Anti-Acne, Antioxidant and Cytotoxic Properties of *Ludwigia peploides* Leaf Extract

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ABSTRACT

Objective: This work is the first to investigate the *in vitro* anti-acne and cytotoxic activities of the leaves of *Ludwigia peploides*. With its important seasonal biomass production, this plant has great potential in several therapeutic and cosmetic applications. Methods: The antibacterial activity of the extract was screened against a consortium of skin microorganisms that cause acne vulgaris disease, using disc diffusion and broth microdilution methods. The antioxidant activity of methanolic leaf extract of *L. peploides* was evaluated by DPPH and NBT assays to assess the free radical scavenging activity of *L. peploides*, which in turn has a great importance related to its role in minimizing the oxidative stress linked to the pathophysiology of diseases like acne vulgaris. Its putative cytotoxicity was examined against human macrophage-like monocytic leukemia (THP-1) and human keratinocytes (HaCaT) cell lines. In addition, antiproliferative activity was screened against B16 cancer cell lines. Results: The extract displayed antioxidant effect by DPPH ($IC_{50}= 58 \pm 6.0$ µg mL^{-1}) and NBT ($IC_{50}= 30 \pm 2.8$ µg mL^{-1}) assays, and it was not toxic on HaCaT cells ($IC_{50} > 200$ µg mL^{-1}). A strong inhibitory activity against *Propionibacterium acnes* (MIC = 1.9 µg mL^{-1}) was registered for the extract, which had a bactericidal effect against *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Salmonella enterica*. Finally, the extract was shown to have an antiproliferative activity ($IC_{50}=5.5 \pm 2.3$ µgmL^{-1}). Conclusion: The extract displays antioxidant and anti-acne effects as well as inhibition potential of B16 melanoma cells proliferation.

Keywords: *Ludwigia peploides*, antioxidant, anti-acne, antiproliferative properties.

INTRODUCTION

Aquatic macrophytes belonging to *Ludwigia* genus (*Onagraceae* family) are invasive plants that perturb the functioning of aquatic habitats[1,2]. Several species of this genus are known for their antioxidant activity[3] and for their ability to synthesize allelopathic molecules[4], which confer medicinal properties such as antimicrobial[5,6,7] and antiproliferative[8] activities. In this context, we examined the putative medicinal properties of *Ludwigia peploides*, which can produce, from June to September, large amounts of biomass, reaching 2-3 kg dry weight m^{-2} [2] on a surface of several hectares in the south of France. Smida *et al.* (2015)[7] have demonstrated the antibacterial properties of this plant that were superior in leaves and flowers.

 Among the large number of studies on the antimicrobial properties of plants, several ones have reported their therapeutic effects in the treatment of the acne vulgaris[9,10]. The latter has an important impact on the social, emotional, and psychological comportment of 650 million people around the world[9]. Acne vulgaris appears as inflammation in skin areas. This disease is due to an oxidative stress in the pilosebaceous unit that creates an anaerobic environment. That is the best suited condition for the development of a Gram-positive obligate anaerobic bacterium, *Propionibacterium acnes*, which inhabits under the human skin and populates the androgen stimulated sebaceous follicles[11]. It is often associated to *Staphylococcus aureus* and other microorganisms such as *Staphylococcus epidermidis*, *Salmonella enterica*, *Escherichia coli,* and *Candida albicans,* which can also be responsible for skin infection diseases. The antibiotics used for its treatment are not specific and can generate resistant bacteria[12]. *P. acnes* seems to be responsible for the production of reactive oxygen species, which provoke an inflammation in the pilosebaceous skin structure. The capability of plants to inhibit this phenomenon is, to some extent, due to the fact that they contain non-enzymatic antioxidants such as polyphenols, which are able to reduce reactive oxygen species[13,14]. Saric *et al.* (2017)[15] reported that the polyphenols found in tea can reduce sebum

secretion and are effective for the treatment of acne. These antioxidant properties are complementary to antibacterial activity[16], in particular in acne disease. Polyphenols have also been described, particularly those found in green tea, as putative anti-tumor agents through their antioxidant activities, which inhibit the growth of tumor cells and favor apoptosis[17,18], the effect of active oxygen radicals being also known to intervene in the carcinogenesis phenomenon[19,20]. Because polyphenols are found in several *Onagraceae* species[21] and in extracts of *L. peploides*[22], we investigated the antioxidant properties in this plant. Thus, in our study, we have measured the total polyphenolic (TP), flavonoid (FLA), tannin (TAN) and anthocyanin (ANT) compounds from a methanol leaf extract of *L. peploides* and have assessed its antioxidant property. Furthermore, its anti-acne and antiproliferative activities were studied and compared to that of classical antibiotic and anti-cancer compounds. The cytotoxicity tests were conducted to ensure the safety of this extract.

