ISSN : 0973-7847

PubMed

Vol 12 / Issue 23 / January-June 2018

Pharmacognosy Reviews

Official Publication of Phcog.Net

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REVIEW ARTICLE

Antimicrobial and Anticancer Potential of *Petiveria alliacea* L. (Herb to "Tame the Master"): A Review

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ABSTRACT

Petiveria alliacea is a perennial Amazonian shrub used in traditional medicine for many purposes worldwide, including as antirheumatic, antispasmodic, antifungal, and analgesic for pain relief. Herein, this review aimed to provide a concise overview of the phytochemistry and antimicrobial, anticancer, and immunomodulatory properties reported in the literature for *P. alliacea*. The herb is rich in sulfur-containing compounds that possess a broad-spectrum of *in vitro* antimicrobial activity against pathogenic fungi and bacteria at low concentrations. *P. alliacea* also showed cytotoxicity and antiproliferative activity against cancer cell lines through sophisticated machinery of cellular damage *in vitro*. Other compounds such as flavonoids, terpenoids, and benzenoids are commonly identified in *P. alliacea* extracts, and they may also justify these activities. Despite the great pharmacological potential, clinical trials are required to ensure its effectiveness and safety. This review may raise new trends on the studies as well as contribute to the community by offering data for decision-making with regard to its use in treating diseases.

Key words: Anticancer activity, antimicrobial activity, cytotoxicity, *Petiveria alliacea,* sulphur-containing compounds

INTRODUCTION

Petiveria alliacea L. (*Phytolaccaceae*) is a wild and perennial shrub that grows throughout tropical areas in South and Central America, Caribbean, and Africa.[1] The herb was first identified by Carl Von Linnaeus and published in his book *Species Plantarum*.[2] The genus name of this herb is derived from Jacob Petiver, who dedicated his work to medicinal plant study, while the epithet is related to the pungent smell of garlic released after tissue disruption.[3]

In the 17th century, African slaves used to use preparations obtained from *P. alliacea* to make their masters lethargic, and for this reason, *P. alliacea* is widely known in Brazil as the herb to "tame the master."[1,3,4] The plant is also called mucuracaá, tipi, guiné, pipi, apacin, herbe aux Poules, anamu, and embayayendo.[5] Nowadays, herbal medicines derived from *P. alliacea* are available on the market in Paraguay, Cuba, and Japan.[4,6]

P. alliacea has been used in traditional medicine with different purposes in many countries, such as antirheumatic, analgesic, and to treat respiratory conditions.[7-11] Pharmacological investigations have highlighted the therapeutic potential of *P. alliacea* as an immunomodulator, analgesic, antimicrobial, and anticancer.[12-18] A large number of papers of *P. alliacea* published in the last decades has motivated our group to gather these data to allow a concise overview for the scientific community. Hence, this paper provides a critical review on phytochemistry and pharmacology of *P. alliacea*, regarding its antimicrobial, anticancer and immunomodulatory activities.

BOTANICAL CONSIDERATIONS

P. alliacea is a perennial subshrub, sub Woody, erect, and branched with long branches, which are delicate and ascending, measuring up to 1 m in height. The leaves are 5–10 cm long and 2–6 cm in width, discolor, oblong-lanceolate, acuminate, with a cuneiform base and short petioles, its texture ranges from membranaceous to herbaceous, with prominent midrib in the abaxial face and secondary arqued veins.[19]

P. alliacea roots are pivoting type and may reach 30 cm in length and 1 cm in diameter in the base; it has a yellowish–brown surface, tortuous, pale externally, and bright whiteness internally, with an acre flavor and a garlic-like odor.[20]

The botanical synonymies are *P. alliacea* var. *grandiflora* Moq., *P. alliacea* var. *octandra* (L.) Moq., *Petiveria corrientina* Rojas, *Petiveria foetida* Salisb., *Petiveria hexandria* Sessé and Moc., *Petiveria ochroleuca* Moq., *Petiveria octandra* L., *Petiveria paraguayensis* D. Parodi.[21]

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Access this article online

Quick Response Code:

Website: www.phcogrev.com

DOI: 10.4103/phrev.phrev_50_17

Cite this article as: Silva JP, do Nascimento SC, Okabe DH, Pinto AC, de Oliveira FR, da Paixão TP, *et al*. Antimicrobial and anticancer potential of *Petiveria alliacea* L. (Herb to "tame the master"): A review. Phcog Rev 2018;12:85-93.

CHEMICAL CONSTITUENTS

Polysulfides

Polysulfides, a class of organosulfur compounds with a wide range of biological activities, represent the major constituents isolated from *P. alliacea* [Figure 1]. The first biologically active sulfur-containing compound isolated from *P. alliacea* was benzyl-2-hydroxyethyl trisulfide (3).[22] Afterward, bioassay-guided fractionation from *P. alliacea* roots yielded benzyl-2-hydroxyethyl disulfide (2).[23] Dibenzyl trisulfide (DTS, 6) have been often isolated from various preparations obtained from *P. alliacea*, mainly from the roots.[18,23-27] DTS possess a large number of biological activities reported, such as anticancer and antimicrobial.[28] In addition, dibenzyl disulfide (DBDS, 5) and other polysulfides, including benzyl hydroxymethyl sulfide (1), dibenzyl sulfide (4), dibenzyl tetrasulfide (7), di (benzyltrithio) methane (8), and dypropyl disulfide (9), were also commonly identified in extractive preparations obtained from the species.[12,17,24,27,29]

