al., 1997.
Entire plant[1]	Nicaragua	Not stated	Liver disorders	[1]Barrett, 1994.
Entire plant[1]	Trinidad and Tobago	Not stated	Kidney disorders	[1]Barrett, 1994; [2]Lans, 2006; [3]Souza et al., 2014.
Leaf[2]	Trinidad and Tobago	Not stated		
Leaf[3]	Brazil	Green leaf OR dry leaf		
Not stated[1]	Brazil	Not stated	Allergy	[1]Medeiros et al., 2013; [2]Oliveira et al., 2010.
Leaf[2]	Brazil			
Root[1,2]	Brazil	Tea and baths	Osteoporosis	[1]Alves et al., 2007; [2]Ferraz et al., 1991b.
Not stated[1]	Brazil	Not stated	Arthrosis	[1]Medeiros et al., 2013; [2]Oliveira et al., 2010.
Leaf[2]	Brazil	Pomade and tincture		
Not stated[1]	Brazil	Not stated	Rheumatism	[1]Medeiros et al., 2013; [2]Oliveira et al., 2010; [3]Silva, 2002; [4]Ferraz et al., 1991a; [5]Bandoni et al., 1976.
Leaf[2]	Brazil	Pomade and tincture		
Leaf[3]	Brazil	Bath associated with		
Entire plant[4]	Brazil	other plants		
Not stated[5]	Argentina	Infusion oral		
		Oral		
Roots[1]	Dominican Republic	Alcoholic tincture oral	Arthritis	[1]Yukes and Balick, 2010.
Roots[1]	Dominican Republic	Infusion oral	Gynaecological disorders	[1]Ososki et al., 2002; [2]Yukes and Balick, 2010.
Leaf and/or roots[2]	Dominican Republic	Infusion oral		
Leaf[1]	Cuba	Not stated	Cancer	[1]Green Reinoso et al., 2014; [2]Ruffa et al., 2002; [3]Lores and Pujol, 1990; [4]Rossi et al., 1990.
Leaf and stem[2]	Cuba	Not stated		
Entire plant[3]	Cuba	Decoction		
Leaf [4]	Brazil	Oral		
Leaf[1]	Brazil	Ointment	Worms	[1]Branch and Silva, 1983.
Leaf[1]	Guatemala	Decoction external	Fevers	[1]Comerford, 1996; [2]Loustalot and Pagan, 1949; [3]Cifuentes et al., 2001; [4]Girón et al., 1991.
Leaf and stem[2]	Puerto Rico	Oral		
Roots[3]	Brazil	Infusion oral		
Roots[4]	Paraguay	Decoction oral		
Leaf and stem[1]	Guatemala	Powder inhalation	Sinusitis	[1]Girón et al., 1991.
Roots[1]	Paraguay	Decoction oral		

lipids and triterpenes, among others (Benevides et al., 2001; Cuervo, 2011).

3.1. Sulphur compounds

Unique to *P. alliacea*, these compounds are mainly localized in the roots, and are known as azufre derivatives (Fig. 3). The presence of cis-3,5-diphenyl-1,2,4 trithiolane (1) and elevated amounts of elementary sulphur was first identified by Adesogan (1974). When submitted to column chromatography, the petrol root extracts of *P. alliacea* afforded dibenzyl trisulphide (DTS) (2), isolated as a viscous and pungent-smelling oil (De Sousa et al., 1990). This finding revealed DTS as a natural product, which has thus far been described as a synthetic product. It is a significant representative of sulphur.

Benevides et al. (2001) evaluated the antifungal activity of methanolic extracts of *P. alliacea*, reporting the highest activity with the root extracts. Thus, they submitted this extract to successive liquid/liquid partitioning in different solvents. The resultant residues were concentrated and posteriorly subjected to a new step of fractionation. This procedure resulted in six bioactive fractions, which afforded five new polysulphides. Thus, yellow and colourless oily substances called dibenzyl sulphide (3) and benzyl hydroxymethyl sulphide (4), respectively, were identified.

Other compounds included the yellow amorphous solid dibenzyl disulphide (5), as well as dibenzyl tetrasulphide (6) and di (benzyltrithio) methane (7), both being identified as orange amorphous solids. Finally, Benevides et al. (2001) described two sulphur compounds that were already registered: di-*n*-propyl disulphide (8), a colourless volatile oil with a strong odour of garlic, and DTS (2), described as a yellow amorphous solid.

Additionally, Kubec and Musah (2001) reported on the isolation and identification of non-volatile phytochemicals. Disruption of the *P. alliacea* tissue may have produced these compounds, because

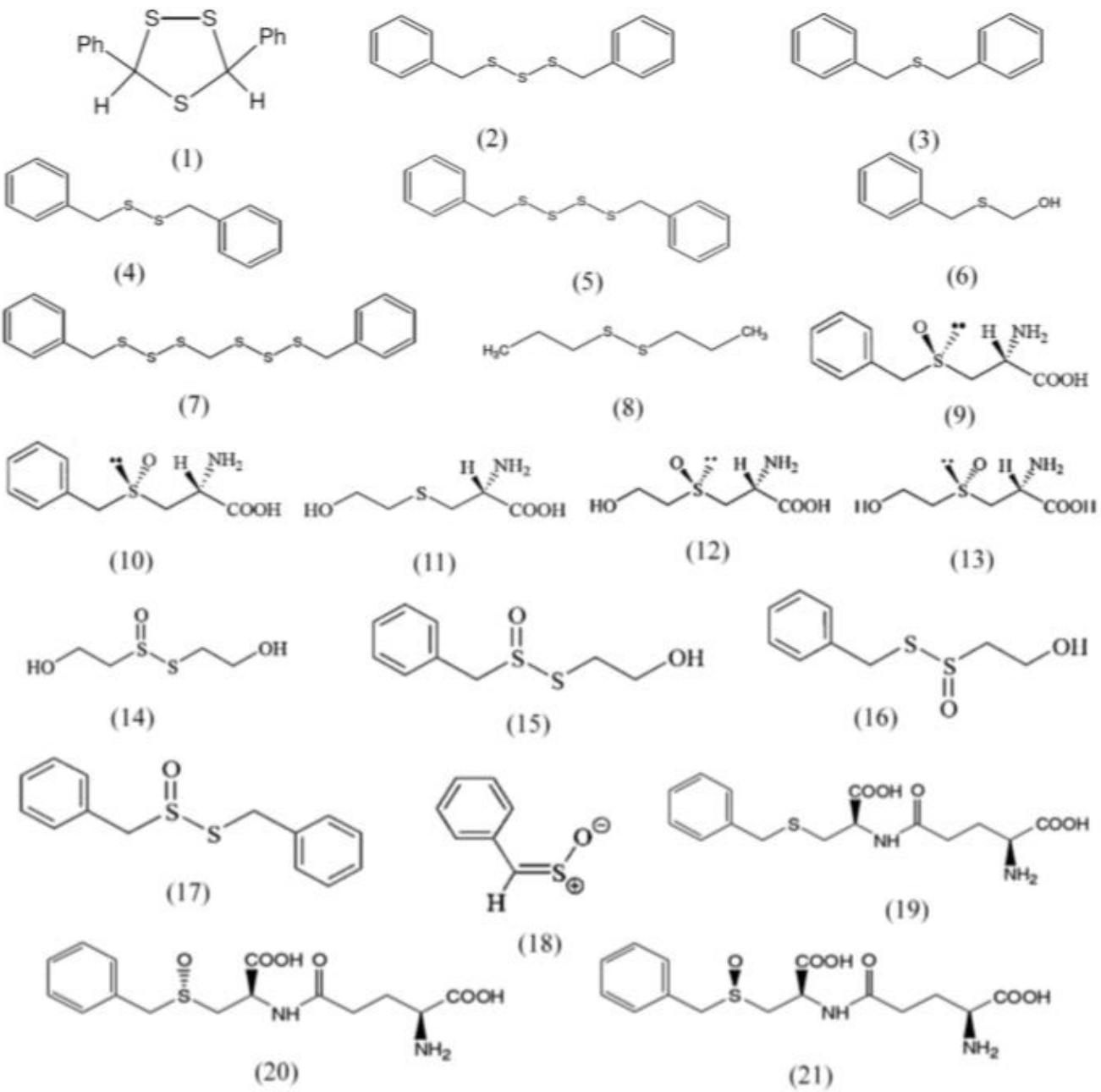

Fig. 3. Sulphur compounds from *Petiveria alliacea*: cis-3,5-diphenyl-1,2,4 trithiolane (1), dibenzyl trisulphide (2), dibenzyl sulphide (3), dibenzyl disulphide (4), dibenzyl tetrasulphide (5), benzylhydroxymethyl sulphide (6), di(benzyltrithio) methane (7), di-*n*-propyl disulphide (8), (RsSc)-S-benzyl-L-cysteine sulphoxide (petiveriin A, 9), (SsRc)-S-benzyl-L-cysteine sulphoxide (petiveriin B, 10), (R)-S-(2-hydroxyethyl) cysteine (11), 6-hydroxyethiin A (12), 6-hydroxyethiin B (13), S-(2-hydroxyethyl) (2-hydroxyethane)-thiosulphinate (14), S-(2-hydroxyethyl) phenylmethane-thiosulphinate (15), S-benzyl (2-hydroxyethane)-thiosulphinate (16), S-benzyl phenylmethane-thiosulphinate (17), (Z)-thiobenzaldehyde S-oxide (18), ($S_{C2}R_{C7}$)-c-glutamyl-S-benzylcysteine (19), γ-L-glutamyl-petiveriin A (20) and γ-L-glutamyl-petiveriin B (21). Adapted from Bezerra (2006), Kubec and Musah (2001), Kubec et al. (2002), Kubec and Musah (2005) and PubChem Compound Database (2015).

most of these elements are not present in the fresh tissue. These non-volatile constituents may serve as precursors of the phenyl/benzyl-containing compounds mentioned previously. In this study, fresh roots were extracted in boiling methanol (MeOH). The sequential addition of 3% hydrochloric acid (HCl) promoted the precipitation of the same material. This precipitate was filtered and subjected to cation exchange to separate the amino acid fraction. This fraction was eluted in ammonium hydroxide (NH_4OH), concentrated and subjected to high-performance liquid chromatography (HPLC). From this process, two diastereoisomers of the sulphoxide of S-benzylcysteine were obtained (Kubec and Musah, 2001); one appeared as small white plates and the other resembled long tiny white needles. According to the absolute configuration of the isolated amino acid, they were designated as (RsSc)-S-benzil-L-cysteine sulphoxide (petiveriin A) (9) and (SsRc)-S-benzyl-L-cysteine sulphoxide (petiveriin B) (10), respectively.

Subsequently, three additional amino acids, S-methyl-, S-ethyl- and S-propylcysteine derivatives, were detected in the roots of *P. alliacea* (Kubec et al., 2002). The method used in the previous study was modified to achieve these amino acids (Kubec et al., 2001). Thus, this work identified three white solid compounds as S-substituted cysteine, named (R)-S-(2-hydroxyethyl) cysteine (11), 6-hydroxyethiin A (12) and 6-hydroxyethiin B (13) (Fig. 3). Furthermore, Kubec and Musah (2002) isolated four thiosulphinates: S-(2-hydroxyethyl) (2-hydroxyethane)-thiosulphinate (14), a yellow oil, as well as S-(2-hydroxyethyl) phenylmethane (15),

S-benzyl (2-hydroxyethane) (16) and S-benzyl phenylmethane (17) thiosulphinates, which were white solids. The authors indicated these compounds to be products of the enzymatic breakdown of S-substituted cysteine derivatives (Kubec et al., 2002).

