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Polyphenolic composition of *Lantana camara* and *Lippia chevalieri*, and their antioxidant and antimicrobial activities

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Abstract

In the present study, the antioxidant and antibacterial activities of methanolic extracts of two Verbenaceae (*Lantana camara* L. and *Lippia chevalieri* Moldenke: aerial part) were investigated. Their polyphenolic composition in the ethyl acetate and aqueous fractions were characterized by HPLC-DAD. The antioxidant capability of the methanolic extracts was assessed by the Ferric Reducing Antioxidant Power (FRAP) and the scavenging activity of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The Minimum Inhibitory Concentrations (MIC) of the methanolic extracts (25 µg mL^{-1}), and the minimum bactericidal concentrations (MBC) (12.5 µg mL^{-1}) against 13 pathogenic bacteria and four serotyped bacteria from the American Type Culture Collection (ATCC) were also determined by the solid agar dishes diffusion method. The results indicated that the *L. chevalieri* extracts are rich in phenolic compounds (among the 27 polyphenolic compounds detected, 20 belong to *L. chevalieri*) and showed the highest antioxidant activities, simultaneously on iron (III) to iron (II)-reducing activity and the radical scavenging activity. However, *L. camara* displayed the best and the broadest antimicrobial spectrum, especially on *Shigella flexneri* and *Pantoea* sp. (two Gram-negative strains of bacteria). The nature of polyphenolics compounds detected (phenol acid and flavone) in *L. camara* can justify this activity.

Keywords: Verbenaceae, HPLC-DAD, antioxidant activity, antimicrobial activity, polyphenolic compounds

Introduction

The health care practices based on the use of the natural resources constitute a significant element of public health in many African countries due to historic, cultural and social reasons [1, 2]. According to the World Health Organization (WHO) [3], traditional medicine is largely more available than modern medicine. Nowadays, approximately 50 drugs result from the tropical plants [4]. Fifty percent of the products prescribed in several countries of Europe and America are natural products or their derivatives [5, 6]. In Africa, WHO [3] estimates that 80% of the population use traditional medicine to meet its needs for health. At the present, the medicinal plants still remain as the first tank of new drugs.

Lantana camara and *Lippia chevalieri*, two species of Verbenaceae, are widespread in the world, and are used to treat several diseases [7-10]. *Lantana camara* (leaves and flowers) is traditionally used against the fever, the influenza, the evils of stomach, bronchitis, rheumatism, asthma, hypertension, leprosy, scale, wounds, cancers, tumors, chicken pox, measles, ulcers and parasitic diseases [7,9,11-16]. *Lippia chevalieri* is used in traditional medicine to treat the respiratory diseases, the diarrhoeas, arterial hypertension, rheumatisms due to gout, painful and infected wounds, pathologies of the liver, bucco-anal and digestive candidiasis, anemia, malaria, painful menstruate, insomnia, nervousnesses and fevers [8,9,17, 18].

To our knowledge, few studies about the polyphenolic composition of *Lantana camara* and *Lippia chevalieri* have been reported. The phytochemical investigations of these two species have been mainly concerned to the essential oils composition [17, 8, 10]; however, some studies focused to the phenol composition and other secondary metabolites can be found. One of them is that of Ghisalberti [8], who isolated six flavonoids, such as 3-methoxy-, 3,7-dimethoxy- and 3,7,4'-trimethoxyquercetin, pectolinarigénin 7-*O*-β-D-glucoside, hispiduline and a camaraside glycoside, from the acetone extracts of leaves and stems of *L. camara*; traces (0.00004 – 0.0002%) of euphane, triterpenes and lactones in the methanolic extracts of roots; and furanonaphthoquinones in the hexane extracts. These groups of compounds are known for their anti-inflammatory properties

(triterpenes) and antimicrobial properties (pentacyclic triterpenes), especially against the gram-positive bacteria (furanonaphthoquinones) [7]. In the present study, the antioxidant and antimicrobial capacities of methanolic extracts of *L. camara* and *L. chevalieri* were assessed and compared in terms of their phenolic composition determined by HPLC/DAD.