Flavonoids

Flavonoids and flavonoids derivatives were found in an ethanol extract from aerial parts of *P. alliacea* [Figure 1], namely 6-formyl-8-methyl-7-*O*-methylpinocembrin (leridal, 10), 6-hydroxymethyl-8-methyl-7-*O*-methylpinocembrin (leridol, 11), 6-hydroxymethyl-8-methyl-5,7-di-*O*-methylpinocembrin (5-*O*-methylleridol, 12), in addition to 3-*O*-rhamnosides of dihydrokampferol (engeletin, 13), dihydroquercetin (alstibin, 14) and myricetin (15).[30,31] Furthermore, the fractionation of the ethanol extract led to the isolation of 7-demethylleridal (16), leridal-chalcone (17), petiveral (18) and 4-ethylpetiveral (19).[31]

Terpenoids

P. alliacea presents mainly mono-and triterpenoids in its composition [Figure 2]. The monoterpenes borneol (20), carvacrol (21), cumin alcohol (22), geraniol (23), geranyl acetate (24), palustrol (25) have been identified in essential oils of root, stems, leaves, and flowers.[32-34]

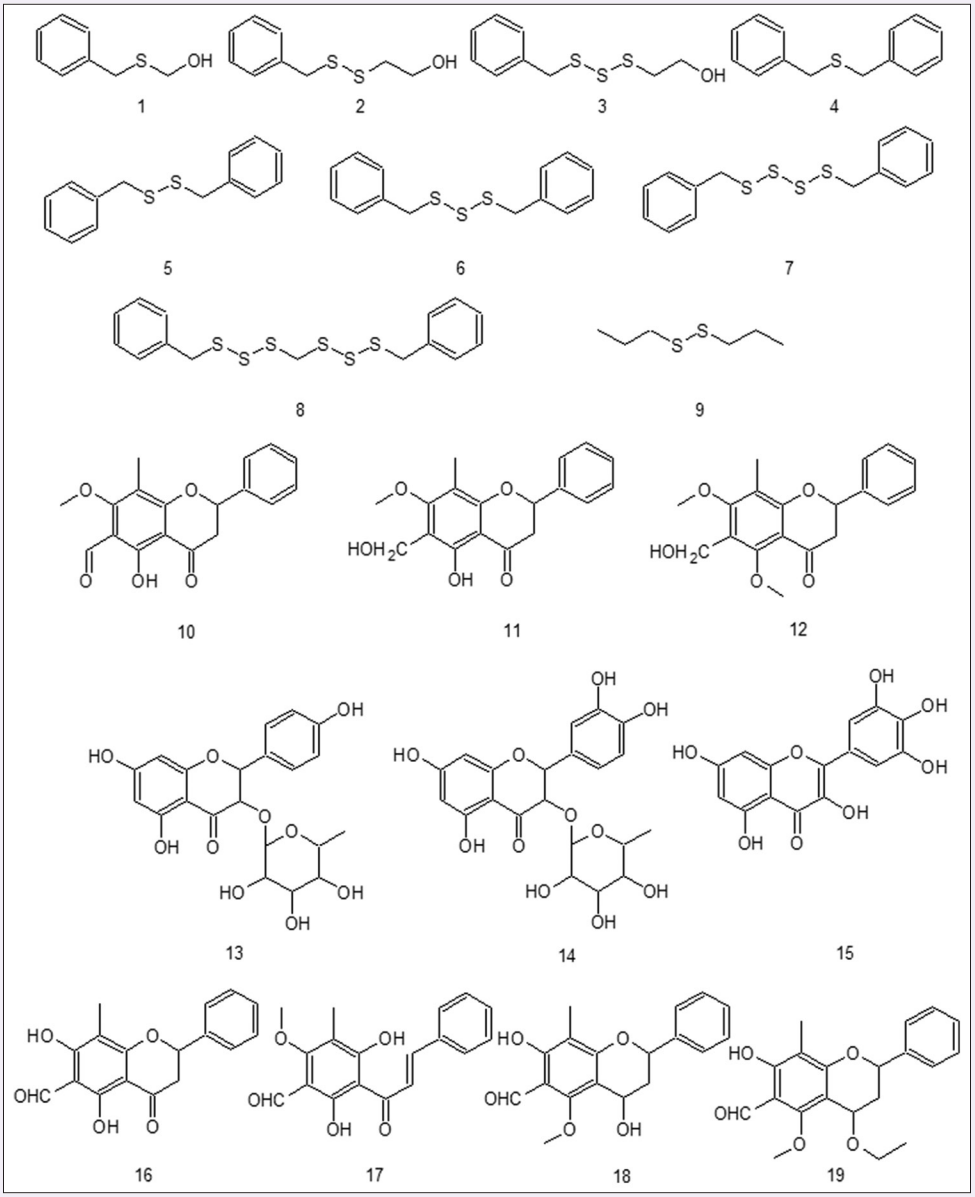

Figure 1: Chemical structures of polysulfides and flavonoids obtained from *Petiveria alliacea*