Sensory observations during experiments on *P. alliacea* led Kubec et al. (2003) to investigate these properties. On conducting the analysis, the disrupted tissue emitted a strong garlic odour, irritating the nasal and ocular mucosae, leading to a serious nasal discharge and lachrymation, respectively. A fresh homogenate from *P. alliacea* roots was submitted to chromatographic methods. This process isolated (*Z*)-thiobenzaldehyde S-oxide (18), a yellow sulphine pungent oil. This sulphine gave off an intense alliaceous odour, typically when the plant was bruised or cut. By contrast, none of the previously isolated thiosulphinates showed strong odour or lachrymatory effects. Therefore, the authors implicated this metabolite in determining these features of the plant.

The presence of γ-glutamyl dipeptides, which possibly act as a reserve of nitrogen and sulphur, was reported. Among other functions, these elements serve as intermediates in the biosynthesis of S-alk(en)ylcysteine sulphoxides (Kubec and Musah, 2005). ($S_{C2}R_{C7}$)-c-glutamyl-S-benzylcysteine (19) and two diastereomeric sulphoxides were termed ($S_{C2}R_{C7}R_S$)-c-glutamyl-S-benzylcysteine S-oxides or γ-L-glutamyl-petiveriins A (20) and B (21), respectively (Kubec and Musah, 2005) (Fig. 3), all three of which were white hygroscopic solids.

Finally, sulphur compounds have been detected in *P. alliacea* roots, at elevated concentrations and diversity. Thus, most studies on these compounds focus on the roots of the plant. It is worth noting that DTS (5) and DTS (2) were recently identified in the stem and leaves of *P. alliacea* (Hernández et al., 2014). Moreover,

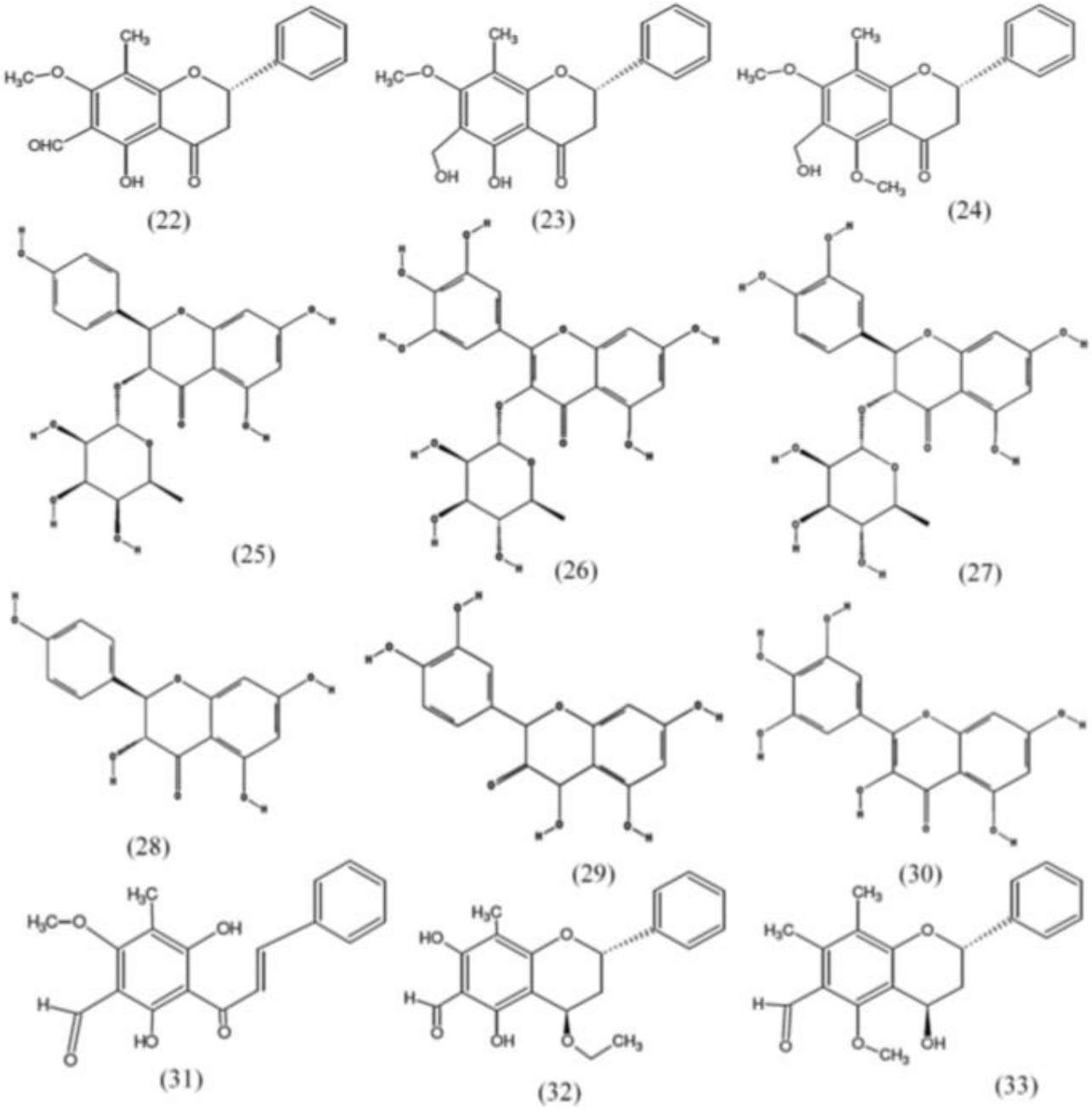

Fig. 4. Flavonoids and derivates from *Petiveria alliacea*: 6-formyl-8-methyl-7-O-methylpinocembrin (22), 6-hydroxymethyl-7-O-methylpinocembrin (leridol, 23), 5-O-methyl ether (5-O-methylleridol, 24), engeletin (25), astilbin (26), myricitrin (27), dihydro-kempleferol (28), dihydroquercetin (29), myricetin (30), leridal chalcone (31), petiveral (32) and petiveral 4-ethyl (33). Adapted from Bezerra (2006) and PubChem Compound Database (2015).

organosulphur compounds were detected in the hydroalcoholic extracts of leaves by thin-layer chromatography (TLC) (Silva et al., 2015). Taken together, these data are indicative of the presence of these compounds in other plant parts.

3.2. Flavonoids and derivatives

Flavonoids and derivatives have been detected in the APs of *P. alliacea*, particularly in the leaves (Monache et al., 1996; Di Stasi and Hiruma-Lima, 2002; Blainski et al., 2010; Audi et al., 2001). The flavanones 6-formyl-8-methyl-7-0-methylpinocembrin (22), 6-hydroxymethyl-7-0-methylpinocembrin (leridol) (23) and 5-O-methyl ether (5-O-methylleridol) (24) were identified in the chloroform fraction of the leaf extract (Monache and Suarez, 1992). Moreover, the ethyl acetate fraction of the same extract was found to contain the flavonoids engeletin (25) and astilbin (26), as well as the flavonol myricitrin (27) (Fig. 4).

The acid hydrolyses of the leaf extract afforded dihydro-kempleferol (28), dihydroquercetin (29) and myricetin (30) (Monache and Suarez, 1992). Previous research identified the AP extracts of *P. alliacea* as the source of the flavonoid leridal chalcone (31), as well as the flavanones petiveral (32) and o-petiveral-4-ethyl (33) (Monache et al., 1996) (Fig. 4). Recently, the ethyl acetate fraction of *P. alliacea* obtained from leaves and stems was found to contain leridol, myricetin, petiveral and petiveral-4-ethyl (Hernández et al., 2014).

3.3. Other compounds

Some phytochemical screenings were performed to identify secondary metabolites in *P. alliacea*. As expected, different fractions collected from different regions exhibited different phytochemical profiles. For example, in a TLC analysis of *P. alliacea* roots, a significant amount of coumarins, but not alkaloids, was detected (Rocha and Silva, 1969). However, in a study investigating medicinal plants collected in Argentina, alkaloids were detected in the leaves and roots of *P. alliacea* (Bandoni et al., 1976).

Fontoura et al. (2005) performed a phytochemical prospection of the ethanolic extract of *P. alliacea* leaves. The aqueous and chloroformic fractions of this extract were also studied. A colourimetric reaction was used to identify these compounds in the ethanolic extract. The results expressed the intensity of the reaction presented by each metabolite. A TLC analysis helped to identify the metabolite classes in the fractions. Thus, the ethanolic extract and the aqueous fraction showed a strongly positive reaction for steroids and coumarins, but not for tannins. The chloroformic fraction was found to present coumarins and tannins, but in lower concentrations.

Oliveira (2012) conducted a phytochemical prospection using a similar methodology to that used by Fontoura et al. (2005). This work evaluated the variation in the chemical composition of the powder and hydroalcoholic extracts of the APs and roots of *P. alliacea*. The plant material was collected in two different seasons (dry and rainy). The powder and AP extract were found to contain spumidic saponins in equal proportions in both periods. In the root samples, only the extract showed a major proportion of these saponins independently of the season of collection.

The intensity of the alkaloids' reactions varied with the detection technique used. The roots extract prepared from material collected during the rainy season showed a strong reaction in the Dragendorff test. Other preparations under study showed a similar profile of alkaloids to that in APs. Sugar reduction was observed in the roots and APs. Only the roots were found to contain sesquiterpene lactones, depsides and depsidones, but in low concentrations in both periods (Oliveira, 2012).

In their study, De Sousa et al. (1990) investigated the chemical constituents of *P. alliacea*. The plant material was collected, air-dried and separated into roots, stems, leaves and inflorescences. These parts of the plant were ground first with petrol and then with ethanol. The known chemical constituents were detected through standard physical and spectrometric methods and compared with authentic samples, whereas unknown compounds were identified based on their microanalysis and spectral data.

Thus, the inflorescence extract included the carbohydrate pinitol (34), as well as other unknown plant compounds, such as the steroid β-sitosterol (35), which is isolated from the roots and stem (Fig. 5). Potassium nitrate (KNO_3) was precipitated from the extracts of roots, stem and leaves. An elevated amount of nitrate was found to induce methaemoglobinaemia in cattle (Trheebilcock et al., 1978). This effect had already been identified in experimental murine models (Andrade et al., 2012), and it may be toxic to humans (De Sousa et al., 1990).

In the same study, De Sousa et al. (1990) described two alkaloids from *P. alliacea*: an unpublished highly polar alkaloid isolated from the stem and termed trans-N-metil-4-methoxyproline (36), and allantoin (37), which is found in the stem and leaves. Some lipids and the triterpene α-friedelinol (38) were also detected in the leaves. Segelman and Segelman (1975) isolated the triterpenes isoarborinol (39) and isoarborinol-acetate (40) from the leaves of *P. alliacea*. Two other triterpenes were obtained from the AP extracts of the plant, babinervic acid (41) and 3-epiilexgenin A (42) (Monache et al., 1996) (Fig. 5).

3.4. Essential oil

The essential oil can be obtained from the leaves, stem, roots and inflorescences of *P. alliacea*, which is yellow in colour, with a strong and unpleasant odour due to allyl sulphide (Domínguez, 1928). *P. alliacea* also contains a powdery amorphous component known as petiverine, which is odourless, bitter, spicy and soluble in alcohol and ether. It is slightly soluble in acidic water solutions at 100 °C (Matta, 1913; Peckolt and Peckolt, 1900).

Bezerra (2006) conducted a comparative study of the volatile constituents of *P. alliacea* roots from two cities in the north-east of Brazil (Maranguape and Apuiarés) with gas chromatography coupled to mass spectrometry (CG/MS). The root essential oil from Manguarape was found to contain benzaldehyde (61.5%) (43), dibenzyl disulphide (18.1%) (5), trans-stilbene (14.1%) (44) and cinnamaldehyde (6.5%) (45), corresponding to 100% of the peaks obtained on the chromatogram. However, only benzaldehyde (53.8%), dibenzyl disulphide (29.7%) and trans-stilbene (3.3%) varied in the quantity of metabolites between the two distinct origins (Bezerra, 2006) (Fig. 6).