Materials and methods

Chemicals

Acetonitrile (HPLC grade), water (HPLC grade), ethanol (HPLC grade) were purchased from J. T. Baker (Xalostoc, México).The references rosmarinic acid, gallic acid, p-coumaric acid, ferulic acid, syringic acid, p-hydroxibenzoic acid, quercetin, quercetin-3-O-rhamnoside, myricetin-3-O-rhamnoside, hesperidine, luteolin-7-glycoside, naringenin came from Aldrich, Sigma, and Fluka. 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, potassium persulfate, methanol, n-hexane, ethyl acetate, dichloromethane (all of them analytical grade) were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [$K_3Fe(CN)_6$] (analytical grade) was purchased from Prolabo (Paris, France). Ascorbic acid and iron trichloride (both analytical grade) were supplied by Labosi (Paris, France).

Plant materials

Aerial parts (stems and leaves) of *Lantana camara* and *Lippia chevalieri*, from Burkina Faso, were collected in June 2010 at UFR-SVT (Unité de Formation et de Recherche en Sciences de la Vie et de la Terre), and in November 2009 at Gonsè, 30 Km East from Ouagadougou, respectively. The plants were botanically identified by Professor Millogo-Rasolodimby from the Plant Biology Department of the University of Ouagadougou. The Voucher specimens (BK-lc2793 and BK-la2775) were deposited in the herbarium of the Laboratory of Biology and Plant Ecology, UFR-SVT of the University of Ouagadougou.

Preparation of plant extracts

Tissue samples (aerial parts) of *Lantana camara* and *Lippia chevalieri* were dried at room temperature and ground to fine powder; using a grinder. The extractions were carried out using ten gram of each sample in 3x100 mL methanol (100%), by a technical methanol steeping during one night. The extracts were individually filtered and concentrated under vacuum to dryness, and then dissolved in 1% methanol (v/v); aliquots were taken to be used in the evaluations of the antioxidant capacity and the antibacterial properties.

Biological activity

Iron (III) to iron (II)-reducing activity (FRAP)

The total antioxidant capacity of the plant extracts were determined by the iron (III) reduction method [19]. The diluted aqueous solution of plant extract (1 mL at a concentration of 100 µg mL^{-1}) was mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (2.5 mL). After 30 minutes of incubation at 50°C, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 2000 rpm for 10 minutes. Then, the upper layer solution (100 µL) was mixed with water (100 µL) and aqueous $FeCl_3$ (0.1 %) solution (20 µL). The absorbance was registered at 700 nm Ascorbic acid was used to produce the calibration curve (0-100 mg mL^{-1}, R^2 = 0.998). The iron (III) reducing activity determination was performed in triplicate for three independent pools of samples, and expressed in mmol ascorbic acid equivalents per gram of extract. The references quercetin and gallic acid were analyzed in the same manner.

Free radical scavenging activity

The capability of the extracts to scavenge DPPH* (2,2-diphenyl-1-picrylhydrazyl) radical was determined according to the method of [20] with some modifications. Briefly, 1.5 mL of freshly prepared methanolic solution of DPPH (0.02 mg mL^{-1}) was mixed with 0.75 mL of extract solution. After 15 minutes of incubation in the dark, at room temperature, absorbencies were registered at 517 nm against a blank sample prepared with 1.5 mL of methanol and 0.75 mL of extract. A standard calibration curve was plotted using ascorbic acid (0-200 mg mL^{-1}, R^2 = 0.9989). Quercetin and ascorbic acid were used as positive controls. The antioxidant activity is expressed in inhibiting concentration 50 (IC_{50}) i.e. the concentration of the extract likely to cause 50% of inhibition. The free radical scavenging activities were expressed in microliter per liter. The analysis was done in triplicate for three independent pools of samples.