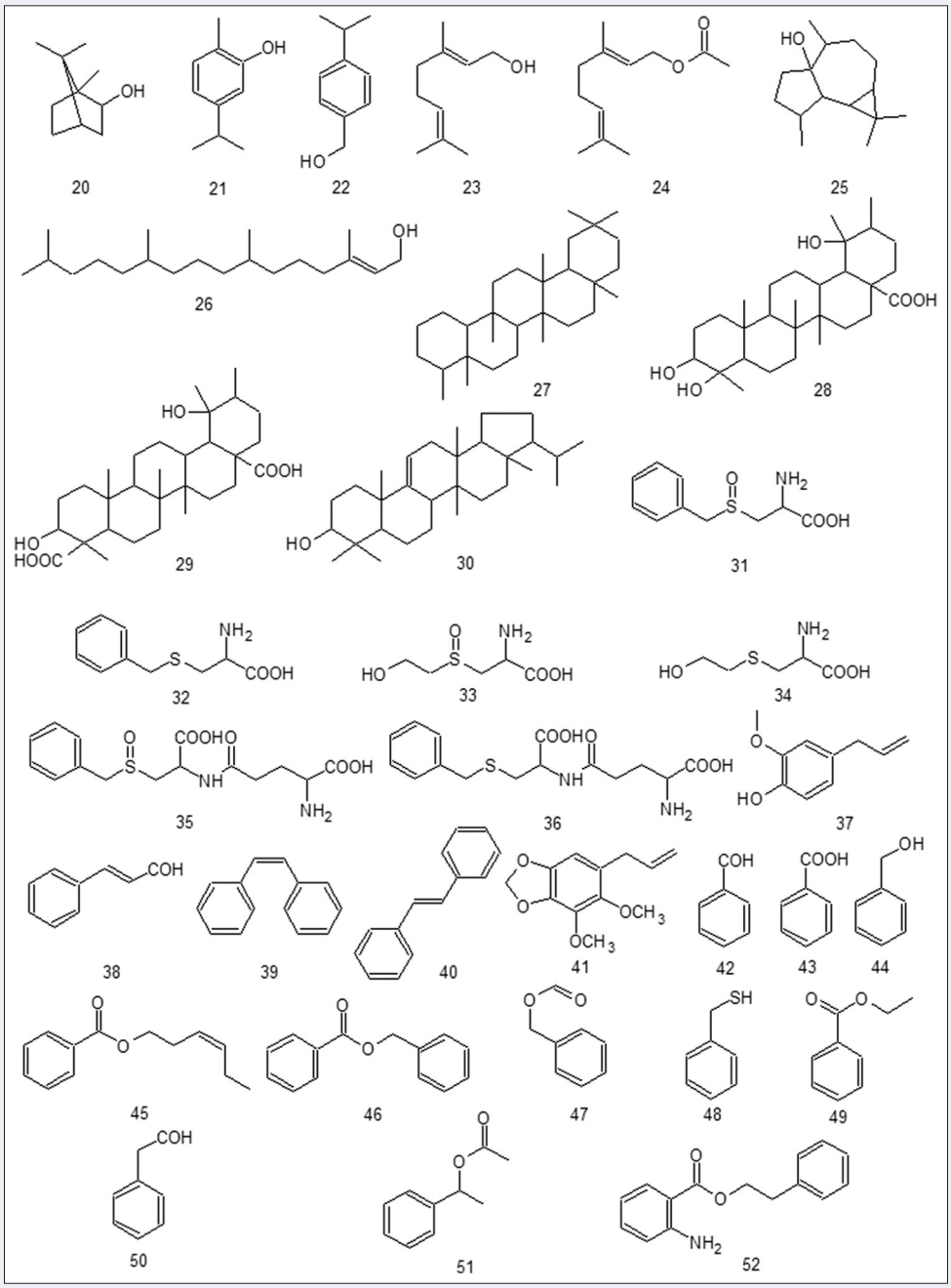

Figure 2: Chemical structures of terpenoids, amino acids derivatives, and volatile compounds found in *Petiveria alliacea*

The diterpene phytol (26) has been identified in hydroalcoholic extracts from leaves and roots.[23,32] Regarding the triterpenes, α-friedelinol (27) was the first isolated of a petroleum ether extract.[28] Barbinervic acid (28) and 3-epiilexgenin A (29) were isolated from an ethanol extract of *P. alliacea*. Segelman and Segelman[35] have identified isoarborinol (30) and their derivatives in the leaves of the species.

Amino acids derivatives

Two diastereomers of *S*-benzyl-*L*-cysteine sulfoxide, petiveriin A ((R_CR_S)-*S*-benzyl-l-cysteine sulfoxide and petiveriin B ((R_CS_S)-*S*-benzyl-l-cysteine sulfoxide (31), were isolated of an amino acid fraction obtained from fresh roots, in addition to *S*-benzylcysteine (32).[36] Gas chromatography analysis (GC) revealed the presence of cysteine derivatives, namely 6-hydroxyethiin A and B (33), and *S*-(2-hydroxyethyl) cysteine (34).[37] The authors also identified in trace amounts *S*-methyl-, *S*-ethyl-, *S*-*n*-propyl- and *S*-(2-hydroxyethyl) cysteine (structures not shown). In addition, γ-glutamyl-petiveriins A and B (35), and ($S_{C2}R_{C7}$)-γ-glutamyl-*S*-benzylcysteine (36) were isolated from roots of *P. alliacea*.[38] The chemical structures of amino acids derivatives obtained from *P. alliacea* are shown in Figure 2.

