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Antimicrobial Activity of *Piper aduncum* sub sp *ossanum* essential oil

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Abstract

Plants are an important source for new antimicrobials. *Piper aduncum* L. (*Piperaceae*) is a medicinal plant use traditionally in South America and The Caribbean Basin. In Cuba, *P. aduncum* subsp. *ossanum* (C.CD.) Saralegui has ethnomedical reports as haemostatic, diuretic and, in urinary infection and skin infection. Ethanolic extract and essential oil obtained from this subspecie was tested by agar disc diffusion method against four strains of *Escherichia coli* (two uropathogenic clinical isolated), *Staphylococcus aureus* and *Candida albicans*. In all microorganisms a moderate or poor antimicrobial effect was exerted; but some activity was found against resistant sulfametoxazol or vancomicine *E. coli* strains. *S. aureus* and *C. albicans* were noted as the most sensitive microorganisms. Monoterpene camphor, present in the essential oil as main compounds could be responsible of the activity. These findings may support traditional use of this subspecie as antiseptic in Cuba.

Keywords: *Piper aduncum* subsp. *ossanum*, essential oil, camphor, camphene, antimicrobial, Cuba.

Introduction

Plant Kingdom is a good source for antimicrobials search [1]. To investigate medicinal plants different approaches concerning selection criteria, plant extraction and bioassay can be followed [2,3]. *Piperaceae* is a family of ten genera and about two thousand species, in their leaves are cells containing essential oil (e. o.). *Piper* is one of the largest genera of this family with more than 1000 species growing in tropical and subtropical regions. In Cuba are 17 species and, most of them are endemic [4]. *Piper aduncum* L. (*P. angustifolium* Lam.) is distributed in Mexico, Central America, South America and in The Antilles. It is a shrub very common in Cuba [4,5]. In Pacific Ocean Islands, where it was introduced, it is consider as an important invasive plant [6]. Latest studies on the taxonomy of *P. aduncum* in Cuba led to the conclusion that there were two specific taxa of this species: *P. aduncum* subsp. *ossanum* (C. CD.) Saralegui, and *P. aduncum* L. subsp. *aduncum*. The former is an endemic distributed in the West and Central region of the country, up to Camagüey, including Isla de la Juventud; while *P. aduncum* subsp *aduncum* is found in the East region of the island [4], which is why to study this variable plant it is important to consider its pharmacobotany, pharmacogeography, and pharmacoetimology [7].In Cuba, as in other countries, this specie has value in traditional medicine, mainly on infectious diseases of skin, urinary tract infections (UTI), leucorrhea and gastrointestinal disorders; another common uses are: haemostatic, astringent and diuretic [8-11]. Previous studies of the chemical composition of *P. aduncum* from Brazil [12-13), Costa Rica [14], Cuba [15] and, Pacific Ocean islands [16] have focused on the volatile compounds of the leaves that yield dillapiol, a compound of interest as insecticide. Essential oil of *P. aduncum* from different geographical locations in the world has been reported as antimicrobial against bacteria and fungi [12,17,18]. Non-volatile compounds like vitexin flavonoids have been reported in the Cuban subspecie *ossanum*, showing anti-inflammatory and antiulcerogenic activity [19-21]. Essential oil of *P. aduncum* subsp. *ossanum* collected in the same location than for this study, (Ciego de Avila Province, Cuba), was analyzed (GC-MS) and 83 compounds were characterized [22], resulting monoterpenes camphor (18.1%) and camphene (15.6%) as major compounds; another volatiles founded were limonene (6.6%), pinene (1%), β-caryophyllene (3.3%), germacrene D (2.0%), globulol (3.8%) and spathulenol (2.9%); dillapiol, found in *P. aduncum* collected in Pinar del Río Province [15], was not present. The aim of the present study is to determine antimicrobial activity of ethanolic extract and e.o. of Cuban infraspecific taxa *P. aduncum* subsp. *ossanum* collected in Ciego de Avila Province.

Materials and Methods

Plant material

Leaves of *P. aduncum* subsp. *ossanum* (C.CD.) Saralegui were collected in Ciego de Ávila Municipality, Ciego de Ávila Province, located at the central-eastern part of Cuba. It was authenticated by Eddy Martínez and Rafael del Risco, curators of the Herbarium of the Centro de Investigaciones del Medio Ambiente de Camagüey and the Herbarium of the Universidad Pedagógica "José Martí" respectively, where a voucher specimens were deposited. Crude drug was obtained by air-drying.

Oil distillation

From 200 g of crude drug, e.o. (0.66% v/w dry basis) was obtained by hydro-distillation in a Clevenger-type apparatus. The oil obtained was dried over anhydrous sodium sulfate and stored in a dark glass bottle at 4 C.

Extract

Plant extract was made by extracting 20 g of crude drug in 100ml of 90º ethanol for 24h, were dried and at the time of bioassay dilute in 5% dimethyl sulfoxide (DMSO).