In 1998, Ayedoun et al. investigated the volatile compounds in the essential oil of *P. alliacea* roots from Benin (West Africa). They obtained the oil by boiling the roots in deionized water using pentane as a liquid extractor, and the phytochemicals were detected by chromatographic methods. The analysis identified 13 compounds, which represent 97% of the oil composition. As illustrated in Figs. 5 and 6, the most abundant components were benzaldehyde (48.3%) (43), dibenzyl disulphide (23.3%) (5), DTS (9.4%) (2) and trans-stilbene (6.8%) (44) (Ayedoun et al., 1998). The chloroformic extracts of *P. alliacea* roots were found to contain organic compounds such as benzaldehyde (43), trans-stilbene (44) and benzoic acid (45) (Adesogan, 1974).

Previously, Zoghbi et al. (2002) studied the composition of the inflorescence (3.3 g) essential oil from Belém and Ananindeua (northern Brazil). The oil was subjected to simultaneous distillation–extraction. The drying process was sequentially carried out in anhydrous sodium sulphate, yielding 0.05% w/w. The mass spectra and chromatographic analysis led to the detection of 11 substances (97.9%). The main compounds found were benzaldehyde (54.8%)

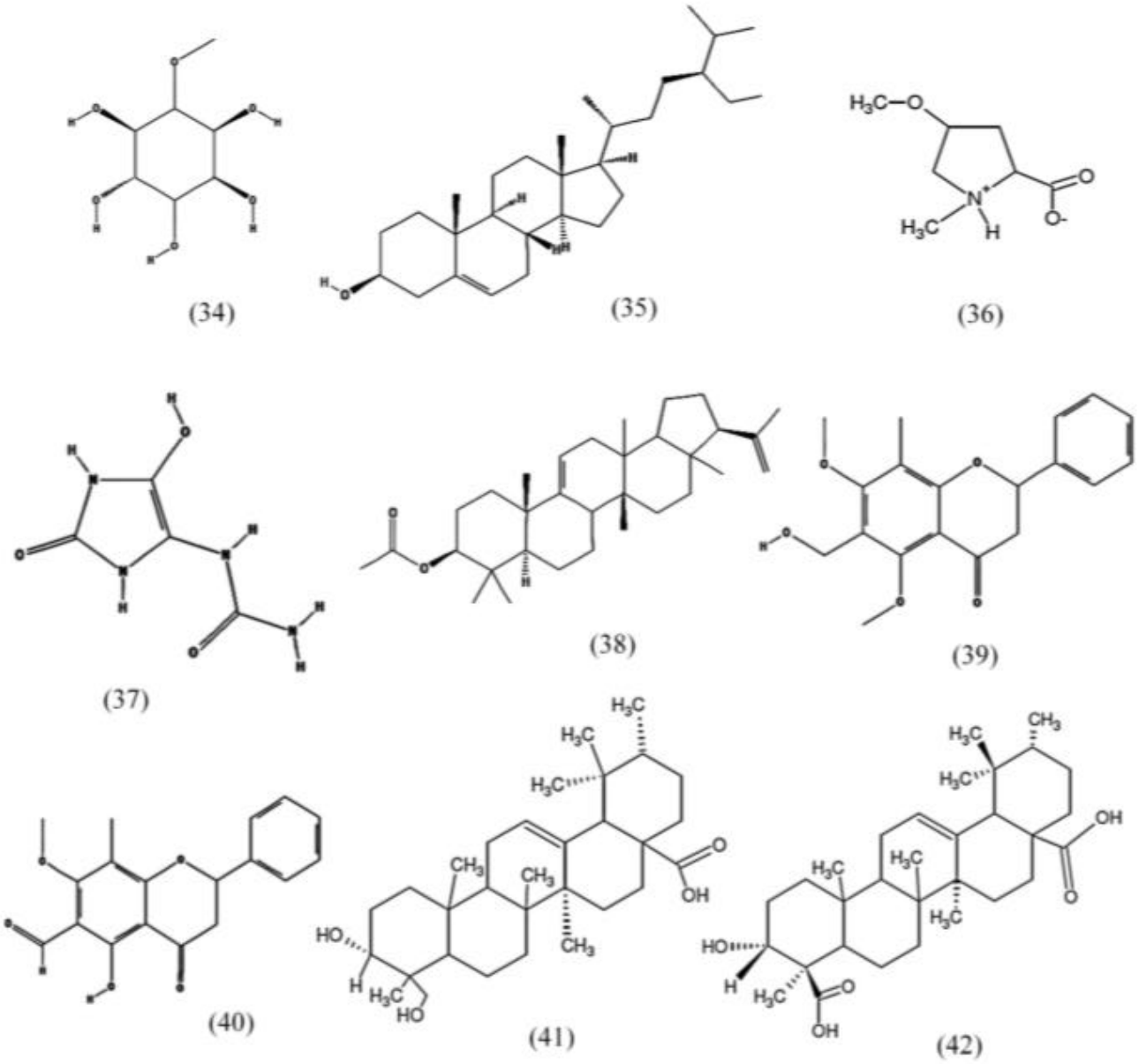

Fig. 5. Structures of other chemical constituents found in *Petiveria alliacea*: pinitol (34), β-sitosterol (35), trans-N-metil-4-methoxyproline (36), allantoin (37), α-friedelinol (38), isoarborinol (39), isoarborinol-acetate (40), babinervic acid (41) and 3-epiilexgenin A (42). Adapted from Bezerra (2006) and PubChem Compound Database (2015).

(43), benzyl thiol (20.3%) (46) and dibenzyl disulphide (18.0%) (2) (Figs. 5 and 6). In their study, Neves et al. (2011) investigated the phytochemical composition of the essential oil from the leaves, stem, roots and inflorescence of *P. alliacea* from Pernambuco (a north-eastern state of Brazil). They followed a similar method of extraction and chemical characterization to that used by Zoghbi et al. (2002).

A better yield (0.12%) of the essential oil was obtained from the inflorescence than from other plant parts (< 0.1%). The root oil contained the majority of compounds such as benzyl alcohol (46.6%) (47), dibenzyl disulphide (19.1%) (5), trans-stilbene (6.2%) (44) and DTS (5.6%) (2). The essential oil from the stem and leaves primarily consisted of carvacrol (stem: 48.3%; leaves: 50.9%) (48), (*Z*)-3-hexenyl benzoate (stem: 9.5%; leaves: 18.6%) (49), dibenzyl disulphide (stem: 23.1%; leaves: 17.6%) and additional benzyl thiol in the stem (9.0%) (46). Inflorescences present (*Z*)-3-hexenyl benzoate (30.5%) (49), carvacrol (29.7%) (48), dibenzyl disulphide (15.7%) (5) (2) and also DTS (5.8%) (2) (Fig. 6).

Further studies have identified benzaldehyde in the essential oils of *P. alliacea* leaves (12.8%), roots (55.1%) and inflorescences (32.5%) from Rio de Janeiro (south-eastern state of Brazil) (Castellar et al., 2014). Leaves were found to contain p-vinyl-guaiacol (24.3%) (50) and/or benzyl thiol (or benzenemethanethiol, 14.5%) (46). The roots presented a lower concentration of pentadecane (7.6%) (51), spathulenol (5%) (52) and heneicosane (4.4%) (53) as well as a small concentration of undecane (14.7%) (54) in the inflorescence (Fig. 6).

Recently, researchers evaluated the volatile components of *P. alliacea* APs from seven distinct regions of Martinique (French West Indies), collected during the dry and rainy seasons (Kerudo et al., 2015). The most abundant among the 51 (89.1–98.1%) components found in the oil of different origins were toluenethiol (2.3–23.0%) (55), phytol (6.4–41.2%) (56), dibenzyl disulphide (13.2–35.3%) (5) and benzaldehyde (0.8–57.1%) (43) (Figs. 5 and 6). Forty of these substances have been identified in previous studies. Kerudo et al. (2015) also noted that the differences in the concentration of major constituents depend on the region of collection and the harvest season. Moreover, some compounds detected

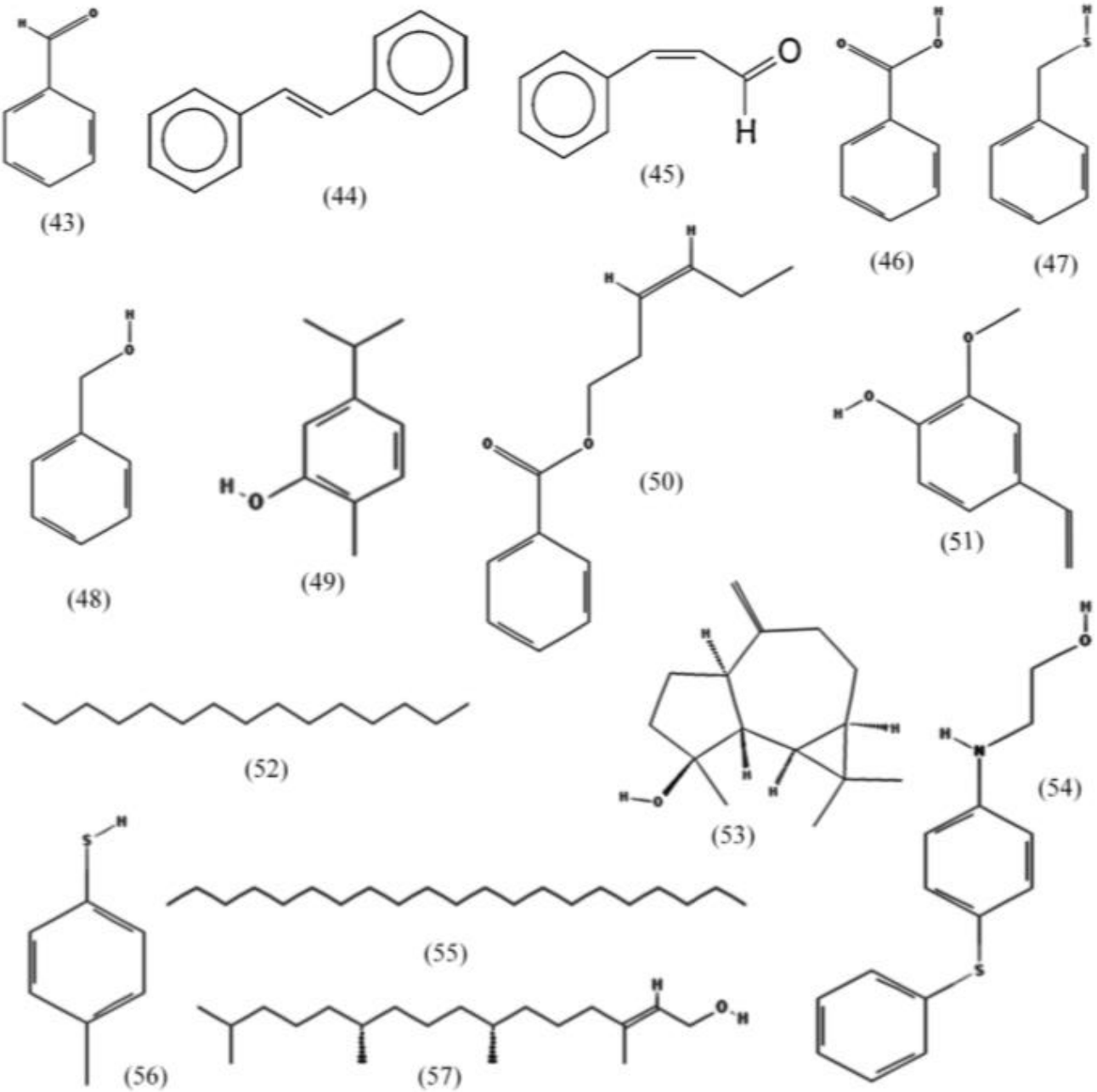

Fig. 6. Components found in the essential oil of *Petiveria alliacea*: benzaldehyde (43), trans-stilbene (44), cinnamaldehyde (45), benzoic acid (46), benzyl thiol (47), benzyl alcohol (48), carvacrol (49), (*Z*)-3-hexenyl benzoate (50), p-vinyl-guaiacol (51), pentodecane (52), spathulenol (53), heneicosanone (54), undecane (55), toluenethiol (56) and phytol (57). Adapted from Bezerra (2006) and PubChem Compound Database (2015).

in some studies were not reported in others, which highlights the differences between origin and phytochemical compositions.