Essential oils

The chemical composition of essential oils obtained from different parts of *P. alliacea* and analyzed by GC revealed the presence of some compounds as phenylpropanoids, terpenoids, and numerous benzenoids [Figure 2]. Five phenylpropanoid have been reported for *P. alliacea* essential oils,

including eugenol (37), cinnamaldehyde (38), cis-and trans-stilbenes (39 and 40, respectively), and dillapiole (41).[25,32-34]

Analysis of essential oils of *P. alliacea* revealed that the benzenoids, including benzaldehyde (42), benzyl alcohol (44), and (*Z*)-3-hexeny benzoate (45), were the predominant components in the root and flower oils.[25,32-34] Others benzenoids identified on essential oils of the species include benzoic acid (43), benzyl benzoate (46), benzyl formate (47), benzyl thiol (48), ethyl benzoate (49), phenylacetaldehyde (50), 1-phenylethyl acetate (51), and 1-phenylethyl anthranilate (52). On the other hand, carvacrol (18) constituted the major compound in the stem and leave essential oils.[33]

Thiosulfinates

Thiosulfinates are molecules synthesized through the oxidation of cysteinyl disulfides and play an important role in redox chemistry of proteins.[39] An homogenate of *P. alliacea* roots afforded various thiosulfinates, namely *S*-(2-hydroxyethyl) 2-(hydroxyethane) thiosulfinate (53), *S*-(2-hydroxyethyl) phenylmethanethiosulfinate (54), *S*-benzyl 2-(hydroxyethane) thiosulfinate (55), and *S*-benzyl phenylmethanethiosulfinate (petivericin, 56) [Figure 3].[37] The rupture of tissues of *P. alliacea* causes the release of strong garlic-like odor and lacrimation due to the irritation of nasal and ocular mucous membranes. The responsible compound for this effect was identified from the roots as (*Z*)-thiobenzaldehyde *S*-oxide (57).[40] Kubec and Musah[36] have observed that many organosulfur compounds commonly identified in *P. alliacea* extracts are degradation products of thiosulfinates by enzymatic activity after tissue disruption.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

Disc-diffusion test, agar-plate, and broth microdilution assays were performed with crude extracts and isolated compounds from *P. alliacea* for testing antimicrobial properties, which revealed promising activity against some bacteria and fungi. Tables 1 and 2 summarize the main data about antimicrobial activity of extracts and compounds isolated from *P. alliacea*, respectively.

Guedes *et al.*[13] evaluated the antimicrobial activity by broth microdilution method of different extracts obtained from *P. alliacea* leaves. In this regard, the hexane extract was more active to inhibit *Staphylococcus aureus* than the polar extract (ethanol 70%), with minimum inhibitory concentrations (MICs) values of 240 μg/mL and 3960 μg/mL, respectively. On the other hand, the methanol extract presented activity against *Enterococcus faecalis* (MIC of 240 μg/mL). Regarding antifungal activity, only the hydroalcoholic extract was active against *Candida parapsilosis* (MIC of 250 μg/mL), *Candida kefyr* and *Candida albicans* (MIC of 760 μg/mL).[13] Illnait-Zaragozí *et al.*[41] also reported the antifungal activity of the hydroalcoholic extract of leaves from *P. alliacea* against several clinical isolates and American Type Culture Collection of *Candida* species with MIC ranging between 8 μg/mL and 64 μg/mL [Table 1].

Several sulfur-containing compounds isolated of *P. alliacea* present a broad-spectrum of antimicrobial activity. The first report on antimicrobial activity of an organosulfur compound isolated from *P. alliacea* is from Szczepanski *et al.*[22] The authors reported the isolation of benzyl-2-hydroxyethyl trisulfide (3), which was active against *S. aureus*, *Mycobacterium tuberculosis*, *C. albicans*, *Trichophyton mentagrophytes*, *Aspergillus niger*, *Penicillium chrysogenum* and *Microsporum gypseum* (MICs values ranged between 0.8 μg/mL and 25 μg/mL).[22]

Bioassay-guided fractionation of an organic extract of the roots afforded the isolation of antifungal polysulfides.[24] Bioautography with *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* yielded dipropyl disulfide (9), dibenzyl sulfide (4), dibenzyl disulfide (5) and dibenzyl trisulfide (6), which posses potent antifungal activity against with inhibition at concentrations between 0.1 μg/mL and 1.0 μg/mL, similar to the positive control Nystatin (1.0 μg/mL).

The lachrymatory principle of *P. alliacea*, (*Z*)-thiobenzaldehyde *S*-oxide possess antimicrobial activity against *C. albicans*, *Klebsiella pneumoniae*, *Escherichia coli*, *S. aureus* and *Streptococcus agalactiae* in a disk diffusion assay.[40] Kim *et al.* (2006)[42] also evaluated the antimicrobial activity of different polysulfides isolated from *P. alliacea*. In this sense, thiosulfinates (54–56) and their degradation products inhibited at low concentrations (MICs values ≤64 μg/mL) the bacteria *Bacillus cereus*, *Mycobacterium smegmatis*, *Micrococcus luteus*, *S. agalactiae*, *S. aureus*, *E. coli*, *Stenotrophomonas maltophila*, *K. pneumonie*, and the fungi *Aspergillus flavus*, *Mucor racemosus*, *Pseudallescheria boydii*, *C. albicans*, *C. tropicalis*, and *Issatchenkia orientalis* [Table 2].