Microorganisms used

Reference microorganisms used, listed in Table 1, include Gram positive: *Staphylococcus aureus* (ATCC 25923), Gram negative: *Escherichia coli* (ATCC 25922, J96/ATCC 700336, 185, MNF) the two later were uropathogenic clinical isolates; and the yeast: *Candida albicans* (ATCC 10231).

Antimicrobial assay

Antimicrobial activity was performed by disc diffusion method [23]. Sterile discs (6 mm diameter) punched out of Whatman No.1 filter paper was made to absorb 5µL of e.o. at 100, 50, 25 and 12.5 %, diluted in 5% DMSO and, ethanolic extract at 1mg/ml. Discs were overlaid on lawn cultures of microorganism (Mueller Hinton Agar (Oxoid)) and incubated at 37 ˚C for 24 hr to read the zone of inhibition. Sulfametoxazol (50 µg disc) and vancomycin (30 µg disc) for bacteria, and amphotericin B (25 µg disc) for yeast were used as reference controls, and 5 % DMSO as negative control, three replicates were made.

Results and discussion

Antibacterial and antiyeast activities of *P. aduncum* subsp. *ossanum* ethanolic extract and e.o. were compiled in Table 1. Antibacterial activity of tested products was moderate or poor in some strains. A concentration dependence nature of the activity was revealed in e.o., mainly in *S. aureus* and *C. albicans,* the most sensitive microorganisms. In *E. coli* uropathogenic clinical isolates was observed a lowest sensitiveness to e.o. than in reference strains, but it is noticeable that in clinical isolates sensitiveness to sulfametoxazol it is null. This could be related to resistance acquired by microbiota exposed to the continuous natural selection that occur in wild conditions and also, by impacts of artificial conditions that create a selection pressured able to generate resistant microorganisms [24].

Table 1: Antimicrobial activity of *P. aduncum* subsp *ossanum* leaves.

Microorganisms	Zone of inhibition (mm)[a]					References[b]		
	% Essential oil (v/v) (5µg/disc)				Ethanol (90º)			
	100	50	25	12.5	1mg/ml	I	II	III
Escherichia coli ATCC 25922	12	8	0	0	8	22	10	-
Escherichia coli J96 (ATCC 700336)	13	10	0	0	7	20	0	-
Escherichia coli 185[c]	10	8	0	0	8	0	10	-
*Escherichia coli*MNF[d]	9	7	0	0	7	0	8	-
Staphylococcus aureus ATCC 25923	15	11	9	8	10	25	19	-
Candida albicans ATCC 10231	13	9	8	7	8	-	-	11

[a] Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates.
[b] I: Sulfametoxazol (50 µg disc) and II: Vancomycin (30 µg disc), for bacteria and, III: Amphotericin B (25 µg disc) for yeast.
[c, d] uropathogenic clinical isolates.

Most sensitive bacteria to ethanolic extract were also *S. aureus*. This is consistent with the prone nature of Gram positive cell wall to be more susceptible to antimicrobials effects, and the more resistant outer membrane of Gram negative, a fairly effective barrier for amphipathic compounds and with a set of multidrug resistance pumps (MDRs) that extrudes amphipathic toxins across the outer membrane [25, 26].
Also in ethanolic extract was evident the e.o. pattern of activity in sulfametoxazol resistant *E. coli* uropathogenic clinical isolates.

In other hand, effect on *C. albicans* was noted in all tested concentration of e.o. and ethanolic extract, this could be related too to cell wall structure, since it is reported that yeast achieve more similarities to Gram Positive bacteria than Gram Negative bacteria [27, 28].Previous research of antimicrobial activity of *P. aduncum* leaves shows a good activity of ethil acetate, ethanol 95%, hexane and methanol fractions against *S. aureus*; while except methanol fraction, no effect was present in *C. albicans* (29). Cáceres et al in 1991, also reported no activity of ethanol 60% in *C. albicans* [30].

Effect of this particular e.o. tested could be due to some known antibacterial volatiles determined in this subspecie: camphor, borneol, caryophyllene, limonene, pinene [11,22]. To corroborate the traditional use of this plant as antiseptic, another mechanism of action must be taken into consideration in the total antimicrobial effect: the possibility of addition or synergy between some described compounds of e.o. and other kind of leaf metabolites isolated in non-volatile fraction of the plant [29, 31-32]. Combination of mechanisms of action should be consider too as a way of explanation of how to avoid resistance of microorganisms, probably by non-conventional antimicrobial mediated mechanisms were effects against bacterial virulence factors are involved [33-36] or by involving activity against bacterial virulence factors, as some known plant metolites capable to inhibit MDRs [26]. Synergism is probable the tool by which e.o. component and the rest of metabolites of this plant acts successfully against germs.

Conclusions

Essential oil and ethanolic extract of *P. aduncum* subsp. *ossanum* have some antimicrobial activity against bacteria and yeast, even on resistant uropathogenic strains. These findings could support traditional use of this subspecie as antiseptic in skin infections and urinary infectious complains. Considering limitations of agar diffusion method to some plant extract and e.o. [4], further antimicrobial studies are needed on e.o. of this plant that could explain traditional uses as antiseptic in skin infection and UTI.

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