4. Neuropharmacological activities

As described before, *P. alliacea* has been widely used in folk medicine to treat CNS disorders (Branch and Silva, 1983; Lima et al., 1991). The following sections of the present review will address the available data obtained in experimental studies using laboratory animals to highlight the promising neuropharmacological effects of *P. alliacea* as well as its isolated fractions and compounds.

4.1. Antinociceptive activity

P. alliacea is used across Latin America to relieve several types of pain, such as toothache and headache (Lima et al., 1991) as well as 'pain in the legs' (Albuquerque et al., 2012). In this regard, Gomes et al. (2005) investigated the antinociceptive effects of acute administration of acetate (FA), hexanic (FH), hydroalcoholic (FHA) and precipitated hydroalcoholic fractions (FHAppt) of extracts from *P. alliacea* roots on female Swiss mice and the putative involvement of CNS mechanisms. The intraperitoneal (i.p.) administration of all tested fractions of *P. alliacea* (at doses of 100 and 200 mg/kg) attenuated neurogenic pain induced by the chemical stimuli with acetic acid (0.6%, 10 ml/kg, i.p.) (Gomes et al., 2005).

The antinociceptive effects of the *P. alliacea* fractions were also observed in the formalin test (formalin 1%, 20 μl, i.p.). FHA (100

and 200 mg/kg) induced a significant inhibition of pain responses in both the first (51.4% and 55.4%) and second (57.9% and 97.9%) phases of the test (Gomes et al., 2005). In addition, FH (200 mg/kg) and FHAppt (200 mg/kg) elicited antinociception selectively in the second phase of the formalin test, similarly to that observed for morphine (10 mg/kg, 89.6% inhibition), used as the positive control (Gomes et al., 2005).

Interestingly, the subcutaneous (s.c.) administration of the opioid receptor antagonist naloxone (2 mg/kg) prior to the formalin test was unable to prevent the antinociceptive effects of FH (200 mg/kg in both phases), FHA (200 mg/kg in the first phase) and FHAppt (200 mg/kg in the second phase) (Gomes et al., 2005). These results suggest a non-opioidergic mechanism mediating the antinociceptive effects of acute administration of different fractions of extracts from *P. alliacea* roots in mice. However, it must be emphasized that the methodological tools used by the authors to assess the antinociceptive effects (writhing and formalin test) are not adequate to identify a specific mechanism of action (Silva et al., 2013; Trevisan et al., 2012, 2014).

As the two phases of the formalin test are sensitive to centrally acting drugs, these results may suggest that some substances presented in the fractions of *P. alliacea* root extracts might induce antinociception via other CNS mechanisms. Nevertheless, peripheral mechanisms may also account for the diverse antinociceptive effects exerted by different fractions of *P. alliacea* (Le Bars et al., 2001).

In accordance with this view, during the hot-plate test, mice previously treated with *P. alliacea* fractions (100 and 200 mg/kg, i.p.) did not display increased latency in response to the thermal stimulus in comparison to animals treated with morphine (10 mg/kg, i.p). As the behaviours (hindpaw licking and jumping) assessed in the hot-plate test are primarily mediated supraspinally (Le Bars et al., 2001), these findings suggest peripheral mechanisms as underlying the antinociceptive effects of the various fractions of *P. alliacea* root extracts.

Additionally, myricitrin is a flavonoid glycoside also found in *P. alliacea* that was reported to have antioxidant, analgesic, anti-inflammatory and antinociceptive properties (Meotti et al., 2006; Schwanke et al., 2013; Domitrović et al., 2015). In recent years, several studies have reported the antinociceptive effects of this flavonoid, which are associated, at least in part, with the following mechanisms: i) inhibition of the protein kinase C (PKC) and PI-3 kinase activities, ii) decrease in the nitric oxide (NO) production and activation of nuclear factor kappa B (NFκB), iii) activation of the protein Gi/0 pathway, iv) increase in the K^+ efflux, v) and decrease in intracellular Ca^{2+} influx (Gamet-Payrastre et al., 1999; Meotti et al., 2006, 2007). Therefore, myricitrin may represent one of many active compounds found in *P. alliacea* that can be responsible for pain relief in both humans and laboratory animals.

4.2. Anxiogenic/anxiolytic activity

As mentioned previously, *P. alliacea* has been used in traditional medicine to treat anxiety (Garcia et al., 2010). Thus, some studies were conducted to assess the validity of this popular use scientifically. Gomes et al. (2008) investigated the acute effects of root extract fractions of *P. alliacea* (FA, FH, FHA and FHAppt) at doses of 100 and 200 mg/kg (i.p.) on the anxiety-related behaviours of female Swiss mice evaluated in the elevated plus maze (EPM) test, a well-validated paradigm for the screening of anxiolytic/anxiogenic compounds. FA (100 and 200 mg/kg, p.o.) reduced the number of entries and the time spent in the open arms of the EPM indicative of an anxiogenic profile. In contrast, FA, FH and FHA (100 and 200 mg/kg, i.p.), as well as FHAppt (200 mg/kg, i.p.), significantly reduced the open arms time. Overall, these results indicate that, contrary to popular belief, *P. alliacea* (at least the roots) does not exert anxiolytic effects (Gomes et al. 2008).

In addition, Blainski et al. (2010) addressed anxiety-related effects of the whole plant (WP), AP and root (R) lyophilized crude extracts of *P. alliacea*. They observed that acute oral administration of WP (300 and 600 mg/kg), AP (600 and 900 mg/kg) and R (300, 600 and 900 mg/kg) did not reduce the anxiety-like behaviour of male Swiss mice subjected to the EPM test. In addition, AP (300 mg/kg) significantly decreased both the number of entries and time spent in the open arms of the EPM, once again indicating an anxiogenic-like profile.

On the other hand, Blainski et al. (2010) reported anxiolytic-like effects of WP of *P. alliacea* (300 and 900 mg/kg, p.o.) on male mice subjected to EPM. Corroborating these findings, also demonstrated that the WP extract of *P. alliacea* (900 mg/kg, p.o.) induced anxiolytic-like effects in female Wistar rats assessed in the open field test. In this study, the WP extract of *P. alliacea* was able to increase the total number of crossings and central quadrants crossed, indicative of an anxiolytic-like effect.

Audi et al. (2001) also evaluated the putative anxiolytic activity of a lyophilized hydroalcoholic extract of *P. alliacea* APs. Male Wistar rats were acutely administered with EBG (200, 400 and 600 mg/kg, p.o.) and were evaluated using the EPM test. EBG (600 mg/kg) significantly increased the percentage of entries in the open arms of the apparatus. However, it did not alter the percentage of time spent in the open arms or the number of entries in the enclosed arms of the EPM. Overall, these data indicated that EBG exerted a selective anxiolytic effect, with no effects on the spontaneous locomotor activity of the animals.

Taken together, evidence from animal models indicates that our comprehension of the *P. alliacea* effects on anxiety is still far from complete. Further studies combining data from different fractions and compounds isolated from *P. alliacea* in a range of behavioural paradigms of anxiety will enable a more conclusive view.

4.3. Antidepressant activity

There is no clear description in literature about the antidepressant use of *P. alliacea* in folk medicine. However, the leaves and roots of *P. alliacea* are used as stimulants in various regions across Brazil and Trinidad (Lans et al., 2001; Muñoz et al., 2000; Negri and Rodrigues, 2010). Depressive disorders have a set of symptoms, such as depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration (WHO, 2015). Thus, the use of *P. alliacea* as a stimulant could be a palliative treatment for depressive symptoms, since stimulant drugs frequently act in monoamine neurotransmission (noradrenaline, serotonin and dopamine), which is also the main pharmacological target of classic antidepressants (Ayflegül Yildiz et al., 2002).

The antidepressant effect of *P. alliacea* was initially investigated by Gomes et al. (2008). In this study, the authors investigated the effects of FA, FH, FHA and FHAppt of *P. alliacea* (when administered acutely, 100 or 200 mg/kg, p.o. and i.p.) on female Swiss mice evaluated with the forced swimming test (FST). Surprisingly, all fractions (100 and 200 mg/kg) produced depressant-like effects when given p.o. as well as i.p., as indicated by the significant increase in the immobility time of the animals subjected to the FST (Gomes et al., 2008).

On the other hand, observed antidepressant-like effects of the WP extract of *P. alliacea* (900 mg/kg, p.o.) on female Wistar rats evaluated in the FTS. They observed that the extract caused a significant reduction in the immobility time of the rats, thereby suggesting a possible antidepressant activity. The authors speculate that these antidepressant-like effects of WP extract of *P. alliacea* might be associated with the presence of coumarins, which are known to act via serotonergic and noradrenergic transmissions

to modulate mood behaviour (Ariza et al., 2007). These discrepant findings on depressive-like behaviours of the *P. alliacea* could be attributed to methodological differences including the part of the plant and doses administered, as well as the animal's species.

Overall, from these limited results in this field it appears that *P. alliacea* might be particularly useful for the treatment of depression, despite the lack of clear popular indications for the treatment of depression. Therefore, further studies are welcome in order to confirm the antidepressant properties of *P. alliacea*, as well as to investigate the underlying mechanisms of action.

4.4. General CNS depressant activity

In traditional medicine, *P. alliacea* is known to act as a general CNS depressant, thereby earning the title 'Remedy to tame the Master' (Camargo, 2007). Moreover, preparations of *P. alliacea* are popular for their sedative activities (Germano et al., 1993). Lima et al. (1991) described for the first time that the acute administration of an aqueous crude extract of *P. alliacea* roots (500, 1000 and 2000 mg/kg, p.o.) reduced the spontaneous locomotor activity of male Swiss mice in the OFT. Although the high dose utilized by Lima and colleagues, this work was the first report to support the evidence of the popular use by slaves.

In addition, it seems that this effect requires lower doses (100 and 200 mg/kg, i.p. and orally) when using root extract fractions, as shown by Gomes et al. (2008). In this study, the effects of *P. alliacea* fractions on autonomic behaviours, such as locomotor activity, rearing and grooming, in female Swiss mice subjected to the OFT were evaluated. All fractions reduced locomotor activity, but only rearing and grooming parameters were reduced in a similar way to diazepam (2 mg/kg, i.p.). Furthermore, they also demonstrated the potential hypnotic effect of this extract, with the aforementioned fractions increasing the sleep time induced by pentobarbital, thereby supporting the role of *P. alliacea* as a general CNS depressant.

Rodrigues et al. (2008) evaluated the CNS depressant effects of the cigarette 'tira-capeta' in mice. Initially, the extract induced a stimulant response followed by a general depressant state, thereby characterizing a biphasic effect. The animals displayed a reduced latency for sleeping and an increased sleeping time (50, 100 and 500 mg/kg) in the pentobarbital-induced sleeping test. However, no significant motor incoordination was observed in the rotarod test (doses up to 200 mg/kg). The 'tira-capeta' extract (500 mg/kg) also elicited a cataleptic state after 10 and 50 min. As previously mentioned, nine plants comprise the 'tira-capeta' preparation. For this reason, it is difficult to establish the degree of influence of *P. alliacea* on the results shown.