CYTOTOXICITY AND ANTIPROLIFERATIVE ACTIVITY AGAINST CANCER CELL LINES

The anticancer properties of *P. alliacea* were revealed after the Managua story, which occurred in Nicaragua in 1960. It was observed that leukemic cows left to pasture in fields in Nicaragua were healed after

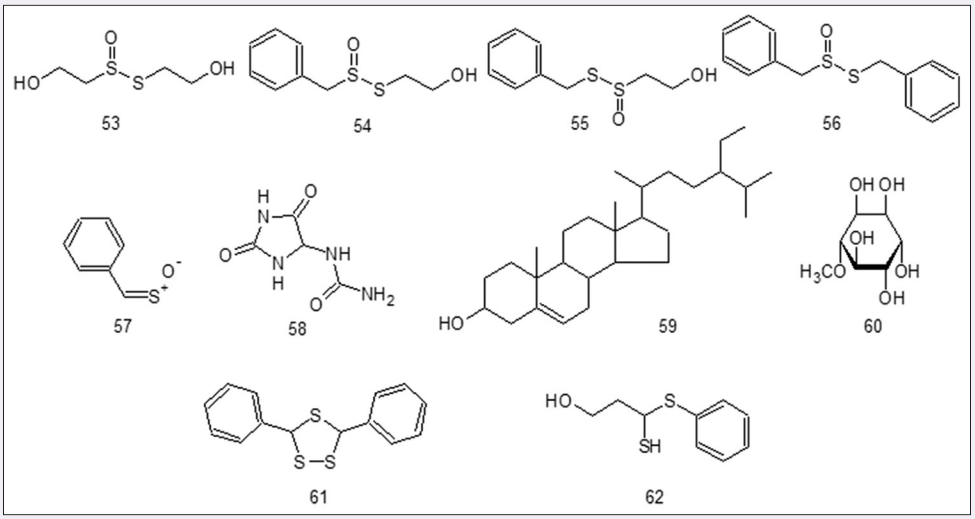

Figure 3: Chemical structures of terpenoids, amino acids derivatives, and volatile compounds found in *Petiveria alliacea*

Table 1: Antimicrobial activities of *Peltaria alliacea* extracts against pathogenic microorganisms

Group	Species	Extracts				Controls		
		n-Hexane[13]	MetOH[13]	EtOH 70%[13]	EtOH 70%[41]	Ampicillin[13]	Fluconazole[13]	Fluconazole[41]
Gram + bacteria	*Staphylococcus aureus*	240	NI	3960	-	0.976	-	-
	Staphylococcus epidermidis	NI	NI	3960	-	0.977	-	-
	Streptococcus mutans	NI	NI	3960	-	0.978	-	-
	Enterococcus faecalis	NI	240	3960	-	0.979	-	-
	Bacillus subtilis	NI	NI	3960	-	0.980	-	-
Gram -bacteria	*Escherichia coli*	NI	NI	3960	-	0.981	-	-
	Pseudomonas aeruginosa	NI	NI	3960	-	0.982	-	-
Yeasts	*Candida parapsilosis*	NI	NI	250	16	-	2	1
	Candida kefyr	NT	NT	760	16	-	16	0.75
	Candida albicans	NT	NT	760	16	-	0.125	8
	Candida tropicalis	NT	NT	-	16	-	-	1
	Candida glabrata	NT	NT	-	16	-	-	16
	Issatchenkia orientalis	NT	NT	-	32	-	-	16
	Candida lusitaniae	NT	NT	-	8	-	-	0.5
	Candida guilliermondii	NT	NT	-	16	-	-	1
	Candida inconspicua	NT	NT	-	64	-	-	16
	Candida ciferri	NT	NT	-	32	-	-	1

The results are expressed as Minimum Inhibitory Concentrations (MIC) values in μg/mL. NI=No inhibition observed, NT=Not tested

consumption of *P. alliacea*.[4] Thus, researchers have performed several biological assays using some tumor cell lines, in the attempt to find the bioactive compounds present in the extracts, as well as their mechanisms of action.

Rossi *et al*.[43] and Jovicevic *et al*.[44] reported *in vitro* antiproliferative activity of ethanol and aqueous extracts of *P. alliacea* leaves on cell lines IM9, DAUDI, Molt4, K562, and MCF7. According to them, aqueous preparations were more active than alcoholic extracts evaluated by colorimetric assay using 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide, which was confirmed by measuring the incorporation of tritiated thymidine. The decoction (1, 10, and 100 μg/mL) inhibited the proliferation by 80%–90% to IM9 in a dose-dependent way, and 50%–60% to MOLT4 and DALDI, and about 20% to K562.

A methanol extract of *P. alliacea* leaves at a concentration of 125 μg/mL inhibited the growth of murine skin (B16), human leukemia (HL-60), breast (MCF-7), and colon (HCT-8) cell lines at 106.1%, 78.4%, 72.4%, and 94%, respectively.[45] Doxorubicin was used as positive control and inhibited the growth of all tumor cell lines evaluated. On the other hand, Ruffa *et al*.[46] observed that the methanol extract of *P. alliacea* leaves was not active against HepG2 cell line in all concentrations tested (15.5–1000 μg/mL).

A bioactive fraction (F4) obtained from the ethanol extract of leaves and stems inhibited the proliferation of A375, Mel Rel, and K562 cell lines at concentrations of 35.2, 36.3, and 32.0 μg/mL, respectively (concentrations ranged between 1.8 μg/ml and 125 μg/ml).[17] Normal fibroblasts and human mononuclear cells without phytohaemagglutinin exhibited IC_{50} of 440 μg/mL and 121 μg/mL, respectively. Cifuentes *et al*.[12] and Hernández *et al*.[47] have revealed cytotoxic activity of ethyl acetate fractions obtained from leaves and stems against K562, NB4, and 4T1 (breast adenocarcinoma cell line) cell lines at a concentration of approximately 50 and 29.3 μg/mL, respectivelly [Table 2].