HPLC/DAD analysis

To determine the phenol composition of the aerial tissues of *Lantana camara* and *Lippia chevalieri*, dried ground samples (1 g) were individually suspended in 10 mL methanol-water (50% v/v), then concentrated to around 5 mL and fractionated twice with 10 mL ethyl acetate. The combined organic fractions (ethyl acetate fraction) and aqueous fraction were concentrated to dryness before being dissolved in 1.5 mL methanol and analyzed (100 µL aliquots) by high-performance liquid chromatographic-diode arrey detection (HPLC-DAD) on a Perkin Elmer HPLC system and a Perkin Elmer Brownlee Analytical C18 column (4.6 x 250 mm, 5 µm), by an acidified acetonitrile-water gradient [21]. Standard chromatograms were plotted at 260 and 340 nm. Spectral data for all peaks were accumulated in the range 200-400 nm using diode-arrey detection (Perkin Elmer Series 200). Direct comparisons of retention time (RT) and UV spectra of resolved compounds with those of standards and with the compilations done by [22] and [23] were used to obtain structural information of the detected phenols.

Antibacterial study

Microorganisms

The microorganisms used in this study consisted of clinical isolated and collection/serotyped strains. The clinical isolated were *Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Pantoea* sp, *Pseudomonas aeruginosa, Salmonella typhi (fish), Salmonella typhi (salad), Shigella flexneri, Staphylococcus aureus, Streptococcus agalactiae, Staphylococcus epidermidis, Vibrio cholerae (water) and Vibrio cholerae (salad).* The following serotyped strains used in this study are: *Bacillus cereus* ATCC9144, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC35659 and *Staphylococcus aureus* ATCC 25923. Before testing, pure cultures were realized with all the strains in Mueller Hinton Agar and Tryptic soy broth. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 Mc Ferland standards.

Antibacterial test

Disc-diffusion method

The effectiveness of the extracts compared to the micro-organisms was evaluated according to the method [24] with some modifications. We made ran 10 mL of solid agar in Petri dishes and put in incubation during 24 hours to check sterility. Sowing is then carried out by the technique by flood. This technique consists in flooding the surface of the solid agar with a bacterial suspension of a density of 10^6-10^7 bacteria/mL. Excess is taken and eliminated. Discs made up of paper Whatman n°1 of 6 mm (sterilized) are deposited on the solid agar. A volume of 15 µL of extract of a concentration of 25 µg mL-1 in methanol 1% is deposited on each disc. The Petri dishes were then put in incubation for 24 hours. The diameters of inhibition zones were materialized by clear zones around the discs are then measured using a ruler. Commercial antibiotic discs of Gentamicin (10 µg/disc), Amoxicillin (10 µg/disc), Ciprofloxacin (5 µg/disc) and Cotrimoxazol (25 µg/disc) were used as positive controls. Methanol 1% was used as a negative control.

Microdilution method

Concerning the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination, broth microdilution method [24] was used with some modification. The MIC was defined as a lowest concentration of the extract at which the microorganism does not demonstrate visible growth after 24 hour incubation at 37°C and MBC was defined as the lowest extract concentration at 99.9 % of the bacteria were killed. 100 µL of broth Müeller Hinton was distributed in sterile 96-well microplates, 100 µL of each extract (25 µL mL-1) to the first wells followed by a dilution in cascade which are added 10 µL of bacterial inocula. For each extract three columns were used. The test columns receive only inocula (control of the culture fertility) and negative control was constituted of culture medium and extract. For each extract three wells receive only extract and inocula. After 24 hours of incubation at 37°C we add p-iodonotrotetrazelium purple (INT) as bacterial growth indicator. The MIC was determined after 30 minutes of incubation at the same temperature. The wells where there are bacterial growths are announced by a red or pink coloring, while those ones which presented clear coloring having know an inhibition of the bacterial growth..

Data analysis

The data are expressed as the means ± standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $P < 0.05$ and linear regression) was carried out with XLSTAT 7.5.2.