Contrasting with these findings, Blainski et al. (2010) described an increase in locomotor activity in male Swiss mice subjected to the OFT when acutely treated with 900 mg/kg of *P. alliacea* root extract. Interestingly, the variability in dose range, extract fractioning and plant part used to prepare the extract may contribute to the very controversial effects of *P. alliacea*. For instance, Cifuentes et al. (2001) observed that root extracts (1250 mg/kg) caused a slight decrease in spontaneous motor activity in mice, whereas leaf extracts induced hyperexcitability at the same dose. Furthermore, both the AP and WP extracts of *P. alliacea* (300 and 900 mg/kg) have been shown to increase locomotor activity (Andrade et al., 2012; Blainski et al., 2010).

Therefore, the CNS depressant activity of *P. alliacea* remains controversial. The conflicting effects described in literature may be associated with differences on the part of the plant, as well as the dose administered. However, it is important to emphasize that both stimulant and depressant effects of *P. alliacea* support the ethnopharmacological use of this plant by slavers and in religious ceremonies.

4.5. Anticonvulsant activity

Lima et al. (1991) were the first to evaluate the possible anticonvulsant activity of *P. alliacea*. Male Swiss mice were acutely treated with an aqueous crude extract of the roots of this plant (500, 1000 and 2000 mg/kg, p.o.), with either pentylenetetrazol (75 mg/kg, i.p.) or maximal transcorneal electroshock (rectangular pulses of 50 mA) being used to induce convulsive behaviour. Animals pretreated with the high extract doses (1000 and 2000 mg/kg) showed a significant increase in convulsive thresholds and a decrease in the duration of convulsion when compared with the control group. These findings support the use of *P. alliacea* for both the prevention and cessation of convulsive episodes. However, these results could not be relevant in the pharmacological practice, since high doses were employed in this study to elicited anticonvulsant effects.

Nevertheless, similar to depressor activity, lower doses of roots fractions also promote anticonvulsant effects. For example, Gomes et al. (2008) reported putative anticonvulsant activity of *P. alliacea* roots fractions (100 and 200 mg/kg, i.p. and p.o.) on pentylenetetrazol-induced seizure model. The authors also observed the anticonvulsant effects of these fractions, in line with the finding of Lima et al. (1991). Although the available scientific evidence supports the use of *P. alliacea* root-derived extracts, the traditional medicine of Latin American communities uses the leaves instead of the roots. Nevertheless, the leaf-based extracts of *P. alliacea* need to be investigated for their anticonvulsant activity (Branch and Silva, 1983; Zamora-Martinez and Pola, 1992).

4.6. Cognitive enhancer activity

As mentioned previously, leaves from *P. alliacea* and other species included in the 'tonic for the brain' category have been used as memory/cognition enhancers (Negri and Rodrigues, 2010; Rodrigues et al., 2008). In folk medicine, preparations containing leaves and roots of *P. alliacea* have been used to improve memory (Mors, 2002). were the first to report the effects of *P. alliacea* extract on the learning and memory processes in laboratory animals. In their study, evaluated learning and memory in female Wistar rats acutely treated with a WP extract (900 mg/kg, p.o.) using an elevated T-maze (ETM) paradigm. Saline (0.9%, 10 ml/kg, p.o.) and caffeine (10 mg/kg) were used as the negative and positive controls, respectively. On analysing the data, they found that WP extract-treated rats showed improvement in long-term memory, but not in short-term memory.

Attributed this effect to the possible presence of dibenzyl trisulphide (DTS) in the WP extract of *P. alliacea*. This chemical component increases the hyperphosphorylation of growth factor-induced mitogen-activated protein kinase (MAPK) (ERK1 and ERK2), which is a critical mechanism associated with long-term memory improvement and neuronal growth (Williams et al., 2007).

In view of the traditional use of the plant (leaves and roots) for memory improvement, Silva et al. (2015) designed a study to investigate the possible effects of *P. alliacea* leaf hydroalcoholic extract (PaLHE) on the learning and memory of male and female Wistar rats. The animals were acutely treated with PaLHE (900 mg/kg, p.o.), caffeine (10 mg/kg, i.p.) or saline (0.9%, 10 ml/kg, p.o.) and subjected to the step-down inhibitory avoidance and Morris water maze (MWM) tests. The results obtained from both tests confirmed the positive effects of PaLHE on long-term memory. These effects were attributed to the chemical constituents of the extract such as flavonoids, steroids, triterpenes, organosulphur compounds (DTS), thiosulphates and polysulphides. These results also identify substances known to have a positive effect on cognitive function (Kubec and Musah, 2001; Williams et al., 2007; Kennedy and Wightman, 2011).

Although research is at a very early stage, the findings reviewed above further highlight the cognitive-enhancing properties of *P. alliacea* evaluated in different behavioural paradigms in laboratory animals, thereby supporting the traditional use of this plant by quilombola adolescents and children to improve cognitive function. Therefore, the investigation of the putative effects of *P. alliacea* in experimental models of learning/memory dysfunction such as Alzheimer's disease and attention deficit hyperactivity disorder (ADHD) represents a very interesting field.

5. Toxicity studies

The toxicity of different extracts obtained from *P. alliacea* remains to be elucidated. In an overview, the acute toxicity of this plant in animal models (up to 14 days) was found to be low. However, in chronic and subchronic exposure, *P. alliacea* was able to induce moderate to high toxicity, including mutagenicity and genotoxicity. In addition, most studies confirmed the diverse acute effects of *P. alliacea* on the CNS, including anxiety, restlessness, confusion, ataxia, tremors and seizures, as mentioned previously. Thus, several studies on animal and human models of the toxicity of this species are described below.

In animal models of acute toxicity, mice exposed once to high levels of crude aqueous extract of *P. alliacea* roots (800–8000 mg/kg) showed reduced locomotor activity during the behavioural tests. Furthermore, mice treated with a dose of 8000 mg/kg presented ptosis and ataxia, although none of the animals died, thereby demonstrating the low toxicity of this extract (Lima et al., 1991). In another study, a hippocratic test was conducted to evaluate the response of female Swiss mice to the acute toxicity of the hydroalcoholic extract of *P. alliacea* roots at an i.p. dose ranging from 500 to 3000 mg/kg; in particular, the oral administration of 100–400 mg/kg of the *P. alliacea* root extract showed low acute toxicity (Gomes, 2006).

Other researchers have also reported the low toxicity of *P. alliacea* extract. For instance, Audi et al. (2001) showed that the AP hydroalcoholic extract (up to 3000 mg/kg) did not induce any sign of toxicity in mice. However, these authors also showed that a dose fivefold that of the tested *P. alliacea* extract was necessary for eliciting an anxiolytic effect and reducing gastric ulcers. In fact, Oliveira (2012) evaluated the oral acute toxicity of *P. alliacea* roots and APs collected during different seasons (dry and rainy) on mice. The animals were orally administered a single dose of 5000 mg/kg and monitored for 14 days. They were analysed for manifestation of signs of toxicity, food consumption and weight change. Later, the mice presented no change in behaviour and food consumption. Only the root extract collected during the dry season led to a reduction in the body weight gain.

Fontoura et al. (2005) reported that the hydroalcoholic extract of *P. alliacea* leaves (500, 1000, 5000 and 10,000 mg/kg) did not cause behavioural or histopathological changes in the liver, kidney, lung and heart. However, 1000 mg/kg of the extract did not result in the same decrease in secretion and intestinal motility as with lower doses (250 and 500 mg/kg). Similarly, a single dose (4000 mg/kg) of the dry crude extract of *P. alliacea* leaves administered to albino rats did not cause mortality or any signs of toxicity after 14 days. However, this dose did alter the leucocyte count, eosinophil differentials, the mean corpuscular volume, mean corpuscular haemoglobin values and haematocrit. In addition, the biochemical results were indicative of hepatic overload (Ximenes, 2008).

In the WP hydroalcoholic extract, the acute toxic effects of the 2000 and 5000 mg/kg doses caused lethargy and drowsiness in mice, but not death (Andrade et al., 2012). Morón (1990) demonstrated the toxicity of a *P. alliacea* decoction at higher oral doses (10,000 mg/kg) in mice without death or signs of toxicity even when administered for seven consecutive days. Germano et al. (1993 and 1995) evaluated the toxic effects of the hydroalcoholic root extract administered topically and orally at a dose of 1 mg/kg (equivalent to 7.7 mg of dry root) for 15 days, reporting no sign of local irritation in the gastric mucosa.

García-González et al. (2006) showed that acute (18 days) and subchronic (70 days) doses of the aqueous extract (1000 and 2000 mg/kg) of *P. alliacea* leaves did not result in the death of mice. Nevertheless, 1000 mg/kg of the extract led to an increase in the blood glucose concentration and a decrease in the haematocrit. For some animal species, high doses of *P. alliacea* may cause intoxication. In this context, Núñez et al. (1983) reported that sheep that consumed this plant daily (3000 or 6000 mg/kg of *P. alliacea*, up to 46 days) presented with initial symptoms of salivation, lachrymation, bradycardia, polyuria, diarrhoea, ataxia and marked inhibition of blood cholinesterase, along with lateral decubitus opisthotonos and atrophy of muscle mass, as well as lesions in the nervous and muscular system. The necropsy presented renal lesions and muscle atrophy with fragmentation and hyalinization of muscle fibres. Other studies reported a lethal dose 50 (DL_{50}) of 360 mg/kg for oral administration of *P. alliacea* leaf extracts in rats and i.p. administration at 1700 mg/kg in mice (Estevez et al., 1976; Delaveau et al., 1980). Moreover, Wistar rats subjected to chronic intoxication (90 days) with an aqueous extract of *P. alliacea* dried leaves at doses of 12.36, 61.8 and 309 mg/kg did not show any signs of toxicity. An increase in urea nitrogen and alanine transaminase is the only indication of a toxic effect (Ximenes, 2008).

In the aerial pathway, histological analyses of the respiratory tract of female Wistar rats showed morphological modifications after the animals were exposed to the steam of *P. alliacea* root (150 g) for a short duration (3 min). After exposure, the animals were sacrificed and aerial pathways were observed for 5–30 min. In the 5–15 min after exposure, significant alterations in the bronchioles, trachea and lungs were noted. Some of the tracheal manifestations included epithelium hyperplasia, signs of increased goblet cell secretion, muscle congestion, mononuclear infiltrate and the absence of epithelium and cilia in some areas. The bronchioles revealed activation of Clara cells, the absence of the epithelium in some areas and the presence of mononuclear cells. In the lungs, thickening of the alveolar septa, an increase in collagen fibres, congestion, extravasation and intra-alveolar exudates were reported (Fletes-Arjona et al., 2013).

These results indicate that the steam of *P. alliacea* roots contains components with an aggressive mechanism of action. Petiverine and coumarin may be attributed to the manifestations observed, based on the increased secretion and induction of vascular congestion associated with the mononuclear infiltration capacity of petiverine and coumarin, respectively. It is important to note that even after 30 min of exposure, anormal bronchiolar features were found with no reversion of the lung damage reported for the shorter durations of steam exposure (Fletes-Arjona et al., 2013).