Bioassay-guided fractionation of the petroleum ether extract of *P. alliacea* roots led to the isolation of biologically active compounds against HL-60 cell line.[23] The petroleum ether extract exhibited effect on HL-60 cell differentiation, with EC_{50} = 3.6 μg/mL (positive control: All-trans-retinoic acid, EC_{50} = 0.1 μg/mL). The fractionation of these extract led to the isolation of the active compounds DTS (6) and 2-[(phenylmethyl) dithio]-ethanol (2) that caused HL-60 cell differentiation at concentrations of 3 and 0.3 μg/mL, respectively. Several studies have reported the antiproliferative and cytotoxic activity of DTS and related compounds obtained by chemical optimization (e.g., bis (*p*-fluorobenzyl) trisulfide) on various tumor cells, and efforts had been made to elucidate their mechanism of action in cancer.[12,14,48-52]

Mechanism of action

In addition to cytotoxic and antiproliferative investigations, some authors also evaluated the possible mechanism for these activities in cell lines. Urueña *et al*.[17] using flow cytometry showed that melanoma tumor cells treated with a *P. alliacea* fraction (concentrations ranged between 7.8 μg/mL and 31.2 μg/mL) induced G2 phase arrest. The fraction disturbed the actin cytoskeleton organization after 24 h of incubation, induced apoptosis in a mitochondria-independent way, and reduced the tumor cell clonogenic survival after 14 days when compared to controls treatment (positive controls: etoposide and vincristine; negative control: ethanol 0.2%). Proteomic techniques were performed to characterize the "protein expression signature" over these tumor cell lines, and they were analyzed through differential protein expression by HPLC-Chip/MS analysis.[17] The proteomic analysis results revealed down-regulation of cytoskeleton proteins, which is related to cytoskeleton disruption and according to the authors, changes in the concentration of some proteins that are involved in translation and transduction processes could explain the decrease of melanoma tumor cells clonogenicity.

DTS, a polysulfide often identified in *P. alliacea*, led to a reversible disassembly of microtubules through the decrease of total expression of tubulin (0.1 μM) and caused a decrease in phosphorylation of erk1/erk2 protein kinases (0.5 μM) in SH-SY5Y cell line.[14] Cytoskeleton proteins, such as tubulin and actin, play important functions in normal and tumoral cell physiology and represent significant targets of emergent anticancer agents.[53,54] Thus, compounds that act in cytoskeleton targets play an important role in cancer therapy, affecting directly proteins that regulate several cellular processes linked to transformation, as cell proliferation and apoptosis.

Apoptosis is an organized cellular event that occurs in normal and pathological conditions. The programmed cell death through apoptosis

Table 2: Antimicrobial activities of sulfur-containing compounds commonly found in *Petiveria alliacea* extracts against pathogenic microorganisms

Group	Species	MIC values of sulfur-containing compounds																	Controls		
		1[42]	2[22]	2[42]	3[33]	4[24,42]	5[24,42]	6[24,42]	7[24]	31[42]	53[42]	54[42]	55[42]	56[42]	57[42]	$C_4H_{10}O_2S_2$[42]	$C_4H_{10}O_2S_3$[42]	$C_7H_8O_2S$[42]	Tetracycline[42]	Amphotericin B[42]	Nystatine[24]
Gram + bacteria	*Staphylococcus aureus*	NI	6.3	NI	256	NI	NI	NI	NT	NI	NI	32	64	64	512	NI	128	64	2	-	-
	Streptococcus agalactiae	NI	NI	NI	256	NI	NI	NI	NT	NI	NI	16	32	16	512	NI	NI	64	1	-	-
	Bacillus cereus	NI	NI	NI	128	NI	NI	NI	NT	NI	128	16	16	16	512	NI	32	128	0.25	-	-
	Micrococcus luteus	NI	NI	NI	64	NI	NI	NI	NT	NI	128	16	16	16	512	NI	128	32	2	-	-
Gram -bacteria	*Stenotrophomonas maltophilia*	NI	NI	NI	NI	NI	NI	NI	NT	NI	128	NI	32	32	NI	NI	128	NI	4	-	-
	Klebsiella pneumoniae	NI	NI	NI	NI	NI	NI	NI	NT	NI	256	NI	128	128	NI	NI	256	NI	1	-	-
	Escherichia coli	NI	50	NI	NI	NI	NI	NI	NT	NI	128	NI	32	32	NI	NI	256	NI	2	-	-
Acid-fast bacteria	*Mycobacterium tuberculosis*	NT	12.5	NT	NT	NT	NT	NT	NT	NI	NT	NT	NT	NT	NT	NT	NT	NI	-	-	-
	Mycobacterium smegmatis	NI	NI	NA	128	NI	NI	NI	NT	NI	128	256	128	128	512	NI	64	128	-	-	-
Yeasts	*Candida albicans*	>512	3.1	128	16	128	256	128	NT	256	>512	16	16	16	128	>512	8	2	-	<1	-
	Candida tropicalis	>512	NI	128	32	128	256	256	NT	256	>512	16	32	32	64	>512	8	4	-	<1	-
	Idastrandia orientalis	>512	NI	128	32	128	256	128	NT	256	>512	16	16	32	64	>512	16	2	-	<1	-
Filamentous fungi	*Trichophyton interdigitale*	NT	0.8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	-
	Pseudallescheria boydii	512	NI	256	32	64	512	64	NT	256	>512	16	32	32	64	32	16	16	-	4	-
	Aspergillus flavus	>512	NI	128	128	256	128	256	NT	256	>512	32	64	32	128	>512	>512	>512	-	4	-
	Aspergillus niger	NT	6.3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	-
	Penicillium chrysogenum	NT	25	NI	NI	NI	NI	NI	NT	NI	NT	NI	NI	NI	NI	NI	NI	NI	-	-	-
	Mucor racemosus	512	NI	256	64	256	256	64	NT	128	512	16	32	32	128	>512	>512	64	-	8	-
	Microsporum gypseum	NI	3.1	NI	NI	NI	NI	NI	NT	NI	NI	NI	NI	NI	NI	NI	NI	NI	-	-	-
	Cladosporium sphaerospermum	NT	NT	NT	NT	1*	0.1*	1*	10*	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	1*
	Cladosporium cladosporioides	NT	NT	NT	NT	1*	1*	1*	10*	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-	1*