Results and Discussion

HPLC-DAD analysis

Each of the different classes of flavonoids has a particular UV spectrum. According to Mabry [22] and Campos and Markham [23], the methanol UV spectrum of a given flavonoid contains enough information to discriminate among flavones and flavonols. According to Kiendrebéogo [25], flavones and flavonols are characterized by a band I at 320-385 nm, and a band II in the interval 250-285 nm, band I of flavones occurring in the range of 304-350 nm. That information, along with the retention times given by the HPLC/DAD analysis allowed the identification of the class of flavonoids found in the aerial tissues of *Lantana camara* and *Lippia chevalieri.*

A total of 27 polyphenolic compounds were resolved, 20 compounds in the extract of *L. chevalieri* and 7 in that of *L. camara* (Table 1). This study revealed 11 cinnamic acid derivatives, 8 flavone, 2 rosmarinic acid derivatives, 3 phenolic acids, 2 luteolin derivatives and 1 saponarin derivative. The absence of flavonols is highlighted. The phenol profile of *Lantana camara* was different to that of *Lippia chevalieri*; this supports the species-specific tendency of these chemical markers previously reported by several authors [26, 27, 28].

Phenolic acids were the most abundant compound in the aerial tissues of both taxa here analyzed. These phenols play important roles in plants such as protection against herbivores and pathogens, cementing material joining phenolic polymers to polysaccharides of cell walls [29], regulation of cell growth and division [30], and inhibition of ion intake by a generalized increase in membrane permeability to inorganic ions as a mode of action of allelopathic compounds [31]; besides, phenolic acids have relevant antioxidant properties [32, 33, 34]. Flavones were the other phenolic compounds found in the tissues of *Lantana camara* and *Lippia chevalieri*, among them, luteolin derivatives, which have important reduction potentials associated to the absence of the 3-hydroxyl group [35].

The phenol composition of *Lantana camara* reported in the present study represents a strong contrast with that of Ghisalberti [7], who reported three quercetin methoxy derivatives in the aerial parts of that species. This discrepancy could be the result of the different systems of phenol extraction carried out by Ghisalberti [7], with acetone, and by us, with 100% methanol.

Table 1: Wavelength characteristic of the phenols acids and flavonoids detected

Nature and type of extract, percentage	Polyphenols	Retention time (min)	λ_{max} (nm) Band II	λ_{max} (nm) Band I
L. camara, ethyl acetate fraction, 12.5%	Phenol acid	35.456	248sh, 284sh	330
	Flavone	46.449	274	329
L. camara, aqueous fraction, 12.5%	Phenol acid	35.002	247sh, 285sh	327
		36.564	247sh, 287sh	325
	Flavone	39.504	275	330
		46.07	275	322, 353sh
		46.537	275	324, 351sh
L. chevalieri, ethyl acetate fraction, 50%	Cinnamic acid derived	34.353	286sh	328
		34.734	289sh	326
		35.306	288sh	328
		35.585	288sh	322
		37.05	286sh	327
		37.8	284sh	326
		38.665	284sh	327
		42.625	285sh	328
	Luteolin derived			
		51.234	273sh	349
		51.894	254sh, 272	347
	Flavone			
		58.318	251, 274	341
		60.416 *	238sh, 277sh	328
		61.266 *	239sh, 276	333
		29.073*	272	334
L. chevalieri, aqueous fraction, 12.5%	Cinnamic acid derived	33.902	248sh, 286sh	325
		36.637	245sh, 284	327
		37.326	244sh, 284sh	328
	Rosmarinic acid derived	34.855	249sh, 286sh	324
		35.097	249sh, 284sh	322
	Saponarin derived			
		28.49	271	333

* : flavone suspect, sh : epaulement

Table 2: Capacity antioxidant values of the methanolic extracts

Sample	FRAP (mmol EAA g^{-1})	DPPH : IC_{50} (µg/mL)
L. camara	$8.17^{d} \pm 0.04$	$16.13^{a} \pm 0.35$
L. chevalieri	$15.16^{b} \pm 0.11$	$6.23^{b} \pm 0.15$
Gallic acid	$18.46^{a} \pm 1.51$	$0.60^{d} \pm 0.05$
Quercetin	$13.19^{c} \pm 2.17$	$0.93^{d} \pm 1.22$
Ascorbic acid	$5.86^{e} \pm 0.51$	$1.8^{c} \pm 0.43$

The values represent the mean and standard deviation for three independent samples
Different letters in the same column (or line) mean significant differences ($p < 0.05$).