On conducting *in vitro* assays, some authors reported that the extract of *P. alliacea* leaves showed low cytotoxicity with a half-maximal inhibitory concentration (IC_{50}) of 1709.77 μg/mL (Oliveira et al., 2013), whereas the *P. alliacea* fraction derived from leaves and stems in normal fibroblasts and peripheral blood mononuclear cells showed an IC_{50} of 440 and 151 μg/mL, respectively (Urueña et al., 2008). In addition, mutagenic and carcinogenic effects have also been reported, with Hoyos et al. (1992) demonstrating the dose-dependent mutagenic and carcinogenic effect of *P. alliacea* extract. They reported DNA damage with the formation of sister chromatid exchanges (SCEs) in human lymphocytes *in vitro* and in mouse bone marrow cells *in vivo*, especially at higher concentrations of 100 and 1000 μg/ml (*in vitro*) and 204 mg/kg (*in vivo*). Our group recently demonstrated the genotoxic effect of *P. alliacea* extract (data not published yet).

According to the Organisation for Economic Co-operation and Development (OECD, 2001), a substance is categorized under level five of acute toxicity (low toxicity) when a few behavioural alterations, but not death, are reported in animals. Based on this categorization and the results of a study on the toxicity of several extracts from *P. alliacea*, the species is known to cause low toxicity; however, at very high doses and prolonged exposure, the plant can exert toxic effects. Moreover, the plant part, season and region of collection may interfere with this toxic effect (Oliveira, 2012).

In humans, acute intoxication was found to cause insomnia, hyperarousal and hallucinations, whereas prolonged use (such as one year of chronic exposure) elicits opposite symptoms such as seizures, weakness, mental retardation and even death, depending on the dose (Peckolt and Peckolt, 1900). Although several studies have reported the low toxicity of the plant, ethnopharmacological reports have indicated death after consumption of high doses (not well established by Peckolt and Peckolt (1900)) for a prolonged period. Moreover, the amount consumed daily in some regions is higher than the dose used in toxicity studies (Ferraz et al., 1991b). Therefore, the toxicity of *P. alliacea* must be investigated further to establish the accurate dose and duration for treatment.

6. Conclusion and perspectives

Popularly known by several different names including 'mucuracaá', 'guiné' and 'pipi', *P. alliacea* is a valuable botanical source because of its many uses and wide range of pharmacological biological activities. Crude extracts, fractions and phytochemical constituents isolated from various parts of *P. alliacea* show a wide spectrum of neuropharmacological activities including anxiolytic, antidepressant, antinociceptive and anti-seizure, and as cognitive enhancers. Phytochemistry studies of *P. alliacea* indicate that this plant contains a diversity of biologically active compounds, with qualitative and quantitative variations of the major compounds depending on the region of collection and the harvest season. Although significant advances have been made in the phytochemistry and pharmacology of *P. alliacea*, information on health effects and clinical value is insufficient. A large number of bioactive compounds have been previously isolated but not tested; therefore, these compounds must be evaluated biologically in more detail. Further *in vitro* and *in vivo* genotoxic tests of *P. alliacea* are also important in order to assess ethnomedical claims. Therefore, in future, detailed and extensive studies are certainly required to improve the knowledge about the mechanisms of action, toxicity and efficacy of the plant as well as about its bioactive compounds before it can be approved in terms of its safety for therapeutic applications.

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IV CAPÍTULO II

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Petiveria alliacea exerts mnemonic and learning effects on rats

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ABSTRACT

Ethnopharmacological relevance: *Petiveria alliacea* L. (Phytolaccaceae) is a perennial shrub native to the Amazon region and other tropical areas such as Central America and the Caribbean. Popularly known as mucuracaá, *P. alliacea* is used in the folk medicine for a broad variety of therapeutic purpose and also in religious ceremonies by slaves as a sedative, which highlights its properties on the Central Nervous System (CNS).

Aim of the study: The present study evaluated the effects of the *P. alliacea* leaves hydroalcoholic extract (PaLHE) on the cognition, including learning and memory.

Material and methods: Three-month-old male and female Wistar rats (n=8–10/group) were administered with 900 mg/kg of PaLHE. The behavioral assays included Step-down Inhibitory avoidance (IA) and Morris Water Maze (MWM) tests.

Results: Consistent with our previous reports, *P. alliacea* improved long-term memory. It also exerted previously unreported effects on short-term and spatial memory improvement, and increased learning in the tasks.

Conclusions: The *P. alliacea* extract elicited mnemonic effects and improved the learning process in both IA and MWM tests. Our results highlight the importance of further studies in order to identify the active substances of the PaLHE and investigate the pharmacological mechanisms that underlies the reported effects.

1. Introduction

The increase in human life expectancy combined with the stressful and competitive routine of today's society has induced a higher prevalence of age-related learning and memory disorders (poor memory, lower retention, and slower recall). These disorders, which have acquired the status of worldwide public health issue, manifest themselves among the aging population even in the absence of neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Li et al., 2014; Vollala et al., 2010). In order to prevent their progression it is important to find new prophylactic and therapeutic agents, which medicinal plants comprise a rich source for potential new drugs. In fact, important marketed drugs that act on the central nervous system (CNS) derive from medicinal plants such as *Atropa belladonna* (atropine), *Papaver somniferum* (morphine), and *Ephedra vulgaris* (ephedrine) (Prakash and Gupta, 2005).

The historical use of medicinal plants emerges as an important tool for the human evolution, once they were the very first therapeutic resource which people relied on (Tomazzoni et al., 2006). Throughout the years, the use of medicinal plants as a source for new drugs has been well established in virtually all cultures (Estrada-Castillón et al., 2014). In 1978, the World Health Organization (WHO) officially recognized the medicinal plants and galenical preparations as valid therapeutic options, recommending their use worldwide (World Health Organization (WHO), 1978). According to WHO reports, more than 80% of the world population makes use of drugs derived from medicinal plants. Studies have

Abbreviations: ALT, Arrival Latency Time; CEPAN-IEC, Comitê de Ética em Pesquisa com Animais from the Instituto Evandro Chagas; CNS, Central Nervous System; ELT, Escape Latency Time; ETM, Elevated T-Maze Test; GPS, Global Positioning System; IA, Step-down Inhibitory Avoidance Test; LIFAMA, Laboratório de Insumos Farmacêuticos da Amazônia; MWM, Morris Water Maze Test; PaLHE, *Petiveria alliacea* leaves hydroalcoholic extract; STM, Short-term Memory; UFPA, Universidade Federal do Pará; WHO, World Health Organization; TLC, Thin Layer Chromatography; VGA, Vanillin-Glacial acid; Rf, Retention Factor

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shown that 91.9% of the Brazilian population have made use of some medicinal plant, such as *Petiveria alliacea* L. (Ethur et al., 2011; Giraldi and Hanazaki, 2010; Oliveira et al., 2010).

The genus *Petiveria* belongs to the Phytolaccaceae, the most archaic family of the Caryophyllales, comprising about 17 genera and 120 pantropical species widely distributed throughout the American continent (Duarte and Lopes, 2005). *P. alliacea* L. is a perennial shrub that reaches up to 70 cm in height and presents small flowers, which form long racemose inflorescences and cuneate achenes bearing spines for dissemination. It is native to the Amazon Rainforest, but can also be found in other tropical areas from Central and South America, the Caribbean, and sub-Saharan Africa. This species is popularly known as guiné, tipi, pênis-de-coelho, mucuracaá, true-tipi, anamú, zorrillo, tame-mister, eye's herb, garlic scented petiveria, verbeine puante, embayayendo, and ouoembo (Andrade et al., 2012; Duarte and Lopes, 2005; Lima et al., 1991).

In traditional medicine, *P. alliacea* L. is associated with a broad variety of therapeutic properties. The use of root, powder, and leaf decoctions or infusions are recommended for their diuretic, antispasmodic, anticonvulsant, emmenagogue, sedative, analgesic and anti-inflammatory, anesthetic, antileukemic, antirheumatic, antihelminthic, antimicrobial, and depurative properties (Duarte and Lopes, 2005; Lima et al., 1991). *P. alliacea* has also been used in religious ceremonies by slaves for its toxic and sedative effects, being called "Remedy to tame the Master" (Gomes et al., 2008; Camargo, 2007). In addition, the weak infusion of the leaves or roots is used to treat poor memory (Mors et al., 2000). To confirm these pharmacological actions and investigate potentially non-described activities, several studies have shown antitumor, anticancer, immunomodulatory, analgesic, anti-inflammatory, antibacterial and antifungal, antinociceptive, anxiolytic, uterine contraction, gastric protective, antidepressant, and motor stimulatory effects (Andrade et al., 2012; Blainsk et al., 2010; Duarte and Lopes, 2005; Hernández et al., 2014; Kim et al., 2006; Lima et al., 1991; Lopes-Martins et al., 2002; Urueña et al., 2008).

Besides having many beneficial pharmacologic properties, *P. alliacea* L. has also been reported for its toxic effects on the CNS among traditional populations that make use of it (Lima et al., 1991). The continuous use of this plant has been linked with the onset of madness. Acute intoxication signs are insomnia, hyperarousal, and hallucinations. On the other hand, long term use elicits contrary symptoms like seizures, weakness, imbecility, and death within 1 year of chronic exposure, depending on the dose (Peckolt and Peckolt, 1900).

There are some studies that have assessed the potential toxicity of different extracts obtained from *P. alliacea*. Lima et al. (1991) performed gross behavioral observation and acute toxicity tests with a crude aqueous extract of the roots (800–8000 mg/kg) in mice that generally presented reduced locomotor activity; besides, ptosis and ataxia were verified when the mice received the 8000 mg/kg dose, and none of the doses given resulted in death. The acute toxicity of the lyophilized hydroalcoholic aerial parts extract (up to 3000 mg/kg) carried out by Audi et al. (2001) in mice resulted in no signs of toxicity. In the study by Fontoura et al. (2005), it was found that the 95% hydroalcoholic extract of the leaves (500, 1000, 5000, and 10.000 mg/kg) did not elicit acute toxicity in mice. García-González et al. (2006) evaluated the acute (18 days) and subchronic (70 days) toxicity of a *P. alliacea* leaves aqueous extract (1000 and 2000 mg/kg), and no mortality nor any signs of toxicity were found at both doses during either experimental periods (18 and 70 days) conducted with mice. More recently, Andrade et al. (2012) investigated the acute toxicity of a whole plant hydroalcoholic extract and observed that the doses of 2000 and 5000 mg/kg induced lethargy and drowsiness in mice, but no death occurred among the treated animals. It is perceptible that the extracts derived from the leaves of *P. alliacea* presented the lower toxicities or even no signs of toxicity. In addition, according to the Organization of Economic Co-operation and Development (OECD, 2001) even though some of the extracts induced a few behavioral alterations, as the extracts did not cause any animal to die, they are classified as a substance with acute toxicity level 5, which is considered as low toxicity.

Some of the behavioral alterations previously reported are supported by the findings on the studies of Gomes et al. (2008), Blainsk et al. (2010), and Andrade et al. (2012), where the administration of the extract from *P. alliacea* L. (root extract fraction—100 and 200 mg/kg; whole plant, aerial parts, and root extracts—300, 600 and 900 mg/kg; whole plant hydroalcoholic extract–900 mg/kg) altered the motor functions, anxiety behaviors, and also elicited a possible CNS depressant effect on both mice and rats. In addition, Andrade et al. (2012) were the first to confirm and give support to the traditional use of *P. alliacea* L. as a brain tonic used to treat poor memory, once an improvement in long-term memory was noted in the elevated *T*-maze test (ETM).

In order to confirm and to expand Andrade et al. (2012) findings, at the same oral dose (900 mg/kg), the objective of the present study was to investigate the effects of *P. alliacea* leaves hydroalcoholic extract (PaLHE) on the CNS of rats through the use of different predictive behavioral tests that evaluate effects on memory and learning processes.