The results are expressed as Minimum Inhibitory Concentrations (MIC) values in µg/mL. NI=No inhibition observed, NT=Not tested. *In this study MIC values are expressed in µg. $C_4H_{10}O_2S_2$=bis (2-hydroxyethyl) disulfide, $C_4H_{10}O_2S_3$=bis (2-hydroxyethyl) trisulfide, $C_7H_8O_2S$=benzylsulfinic acid

is also pivotal in cancer treatment and is considered a common target of cancer therapeutic strategies.[55] Apoptosis via intrinsic pathway represents an important target to cancer treatment and discovery of novel apoptotic inducers is one of the current concerns in anticancer research. Regarding apoptosis, Cifuentes *et al*.[12] revealed that fractions obtained from ethyl acetate extract of *P. alliacea* leaves and stems induced mitochondrial membrane depolarization after 8 h of treatment in different tumor cell lines at a concentration of 31.2 μg/ml using as positive and negative controls, P2Et (precipitate of ethyl acetate fraction obtained from ethanol extract of *Caesalpinia spinosa*) and ethanol, respectively. They also investigated the effects of S2 and S3 at concentration of 6.2 μg/ml on the modulation of heat shock protein 70 (Hsp70). Western blotting analysis of K562 cells treated for 10 h and exposed to thermal stress revealed that S3 fraction decreased the Hsp70 protein expression as compared to positive control (quercetin at a concentration of 100 μM). In normal cells, Hsp proteins protect them from environmental stress damage, and in cancer cells, they promote cell proliferation, as well as inhibit cellular death pathways.[56] Thus, the study of the regulation of Hsp proteins represents an interesting target in the evaluation of medicinal plants with potential anticancer activities.

On the other hand, Hernández *et al*.[47] revealed that different concentrations (7.3, 14.6, and 29.3 μg/mL) of an ethyl acetate fraction induce apoptosis of 4T1 cells, but it not affects mitochondrial membrane depolarization. The fraction also induces the activation of caspase-3 and DNA fragmentation on 4T1 cells. According to the authors, the cytotoxicity activity may be explained by the activation of the glycolytic pathway enzymes.

Santander *et al*.[57] revealed that an ethyl acetate fraction (FAST 8) obtained from *P. alliacea* induced changes in gene expression profile (21 genes were affected) of K562 cells treated for 24 h at a concentration of 25 μg/mL as compared to negative control (ethanol 0.2%). Nevertheless, the authors suggested that the identification of modulated genes treated with *P. alliacea* could provide new targets in cancer therapy.

IMMUNOMODULATORY ACTIVITY

P. alliacea crude extracts and their isolated constituents present good immunomodulating properties through cell mediation and modulation of different cytokines release. Currently, several herbal preparation containing *P. alliacea* are available in the market to boost or support immunity.[6]

The first study about the effects of *P. alliacea* on the immune system was carried out by Delaveau *et al*.[58] The authors investigated the protective effect of the ethanol extract of *P. alliacea* roots and its unsaponifiable fraction on phagocytic activity of the reticuloendothelial system in male Swiss mice infected with a lethal dose of *E. coli* O111:B4. Both extract and fraction at a final concentration of 50 mg/kg (i.p.) showed protective effects due to the enhancement of the reticuloendothelial system that stimulates *E. coli* O111:B4 phagocytosis.

The administration of DTS to mice (11.0 mg/kg/day, i.p.) twice weekly for 3 weeks significantly increased the thymus weight (40.5 ± 2.6 mg, $P < 0.05$), the number of Peyer's patches (9.2 ± 1.3 mg), and the differential cell count value (7.65 ± 0.98) when comparing with control group (19.2 ± 1.5 mg, 5.0 ± 0.9 mg, 2.85 ± 0.73).[18] Lopes-Martins *et al*.[59] related that oral administration of a root hydroalcoholic extract (31.4 mg/kg body wt. and 43.9 mg/kg body wt.) decreased the number of migrating neutrophils, mononuclear cells and eosinophils, evaluated in an animal model of carrageenan-induced pleurisy.

Marini *et al*.[60] reported that a plant decoction of whole plant increased the production of cytokines interleukin-2 (IL-2) and IL-4 (IL-2: 4 IU/mL, IL-4: 4 IU/mL), and interferon (IFN) (30 IU/mL) in murine splenocytes cultures as compared with control group (IL-2: 0 IU/mL, IL-4: 0 IU/mL, and IFN: <5 IU/mL). In addition, the aqueous extract increased at 100% the activity of NK cells after 24 h of treatment, which could be explained by the IFN production. *P. alliacea* decoction also (concentrations ranged between 0.001 μg/mL and 1000 μg/mL) induced cell proliferation in splenocyte cultures from 10 months old mice (>5000 CPM from 10, 100 and 1000 μg/mL; $P < 0.001$) as compared to Con-A control after 48 h of exposure (1458 ± 187 CPM).[61] There was also an increase of IL-2 receptor expression in stimulated mice splenocytes cultures after the treatment with *P. alliacea* decoction.