Table 3: Summary table of the diameters (mm) of inhibition zones

Bacterial strains	*L. camara*	*L. chevalieri*	Ampi (10 µg/d)	Cipro (5 µg/d)	Genta (10 µg/d)	Co-tri (25 µg/d)
Bacillus cereus ATCC9144	11	-	-	25	18	24
Citrobacter freundii	9	-	ND	ND	ND	ND
Escherichia coli (isolate)	-	-	26	33	28	28
Escherichia coli ATCC 25922	11	8	19	23	20	ND
Klebsiella pneumoniae	10	10	ND	ND	ND	ND
Pantoea sp	12	10	ND	ND	ND	ND
Proteus mirabilis ATCC35659	8	8	-	27	20	29
Pseudomonas aeruginosa	11	8	ND	ND	ND	ND
Shigella flexneri	12	-	18	31	34	30
Salmonella typhi (fish)	8	8	22	31	20	ND
Salmonella typhi (salade)	11	-	ND	ND	ND	ND
Staphylococcus aureus ATCC 25923	8	8	-	30	20	ND
Staphylococcus aureus (isolate)	11	9	16	31	27	35
Staphylococcus épidermidis	9	-	-	24	18	ND
Streptococcus agalactiae (isolate)	8	11	ND	ND	ND	ND
Vibrio cholerae (fish)	-	-	8	31	19	ND
Vibrio cholerae (water)	-	-	ND	ND	ND	ND

Ampi: Ampicilline; Cipro: Ciprofloxacine; Genta: Gentamicine: Cotri: Cotrimoxazole; -: Inactive; ND: Non determined.
NB: the insulation of Proteus must be done on agar-well dries. Indeed their growth is slowed down (inhibited) in the presence of methanol or of ethanol deposited on the internal surface of the lid of limps of Petri.

Table 4: Minimum Inhibition Concentration (MIC), minimum bactericidal concentration (MBC), and proportion MBC/MIC.

	Extract	E. c s		K. p.	P.		S. fle.	
***MIC* (µg/mL)**	*L. camara*	12.5	25	25	12.5	25	12.5	25
***MBC* (µg/mL)**	*L. camara*	-	-	-	12.5	12.5	12.5	12.5
MBC/MIC	*L. camara*				1	2	1	2

MIC: Minimum Inhibitory Concentration; MBC: minimum bactericidal concentration; E. c. s: *Escherichia coli* ATCC25922; K.p.: *Klebsiella pneumoniae*; P.: *Pantoea* and S. fle: *Shigella flexneri.*

Antioxidant activity

The reducing power of the different samples here analyzed is showed in Table 2. Significant differences were found between the value calculated for *Lippia chevalieri* (15.16 ± 0.11 mmol AAE g^{-1}) and that calculated for *Lantana camara* (8.17 ± 0.04 mmol AAE g^{-1}). These results show that *Lippia chevalieri*, with a complex phenol composition (20 compounds), reduces almost twice more Fe(III) that *L. camara*, which have a simpler phenol composition (seven compounds), suggesting an association between the reducing capability with the phenol composition complexity.

We note that the antioxydant capacities of *L. camara* (8.17 mmol EAA G^{-1}) and of *L. chevalieri* (15.16 mmol EAA G^{-1}) are respectively better than ascorbic acid (5.86 ± 0.51 mmol EAA G^{-1}), used like reference substance. Compared to quercetin (13.19 ± 2.17 mmol EAA G^{-1}), known like a potent antioxidant [35, 36] and with the gallic acid (18.46 ± 1.51 mmol EAA G^{-1}), the reduction produced of *Lippia chevalieri* was not significantly different. On the other hand these last two compounds of

references showed significantly higher reducing power than that of *L. camara*. The antioxidant activity is expressed in inhibiting concentration 50 (IC_{50}) i.e. the concentration of the extract likely to cause 50% of inhibition.