2. Material and methods

2.1. Collection, identification and preparation of Petiveria alliacea leaves hydroalcoholic extract (PaLHE)

P. alliacea was collected from the village of São Raimundo in Acará city (Pará state, Amazon region) in March, 2010. Geographic coordinates obtained using global positioning system (GPS) equipment, situate the collection are at latitude 01°32.684′ and longitude 048 23.984′. A specialist from the Emilio Goeldi Museum (Pará-Brazil), Mário Jardim PhD, carried out the botanical identification and deposited the sample as a voucher specimen under the code MG94354. According to the folk medicine, approximately 9 g of the dried *P. alliacea* are added to 600 ml of water, boiled, filtered and should be taken orally 3 times a day (Ferraz et al., 1991).

The plant material (leaves) was firstly washed with tap water, and then followed by 10% ethanol solution. After the cleaning process the material was dried at room temperature for two days, at an average temperature of 40 °C in an oven with forced air circulation for six days, and crushed in a knife mill for obtaining the sprayed drug.

The sprayed drug was then macerated for five days in a 70% ethanol solution. The macerate was dried in a rotary evaporator (Laborata 4000 efficient; Heidolph Instruments GmbH & Co. KG) at 45 °C, 1 atm pressure, and 120 rpm. As a way of ensuring total solvent removal, the macerate was submitted to water bath at 40 °C. 96.65 g of PaLHE, corresponding to 1430 g of the dried leaves, were obtained and applied to animal treatment. The extract was prepared at the *Laboratório de Insumos Farmacêuticos da Amazônia* (LIFAMA).

2.2. Thin layer chromatography of PaLHE

Thin layer chromatography (TLC) was performed on precoated silica gel 60 F254 plate (Merck, Germany) for identification of sulfur compounds in PaLHE, as described by Wagner and Bladt (2001). Briefly, aliquots of PaLHE dissolved in dichloromethane (10 mg/mL) were applied on TLC plate, with the help of glass capillary tubes. TLC was developed using toluene–ethyl acetate at a volume ratio of

100:30 (v/v) as mobile phase. Aqueous extracts of *Allium sativum* and *Allium cepa* were extracted with dichloromethane and the nonpolar phase was used as a positive control for the identification of sulfur compounds. After chromatography, the plate was sprayed with 10 mL of vanillin-glacial acid reagent (VGA), heated at 110 °C for 3 min, and then evaluated under visible light. All spots were recorded and the retention factor values (R_f) were calculated using the formula: R_f = distance the spot moved above the origin/distance the solvent front moved above the origin.

2.3. Animals

Three-month-old, male and female Wistar rats (n = 8–10 rats/group, 4–5 males and 4–5 females), weighing between 180 and 200 g, obtained from the Animal Facility of the *Instituto de Ciências Biológicas, Universidade Federal do Pará* (UFPA), were used as experimental models. The animals were kept under standard conditions of temperature, humidity, and a light/dark cycle of 12 h (7:00 a.m. lights on) with water and food ad libitum. Fluorescent lights (12 lx) were used in the rooms where the behavioral experiments were performed.

The research project was approved by the *Comitê de Ética em Pesquisa com Animais* from the *Instituto Evandro Chagas* (CEPAN-IEC) under number 56/2009, and the study was conducted in accordance with the standards set by the Guide for the Care and Use of Laboratory Animals.

Each animal in the treatment group was orally administered (gavage) with 900 mg/kg of PaLHE dissolved in saline solution (0.9% NaCl) an hour before the behavioral tests. The 900 mg/kg dose was chosen considering the fact that in previous studies conducted by Blainsk et al., (2010) and Andrade et al. (2012) such dose was found to have low toxicity and also be capable of eliciting more prominent effects on the CNS. The positive control group was treated with caffeine (CAF: 1, 3, 7-tri-methylxantine; Sigma-Aldrich) at a concentration of 10 mg/kg, the standard dose used for cognitive function evaluation (Andrade et al., 2012), an hour before the experiments. The control group received saline solution (0.9% NaCl).

2.4. Behavioral assays

2.4.1. Step-down inhibitory avoidance (IA) test

Animals were submitted to the inhibitory avoidance apparatus (EP 104R, Insight, Brazil) that was an acrylic box (50 × 25 × 25 cm^3) whose floor consisted of parallel stainless steel bars (1 mm in diameter) spaced 1 cm apart. A platform (7-cm wide × 2.5-cm high) was placed on the floor against the left wall of the box. Using a protocol similar to Maia et al. (2009), on the first day, animals were placed individually on the safe platform of the apparatus, and were allowed to explore the environment for a habituation period of 180 s. On the second day, animals were placed on the platform and the latency to step-down on the grid with four paws was measured with an automatic device that was used as measure of retention (cut off 180 s). During training session, immediately after stepping down on the grid, the animals received a 0.4-mA, 1.0-s scrambled footshock. Then, the animals were immediately removed from the apparatus until next session that evaluated short-term memory (STM), performed 1.5 h after training.

2.4.2. Morris Water Maze (MWM) test

The Morris Water Maze test (Morris, 1981) was used to evaluate learning and memory (spatial and long-term memory). The apparatus consists of a circular water tank (150 cm of diameter, and 60 cm of height), which was filled with water (± 25 °C) up to 45 cm. The water was made opaque through the addition of a non-toxic, water soluble dye. The water tank was then divided into four equal quadrants (Q1–Q4). An acrylic platform (diameter of 10 cm^2) of 43 cm of height was placed in the center of Q4, which was randomly chosen to be the target quadrant in the present study. Each animal was subjected to four consecutive trials (120 s on each trial) on the first day (training session) with five minutes of interval in between each trial, during which they were allowed to remain on the platform for 20 s. The starting position for each trial was modified, following four insertion cardinal points (N, S, W, and E). If the animals did not find the platform within the 120 s, they were gently hand-guided to the platform where they could stay for 20 s. Escape latency time (ELT) to find the hidden platform in Q4 on each trial was noted as an index of acquisition (learning). Animals were subjected to a probe trial 24 h after the last acquisition trial. The platform was removed from the water maze for the probe trial. The animals were allowed to explore the maze in search for the platform during 60 s. Time spent in target quadrant (Q4) and the arrival latency time (ALT) in Q4 were noted as an index of retrieval (long-term/spatial memory) (Juyal et al., 2010; Singh et al., 2013).

2.5. Statistical analysis

All values are expressed as mean ± S.E.M. (n = 8–10 animals per group). Following significant ANOVA, multiple post-hoc comparisons were performed by Bonferroni's test. The accepted level of significance was $p < 0.05$. All tests were performed using the GraphPad Prism® version 5.0 software package (San Diego, CA, USA).

3. Results

As shown in Fig. 1, the nonpolar phase of *A. sativum* and *A. cepa* extracts showed the development of organosulfur compounds spots through TLC analysis as previously reported by Wagner and Bladt (2001). Regarding the PaLHE, after chromatography

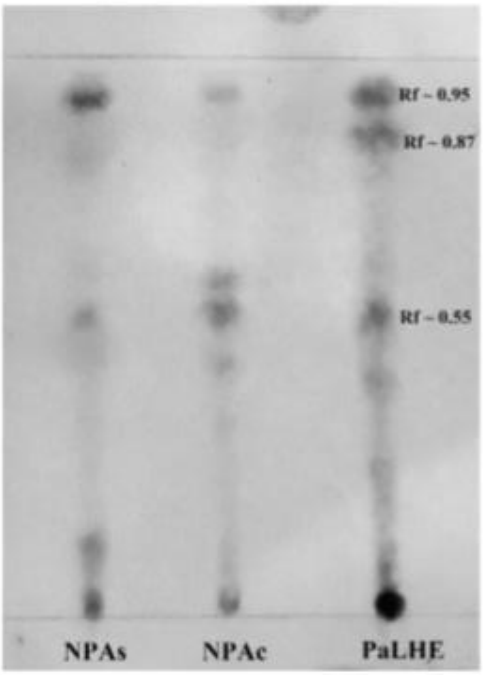

Fig. 1. Thin layer chromatography (TLC) plate shows organosulfur compounds retention factors (R_f) of *Petiveria alliacea* leaves hydroalcoholic extract (PaLHE) according with the positive controls after treatment with vanillin-glacial acid (VGA) reagent. Positive controls: NPAs = Nonpolar phase of *Allium sativum*; NPAc = Nonpolar phase of *Allium cepa*.

and spraying, the TLC plate presented three majority spots. These spots were similar to the positive controls used with R_f of 0.95, 0.87, and 0.55 for PaLHE, indicating organosulfur compounds in the extract.

As illustrated in Fig. 2, both PaLHE-treated rats ($F_{(2,29)}=6.269$; $p<0.001$) and the caffeine group ($F_{(2,29)}=2.546$; $p<0.05$) exhibited increased step-down latency on the IA test. Subsequent post-hoc comparisons using Bonferroni's test revealed that PaLHE ($F_{(2,29)}=4.163$; $p<0.001$) elicited a more significant effect than the one observed on the caffeine-treated group ($F_{(2,29)}=2.546$; $p<0.05$).

In the MWM test, animals pre-treated with caffeine ($F_{(2,29)}=5.137$; $p<0.001$) and PaLHE ($F_{(2,29)}=5.853$; $p<0.001$) presented reduced escape latency time in the fourth training session than the control group (Fig. 3, panel B). This result was not verified during the first training session, where both the caffeine ($F_{(2,29)}=1.900$; $p>0.05$) and PaLHE-treated animals ($F_{(2,29)}=1.905$; $p>0.05$) did not differ from the control group (Fig. 3, panel A).

For the retrieval probe trial, PaLHE ($F_{(2,27)}=4.107$; $p<0.001$), as well as the caffeine group ($F_{(2,27)}=2.937$; $p<0.001$), exhibited a reduced arrival latency time in Q4 (target quadrant) (Fig. 4, panel A). As shown in Fig. 4 (panel B), both caffeine ($F_{(2,27)}=3.829$; $p<0.001$) and PaLHE ($F_{(2,27)}=5.220$; $p<0.001$) treated-groups spent more time at Q4 in search for the hidden platform (previously there) than the control group.

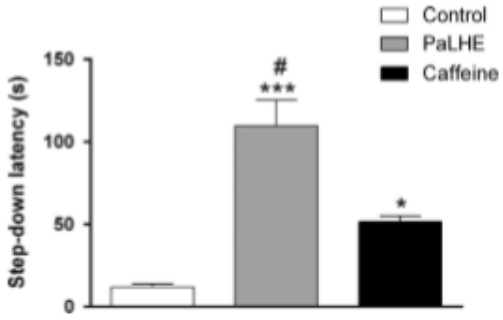

Fig. 2. Effects of *Petiveria alliacea* leaves hydroalcoholic extract (PaLHE) 900 mg/kg, vehicle (0.9% NaCl) and caffeine (10 mg/kg) on step-down latency in rats submitted to the inhibitory avoidance test for three minutes (180 s). Each value represents the mean + S.E.M. of 8–10 animals (males and females). $^{*}p<0.05$ compared to the control group treated with saline. $^{***}p<0.001$ compared to the control group. $^{\#}p<0.001$ compared to the caffeine group (ANOVA, Bonferroni's test).

4. Discussion and conclusions

In the present study, the effects of PaLHE on the cognitive functions (i.e., memory and learning) were evaluated using IA and MWM tests.

TLC analysis of PaLHE was able to identify the presence of organosulfur compounds in the extract. Regarding such compounds, our results were similar to the study performed by Kubec and Musah (2001), in regards to the spot with $R_f=0.55$. In addition, organosulfur compounds like thiosulfinates and other polysulfides may be associated to the subsequent spots visualized in the PaLHE-TLC analysis (Kubec and Musah, 2001). Moreover, several studies have reported the identification of organosulfur compounds in the leaves of *P. alliacea*, including dibenzyl trisulphide (DTS) (Benevides et al., 2001; Cifuentes et al., 2009; Kubec et al., 2010; Rosado-Aguilar et al., 2010; Urueña et al., 2008).