A hydroalcoholic extract of *P. alliacea* roots showed a protective effect against *Listeria monocytogenes* infection.[62,63] Pretreatment of Balb/cj mice with 1000 mg/kg/day (p.o.) of the extract, for 5 days prior the infection, increased the percentage of survivors in 30% ($P < 0.05$) and the number of granulocyte/macrophage colonies (CFU-GM) from 47.60 ± 10.3 in nontreated group to 144.00 ± 11.2 ($P < 0.01$).[62] The extract also increased the production of cytokines IL-2 and IFN-γ after mice infection and enhanced natural killer cell activity, when compared to control groups not treated.[63] Th2 cytokines IL-4 and IL-10 were the same as the noninfected and nontreated groups.

Santander *et al*.[15] evaluated the immunomodulatory activity of the ethyl acetate soluble and aqueous fractions of leaves and stems from *P. alliacea* using human monocyte-derived dendritic cells (DCs) stimulated with lipopolysaccharide (1 μg/mL). The fractions (6–63 μg/mL) induced morphological changes and co-stimulatory expression of CD86 in DC, indicating partial maturation. Moreover, pro-inflammatory cytokines IL-1β, IL-6, IL-8, IL-10, IL-12p70, and tumor necrosis factor-α were secreted, while nuclear factor- kappa B gene expression was upregulated and transforming growth factor β gene expression decreased.

Batista-Duharte *et al*.[64] reported that oral administration of leaves and stems powder of *P. alliacea* for 5 days (400 or 1200 mg/kg) showed immunoprotective effects on 5-fluorouracil-induced myelosuppression in Balb/c female mice. Treatment with *P. alliacea* increased the global leukocyte count, cellularity and number of antibody-forming cells (AFCs) IgG, when compared with control groups treated with a saline solution or 5-FU only.

It appears that *P. alliacea* acts on the immune system through modulation of Th1 response (i.e., enhancing the expression of pro-inflammatory cytokines in bacterial infections). Moreover, there was also the production of anti-inflammatory cytokine IL-10, which regulates inflammatory events by suppression of pro-inflammatory cytokines and other stimulating factors.[65] The expression of IL-10 after administration of *P. alliacea* preparations may justify the anti-inflammatory activity reported by some authors[66,67] and could be related to the finalization of Th1-type responses. Immunomodulation is interesting in the prevention of several infectious diseases and can be used in cancer treatment along with the classic drugs, improving the immune status of patients.

Regarding chemical constituents, triterpenes are a class of secondary metabolites that present activities on the immune system.[68] Some authors had isolated these compounds mainly in essential oils of *P. alliacea*. Alamgir and Uddin[69] related that the compounds β-sitosterol and daucosterol, both present in the plant, exhibit immunomodulatory activity.

CONCLUSIONS

Medicinal plants and their derivatives represent an emerging potential source of discovery of new drugs to treat several disorders at present. *P. alliacea* is a cosmopolitan plant that provides easy access to consumption by population. Till date, data have revealed that *P. alliacea* preparations present in its constitution many biologically

active compounds. In this sense, several sulfur-containing compounds (i.e., polysulfides and thiosulfinates) have revealed higher antimicrobial activity against pathogenic bacteria and fungi at low concentrations.

Furthermore, many investigations conducted with *P. alliacea* showed its promising potential for the treatment of cancer with a mechanism of action gathering sophisticated machinery of cellular damage, mainly by deregulation of the cytoskeleton proteins. In the immune system, the species act in the enhancement of the expression of pro-inflammatory cytokines during bacterial infections and modulating anti-inflammatory responses, with potentiates the chemotherapy treatment during cancer or infections.

Further investigations are needed to elucidate the synergistic/antagonistic mechanisms by which the compounds present in the plant extracts may interact to produce the pharmacological activities.

Acute or chronic preclinical studies have revealed that *P. alliacea* preparations did not produce toxic effects in rodent experimental models with doses ranging between 0.5 mg/kg and 10000 mg/kg.[70-73] However, data in respect of side effects in animal models is contradictory.[74] Thus, more studies are required to ensure the safety and characterize potential side effects after *P. alliacea* administration.

Regarding genotoxicity, Hoyos *et al.*[75] revealed that the plant has mutagenic agents, potentially carcinogenic and that its consumption in large quantities may represent a risk of development of health problems in its users. In fact, our group has revealed that the hydroalcoholic extract of aerial parts possess *in vitro* genotoxic effects affecting DNA. However, there is no *in vivo* genotoxic potential assayed by micronucleus assay in rodents.[76]

This review may raise new trends in the studies with *P. alliacea* as well as contribute to the scientific community by offering data for decision-making with regard to its use in diseases treatment and pharmaceutical technological products development. The species has a world biological interest due to its characteristics; however, its use must be rational to ensure its therapeutic benefits. In addition, clinical trials are required to validate the therapeutic use of preparations obtained from *P. alliacea*, to obtain safe doses, evaluate the potential side effects and treatment schedule.

Acknowledgment

We are very grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for scientific fellowships. We gratefully acknowledge the financial support of Pró-Reitoria de Pesquisa e Pós-Graduação from UFPA (PROPESP/UFPA).

Financial support and sponsorship

We are very grateful for scientific fellowships from Coordination of Superior Level Staff Improvement (CAPES).

Conflicts of interest

There are no conflicts of interest.

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