The estimation of the antiradical capacity of the flavonoids of the different extracts was performed by the value of EC50 (Effective Concentrations), these are shown in Table 2. EC50 for *Lippia chevalieri* was 6.23 ± 0.15 µg mL-1 and for *Lantana camara* was 16.13 ± 0.35µg mL-1. These results showed that *L chevalieri* has an antiradical capacity on DPPH* around twice higher than *L. camara.* Compared to the substances of references, the radical scavenging activity of our two extracts are lowest than these references. The results indicate that the strongest antiradical capacity as well as the highest reducing capacity was showed by the extracts of *Lippia chevalieri.*.

The important antioxidant capacity of *Lippia chevalieri* could be explained by its phenol composition, which was more complex than that of *Lantana camara*. Previous studies reported that the total phenolic content, total flavonoid content, and tannin content of *Lippia chevalieri* were higher than those of *Lantana camara* [37]. Several reports have suggested that there is a correlation between the total phenol content and the antioxidant activity of plant extracts [38, 39, 40, 41, 42, 43]. In this manner, the higher antioxidant properties of *Lippia chevalieri* extracts could result not only from the interactions of phenolic acids and flavonoids but also of tannins.

Our results are in agreement with those from [44] and [45], which showed that there is a good correlation between the antioxidant activities and the phenolic compounds content. Coulidiati [44] reported values R^2 of 0.597, as the weakest correlation, between antioxidant activity and tannin content and total phenolic content, for three species of Combretaceae. The correlation between the flavonoid content and the antioxidant activity has been a controversial issue. Authors like [46] have found that correlation, but some others like [47] have reported no correlation between those two parameters. Several other searchers also showed the implication of certain groups of phenolic compounds on the antioxidant capacities by DPPH* scavenging activity and FRAP [48, 49, 50]. Thus, according to Surveswaran [50], the correlations between antioxidant activity and total phenolic content of 133 species of medicinal plants of Indiana ranged from R^2 = 0.89 to R^2 = 0.97, revealing the implication of the total phenolic content and the tannin content in 83 of the species of plants of their study.

Other authors have reported the relevance of certain secondary metabolites to confer the antioxidant capacity to plant extracts. Thus, it was proven, in an *in vitro* study that the flavonol rutin has an antioxidant activity at 500 ng [51]. Rutin was also detected in the extract of *Lippia chevalieri* by the technique of thin layer chromatography [52]. Among the three plants of the study of Coulidiati [44], *Combretum acutum* Laws, in which rutin was identified, important DPPH scavenging activity and FRAP were detected. Thus, this secondary metabolite could explain the higher antioxidant activity of *L. chevalieri* compared to *L. camara*.

Penchev *et al.* [53] showed that the rosmarinic acid is an antioxidant agent, but, the phenolic acids are, in a general, endowed with the antioxidant capacity. Caffeic acid prevents the lipidic peroxidation of food and the diseases induced by the free radicals, such as cancer, pathological contracting of the arteries, and the ageing of tissues [54, 55, 56, 57]. Phenolic acids, among them caffeic acid, and rosmarinic acid derivatives, were detected, in the present study, in the methanolic extracts of *Lantana camara* and majorly in those of *Lippia chevalieri* [52]. This two species of plants could also owe their antioxidant capacities to the essential oils they synthesize. Mevy [8] reported that β-caryophyllen, 1,8-cineol and the germacren are antioxidant compounds. It was also reported that the geniposides and the verbascoside, isolated from *L. camara* have antioxidant properties [58]. The verbascoside protects the destruction oxidative of hemoglobin, inhibits the aldolase reductase as well as the lipidic peroxidation; this compound presented as well immunomodulatrice activity, immunosuppressive properties and apparently reinforced the anti-tremor effect of the L-DOPA [7]. Barre [59] showed that the ursulate acetic and a derivative of oleanolic acid, which are two pentacyclic triterpenes derivatives, carry on a great antimutagenic activity on mice. *Lippia chevalieri,* which displayed in the present study a significant antioxidant activity, could be regarded as a worthily source of antioxidant compounds.