Passive or inhibitory avoidance is a test in which the behavioral response is obtained from an aversive stimulus (Maia et al., 2009). In this test, normal exploratory behavior in the animal is inhibited by applying an electroshock. Thus, the learning process is expressed by the inhibition of exploratory activity. After analyzing

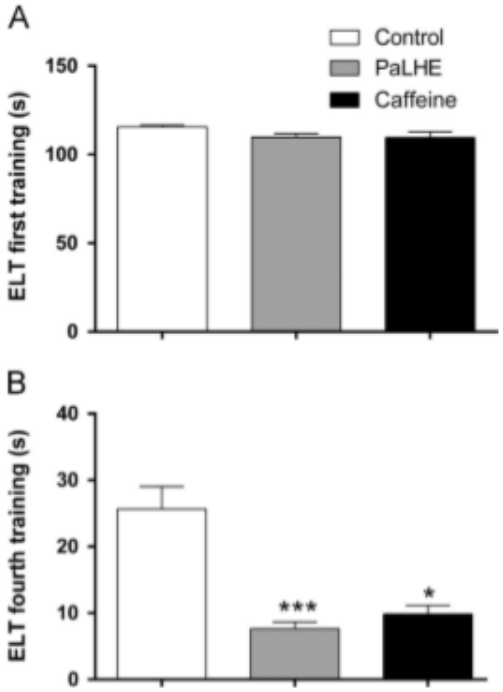

Fig. 3. Effects of *Petiveria alliacea* leaves hydroalcoholic extract (PaLHE) 900 mg/kg, vehicle (0.9% NaCl) and caffeine (10 mg/kg) on Escape latency time (ELT) in rats submitted to the training sessions of the Morris Water Maze test. Panel A – ELT from first training session of the MWM test. Panel B – ELT from the fourth training session of the MWM test. Each value represents the mean + S.E.M. of 8–10 animals (males and females). $^{*}p<0.05$ compared to the control group treated with saline. $^{***}p<0.001$ compared to the control group (ANOVA, Bonferroni's test).

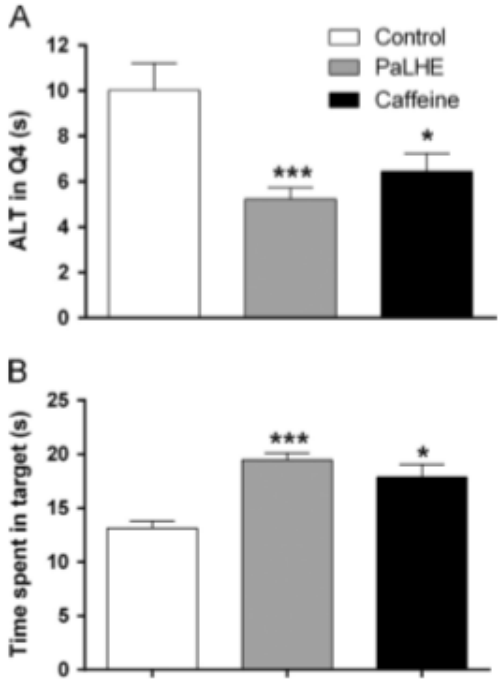

Fig. 4. Effects of *Petiveria alliacea* leaves hydroalcoholic extract (PaLHE) 900 mg/kg, vehicle (0.9% NaCl) and caffeine (10 mg/kg) on the probe trial of the Morris Water Maze test (MWM) in rats. Panel A – Arrival latency time (ALT) in the target quadrant (Q4) during the probe trial of the MWM test. Panel B – Time spent in the target quadrant (Q4) during the probe trial of the MWM test. Each value represents the mean + S.E.M. of 8–10 animals (males and females). $^{*}p<0.05$ compared to the control group treated with saline. $^{***}p<0.001$ compared to the control group (ANOVA, Bonferroni's test).

the parameters considered in this model, it was found that the PaLHE increased learning and STM parameters.

Meanwhile, in a study of the total plant extract of *P. alliacea* L., Andrade et al. (2012) performing the ETM test, reported that the plant extract mediated long-term memory improvement, but not learning and STM. However, several studies described controversial results that could be related to different parts of the plant used in the extract preparation (Audi et al., 2001; Blainsk et al., 2010; Cifuentes et al., 2001). In this sense, our study only collected leaves of the plant which may have modified the extract's compounds.

Several studies involving fractions of plant extracts, especially of the leaves of *P. alliacea*, identified a considerable presence of flavonoids (Blainsk et al., 2010; Kubec and Musah, 2001; Kubec et al., 2002; Williams et al., 2007). According to Spencer (2008), flavonoids promote beneficial effects on the CNS, including on memory and cognitive function. Considering that the expansion of both short and long-term memory is controlled at the molecular level, which in the STM involves covalent modifications of pre-existing proteins, while the long-term memory is mediated by the synthesis of new mRNA and proteins (Carew, 1996), it is known that some phytochemical constituents, especially flavonoids, may exert direct effects on the modulation of cellular proteins and in the signaling pathways of lipid kinases, which are directly related to synaptic plasticity and memory (Bourtchuladze et al., 1994; Williams et al., 2004).

In addition, García-González et al. (2006) have reported the presence of steroids, terpenoids, saponins, polyphenols, alkaloids and tannins in the leaves of *P. alliacea*, while Kumar and Khanum (2012) reviewed the neuroprotective potential of the same phytochemicals, which were able to elicit enhancement not only in memory function but also in learning capacity. In light of this, our findings are consistent with the pharmacological effects of chemical compounds of the *P. alliacea*.

In order to reinforce previous data, in the present study our group performed the MWM test to further evaluate the mnemonic effects of the PaLHE. The MWM test was developed by Morris (1984) and improved by Stewart and Morris (1993) for assessing related forms of learning and memory. The parameters observed for this test are: i) ELT (acquisition trials), which is correlated with learning as the animals tested tend to present reduced ELT throughout the trials; ii) arrival time at the target quadrant (retrieval probe trial); iii) and the time spent at the target quadrant in the searching for the platform that was previously there for assessing long-term/and spatial memory (Brandeis et al., 1989; D'Hooge and De Deyn (2001)).

Results from the acquisition trials on the MWM test showed a reduction in the ELT, suggesting an increase of the animals learning ability. As previously discussed, Andrade et al. (2012) did not observe any effects of *P. alliacea* L. over the learning process. However, our study performed two models of learning with positive results. These contradictory results might be related with the fact that they used a whole plant extract, which has less total flavonoids amounts when compared to the leaves extract (Blainsk et al., 2010). *P. alliacea* L. has also been reported to present triterpenes and steroids (Cuervo, 2011; Urueña et al., 2008), which in addition to flavonoids in other plants have been associated with the improvement of learning capacity (Haque et al., 2006; Kennedy and Wightman, 2011; Spencer, 2008). The present study demonstrates, for the first time, that leaves of *P. alliacea* L. improve the learning process in rats.

On the retrieval probe trial, after 24 h PaLHE exhibited mnemonic activity, indicated by a longer time spent in the target quadrant (Q4) and shorter time to first arrive at Q4. These parameters serve as indications that the extract improved long-term and spatial memory (Brandeis et al., 1989; D'Hooge and De Deyn, 2001). The improvement in long-term memory by *P. alliacea* whole extract was previously reported by Andrade et al. (2012). In agreement with our study, we demonstrated that PaLHE was able to obtain broader mnemonic activities. Our group performed a TLC analysis of PaLHE and was able to qualitatively demonstrate the presence of a class of metabolites known as organosulfur compounds. Taking this into consideration we suggest that the presented effects on memory could be due to the action of dibenzyl trisulphide (DTS), an organosulfur compound previously isolated from *P. alliacea* (Williams et al., 1997). DTS has been demonstrated to mediate the hyper-phosphorylation of growth factor induced MAPKinases (ERK 1 and ERK 2) phosphorylation, a crucial process for the improvement of long-term memory, and neuronal growth (Williams et al., 2007, 2009). In addition, DTS was found to be the major metabolite in *P. alliacea* (Lowe et al., 2015), being also isolated from its leaves (Williams et al., 1997). Therefore, the mnemonic activity elicited by PaLHE could be the result of DTS acting in the MAP-kinase pathway, mediating the improvement in long-term memory.

In regards to spatial memory, our data demonstrated that PaLHE enhanced spatial memory in rats. Previous studies have reported that hippocampal place cells are the main substrate involved with the spatial memory abilities assessed through the MWM test. Also, there is evidence indicating the importance of the hippocampus for both acquisition and retrieval of spatial information, as well as for consolidation/storage (D'Hooge and De Deyn, 2001; Poucet et al., 2000). The TLC analysis of PaLHE reviewed the presence of thiosulfinates and other polysulfides, organosulfur compounds with high liposolubility (Kubec and Musah, 2001; Musah et al., 2009). In light of this, PaLHE's constituents could be crossing the hematoencephalic barrier, reaching and acting on the hippocampus to induce its effects over spatial memory. Therefore, this is the first study that links PaLHE with such mnemonic effect.

Our results provide new evidence that PaLHE promotes learning improvement and reinforces mnemonic activities in rats, which confirms one of its ethnopharmacologic use (i.e. poor memory). It is important highlights that the behavioral effects are more proeminent at higher doses of the *P. alliacea* extracts, that is correlated to the popular use (Ferraz et al., 1991). The exact mechanisms involved in the observed effects remain unclear, and more researche works are needed to elucidate the chemical compounds responsible for the pharmacological responses reported on this study.

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V CONCLUSÃO

5. CONCLUSÃO

De acordo com nossos resultados, o extrato hidroalcóolico de suas folhas reforça a aprendizagem, MCD, MLD e memória espacial em ratos. A metodologia empregada em nosso trabalho para a pesquisa de compostos de enxofre foi capaz de detectá-los no extrato utilizado. Tais achados indicam que a planta oriunda da região amazônica também produz estes metabólitos, que juntamente com os flavonoides e derivados são as classes com maior número de substâncias isoladas na *P. alliacea*.

Quanto aos demais efeitos centrais, a espécie parece possuir propriedades antiepiléticas, antinociceptiva, antidepressiva, depressora, ansiolítica e ansiogênica. Embora controversas, estas atividades podem ser explicadas pela variação química que há entre as partes da planta. Além disso, diferentes condições climáticas, tipo de solo e períodos distintos de coleta também podem influenciar a composição química, e, consequentemente, modificar suas atividades biológicas.

Destaca-se que, pouco se sabe sobre os possíveis mecanismos pelos quais a planta exerce suas atividades, o que indica a necessidade de dar prosseguimentos as pesquisas, para plena compreensão de seu potencial farmacológico.

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ANEXO A – Parecer do CEPAN-IEC

MS - SECRETARIA DE VIGILÂNCIA EM SAÚDE
INSTITUTO EVANDRO CHAGAS

Parecer de Aprovação Nº 056/2009/**CEPAN**/IEC/SVS/MS
Registro CEPAN - Nº 0050/2009

Ananindeua/PA, 11 de dezembro de 2009.

1. Projeto: **"Efeitos de espécies amazônicas sobre o sistema nervoso central: análises comportamentais, fitoquímicas e antioxidantes".**

Pesquisador Responsável: MARCIENE ATAIDE DE ANDRADE

Conforme decisão do Comitê de Ética em Pesquisa com Animais-CEPAN do Instituto Evandro Chagas, cientificamos que o projeto acima **foi aprovado.**

Recomendamos ao coordenador responsável que mantenha atualizados todos os documentos pertinentes ao projeto.

Os relatórios parciais deverão ser encaminhados a este Comitê, anualmente, a partir do início do projeto.

Atenciosamente,

NELSON ANTONIO BAILÃO RIBEIRO
Coordenador do CEPAN/IEC

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