Antimicrobial activity

With regard to the antimicrobial activities, the diameters of inhibition were measured by the method of diffusion of plate described by [24]. This activity was evaluated on twelve bacterial strains with gram-negative and five bacterial stocks with gram-positive. Table 3 indicates the results of bacterial inhibition obtained from methanolic macerated of *Lantana camara* and *Lippia chevalieri*. In the present study, the assayed extract concentration was 25 µg mL^{-1}. The inhibition zones of the Gram-negative bacteria reached values between 8 and 12.33 mm (Table 3). The best activities were obtained with the extract of *L. camara* on *S. flexneri* and *Pantoea sp* with diameters of 12.33 and 12 mm, respectively. The lowest inhibitions (8 mm) were obtained with macerated of the two analyzed species on *P. mirabilis* (ATCC35659), *S. typhi* (fish) and *S. typhi* (salad). The results showed that certain bacteria such as *E. coli* (uroculture); *V. cholerae* (water) and *V. cholerae* (fish) were are not sensitive to any of the extracts at the concentration of 25 µg mL^{-1}.

The test on the Gram-positive bacteria gave inhibition zones ranging between 8 mm and 11.33 mm. The broadest diameters of inhibition were obtained with *L. camara* (11.33 ± 0.35 mm) on *S. aureus* ATCC25923, followed by *L. chevalieri* (10.67 ± 0.35 mm) on *S. epidermidis*. The results indicated that the extracts of *L chevalieri* had a higher bacterial inhibition activity than those of *L. camara* on *S. epidermidis*. However, *L. camara* extracts produced diameters of inhibition with the majority of the bacterial strains (except *E. coli* - clinical, *V. cholerae* (water) and *V. cholerae* (fish). These results reveal that the inhibition zones

vary according to the class or the bacterial species and to the composition of the extract. According to [60] the extracts inducing inhibition zones broader than 3 mm around the disc are regarded as antimicrobial. In the present study the extracts of *Lantana camara* and *Lippia chevalieri* had highest antimicrobial activity on *E coli* (ATCC25922) (11.33 mm), *S. flexneri* (12.33 mm) and *Staphylococcus aureus* (ATCC25923) (11.33 mm).

The Minimum Inhibitory Concentrations (MIC) of the extracts of *Lantana camara* and *Lippia chevalieri* were also estimated. We considered the extracts which had an inhibition zones superior or equal at 9 mm (Table 4). The MIC varied from 12.5 to 25 µg mL^{-1}. The best inhibition activity was obtained by the extract of *L. camara* on *S. flexneri, Pantoea* sp, *K. pneumoniae* and *E. coli* ATCC 25922.We note that these results are obtained on Gram-negative strains. The minimum bactericidal concentration (MBC) varied in the same manner than MIC. Concerning the ratio MBC/MIC, *L. camara* extracts had a bactericidal effect on bacterial strains such as *Pantoea* sp and *Shigella flexneri*. Some authors [61] proposed a classification of plant extracts on the basis of MIC values, as follows: strong inhibition (MIC inferior at 500 µg mL^{-1}); moderate inhibition (MIC vary from 600 to 1 500 µg mL^{-1}); lowest inhibition (MIC superior at 1 600 µg mL^{-1}). According to that, the extracts of *L. camara* had a strong inhibition on the strains indicated. The important antimicrobial activity of *Lantana camara* could be explained by its phenol acid and flavones. Several authors have reported the relation between the phenol composition of extracts and their antibacterial activity. Vaquero et al. [62] showed that quercetin inhibits *P. mirabilis* (MIC: 10 µg mL^{-1}), *K. pneumonia*, and *E. coli* starting from 25 µg mL^{-1}. Ksouri [63] showed that the isoquercetin and the catechin are the major phenolic compounds implied in the antimicrobial activities. The characterization tests [52] made it possible to detected sterols/triterpenes, tannins and the saponosides whose antimicrobials activities were already shown [64, 65, 66]. Indeed, the tannins are suitable for precipitation during the reactions of oxidations, and that could be a factor of toxicity for the micro-organisms [67]. The former studies on the *L. camara* showed that this species of plant accumulated toxic molecules, such as lantadene A to D [7]. The mechanism of toxicity of polyphenols against the micro-organisms could be explained by inhibition of the hydrolytic enzymes (proteases) and carbohydrates or with other interactions for inactivating microbial adhesion, the proteins of transport of the cellular envelope and the non specific interaction with the carbohydrates. Thus, these mechanisms of inhibition are located at several levels, the polymer polyamides of the bacteria can support the reactivity of their proteins, but also the phenolic compounds can be at the base of the deprivation of iron or the hydrogen bonds of vital proteins i.e. the bacterial enzymes. Thus, the strongest antimicrobial activity of *L. camara* could be justified by its content of toxic compounds. In the same way, acetate ursalate (isolated from *L. camara*) has antimicrobial properties, which are similar to those of chloramphenicol [7]. Its activity being more marked against *Staphylococcus aureus* and *Salmonella typhi* at concentrations of 30 µg per disc. The extracts of *Lantana camara* and *Lippia chevalieri* displayed higher antibacterial activity on the Gram-negative strains. The Gram-negative bacteria have structures less rich in peptidoglycane (10% of the lining), meaning less layers of peptidoglycane (1 to 3 layers) and these peptidoglycanes are grooved than those of the Gram-positive bacteria [68]. Indeed, compared to the Gram-negative bacteria, the Gram-positive are very rich in peptidoglycane (50 to 80% of lining), consist of several layers of peptidoglycane and have a significant reticulation [68]. The peptidoglycanes role, without being restrictive, is to ensure the rigidity and the solidity of bacterial ligning as well as to protect the cytoplasmic membrane against osmotic lysis. The complexity of the Gram-positive bacteria could explain the inactivity of our extracts, although non-flavonoid phenols can display antimicrobial activity on the Gram-negative bacterial strains particularly on *E. coli* ATCC 35218 and ATCC25922 [65]. Furanonaphthoquinones are among those phenols having antimicrobial properties on Gram-negative bacteria [25]. In the present study, the best antimicrobial activity was obtained with gallic acid on human *Klebsiella pneumoniae*, at 100 mg ml^{-1}, followed by caffeic acid on *Klebsiella pneumoniae* and *the E. coli* at 200 mg mL^{-1}. A negative MBC and a MIC of 8 % were found by using essential oils of *L chevalieri* on *Escherichia coli* CIP105182 [9].

The inhibition zones of antibiotics tested in the present study showed that the majority of the bacterial strains were sensitivity to Ciprofloxacine and Gentamicin, showing the broad spectrum of activity of those antibiotics. Considering the diameters of sensitivity, superior at 10 mm, the extract of *L. camara* was active on seven bacterial strains particularly on *Shigella flexneri. Shigelles* are Gram-negative bacilli which resist the gastric acidity. Thus after their intrusions, they go through the stomach and reach the intestine, then the enterocytes they multiply and produce toxins [69]. They are invasive bacteria, accused in the bacillar gastroenteritis and dysenteries. The highlighted antimicrobial activity would confirm the therapeutic use of the leaves of *L. camara* in traditional medicine in the treatments of the oral affections, coughs, pneumopathies, diarrhoeas, influenza, and like antibiotic [9], although according to our results, against Gram-positive bacilli [9]. The extracts of *Lantana camara* could be used to cure those diseases, in particular that caused by *S. flexneri*.

Conclusion

An important richness of phenolic compounds (27) was found in the aerial parts of *Lantana camara* and *Lippia chevalieri*. The phenolic acids were among the most abundant compounds in those both species. With 20 compounds, the profile of *Lippia chevalieri* was the most complex; being those complexity apparently associated to its important antioxidant activity. In this study, phenolic acid and flavone contents can justify the best antimicrobial activity of *L. camara* extract. Methanolic extract had shown effectiveness against microorganisms responsible of

infectious diseases, thus justifying the successful use of *L. camara* for the treatment of dysenteries, diarrhea, and pneumopathies in traditional medicine. Further investigations will be performed to isolate and identify the compounds present in the extracts of those both medicinal plants, and taste the toxicity of the pure compounds against pathogenic bacteria and their antioxidant properties, in vivo.

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