MATERIAL AND METHODS

Treatment of the Samples and Preparation of Extracts

Plants were collected alongside the bank of a dead arm of the Durance river (43° 40 17 N, 5° 28 07 E), near Pertuis town (Vaucluse, France). The characteristics of this sampling station were described by Smida *et al.* (2015)[7]. Leaves were immediately separated from plants before being air-dried and then ground into a coarse powder with the aid of a mechanical grinder (IKA Labortechnik type A10). Then, the ultrasound-assisted extraction commenced by soaking one gram of the obtained leaves powder in 50 mL of a methanol: water mixture (80:20 v/v) for 10 min (Bioblock Scientific 88160), then mixed under a vigorous magnetic stirring at 130 tr.min^{-1} and centrifuged at 3354 g at 4°C for 30 min. The supernatant was filtered through a Whatman filter paper (0.22 µm) and concentrated under vacuum and reduced pressure at 40°C using a rotary flask evaporator (Heidolph Laborta 4000). The dry crude hydro-methanolic extract was labeled with Dry Plant Extract (DPE) and stored at 4°C in a sealed sterile glass flask until further use. The extract was dissolved in DMSO for all the biological tests performed.

Chemical Analysis Methods

Determination of Total Phenolic (TP) Content

TP content in the methanol extract was determined using Folin-Ciocalteu reagent according to Ksouri *et al.* (2009)[23] and expressed as mg of gallic acid (GA) equivalent per gram of dry plant extract (DPE) or, taking into account the yield of extraction, per gram of dry plant material (DPM).

Determination of Flavonoid (FLAV) Content

Flavonoid content was estimated according to Ksouri *et al.* (2009)[23] and expressed as mg of catechin (CAT) equivalent per gram of DPE or DPM.

Determination of Total Tannin (TAN) Content

The method to determine TAN content was based on the reaction of tannins with hydrochloric vanillin. Thus, 0.5 mL of diluted (1/100) methanol extract was mixed with 3 mL of a 4 % vanillin solution in methanol. This mixture was vigorously stirred, then let to rest for 15 min before adding 1.5 mL of concentrated HCl (38 %). The

absorbance was measured at 500 nm. Tannin content was expressed as mg CAT equivalent per gram of DPE or DPM.

Determination of Anthocyanin (ANT) Content

The differential pH method described by Giusti and Wrolstad (2001)[24] was used. Anthocyanin content was expressed as mg of cyanidin-3-glycosylated (C3G) equivalent per gram of DPE or DPM.

Antioxidant Assays

Antioxidant activity was performed on the leaf extract using the 1, 1′-Diphenyl-2-Picrylhydrazyl (DPPH) free radical scavenging activity and the superoxide (O_2^{-}) radical scavenging nitro blue tetrazolium (NBT) assays.

DPPH Radical Scavenging Activity

The capacity of the extract to scavenge DPPH radical by its reduction was measured according to a modified method of Tuberoso *et al.* (2007)[25]. In a 96-well plate, a reaction mixture containing 100 µL of DPPH methanolic solution (0.5 mmol.L^{-1}) and 10 µL of the extract was added into each well to give a final concentration range (9.37-600 µg.mL^{-1}). Absorbance was measured at 470 nm after 15 min of incubation in the dark. Gallic acid and quercetin were used as references. The percentage of inhibition at the steady state of each concentration was used to graphically determine the inhibitory concentration 50% (IC$_{50}$), defined as the extract concentration (µg.mL^{-1}) required to obtain 50 % of the DPPH reduction.

The radical scavenging activity was calculated using the following formula:

% inhibition = (absorbance $_{control}$ − absorbance $_{sample}$) / (absorbance $_{control}$) * 100

Superoxide Radical Scavenging Activity

The leaf extract was tested for its free radical scavenging activity using the non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system, which generates superoxide radicals that reduce NBT to a purple-colored formazan. The measurement was performed in 96-well microplates. The reaction mixture consisted of NADH (78 µmol.L^{-1}), NBT (50 µmol.L^{-1}), PMS (10 µmol.L^{-1}), and 10 µL of the extract sample was added into each well to give a final concentration range (9.37–600 µg.mL^{-1}). The reagents were dissolved in a 16 mmol.L^{-1} Tris–hydrochloride buffer at pH 8. After incubation for 5 min at room temperature, the measurement was performed at 560 nm against an appropriate blank to determine the quantity of formazan generated. The percentage of inhibition at the steady state for each concentration was used to calculate the IC$_{50}$ values, i.e. the extract concentration corresponding to 50 % of the NBT reduction. Ascorbic acid was used as reference.

Biological Analysis

Antimicrobial Activity Assay

Microorganisms

Bacterial strains included in this study were the hospital strains, *Propionibacterium acnes* and *Staphylococcus aureus*, *Staphylococcus epidermidis* (CIP 68.21), *Salmonella enterica* serovar "Heidelberg" (*S.* Heidelberg B182), *Escherichia coli* (CIP 53126), and a yeast strain, *Candida albicans* (ATCC 26555). The hospital cultures

were procured at the Department of Microbiology, University Hospital Center of Rennes (France). All strains were maintained on Tryptone soya agar at 4°C.

Disc Diffusion Method

Antibacterial activities were determined according to Smida *et al.* (2015)[7]. The dry extract was deposited at a concentration of 7.5 µg dissolved in 0.15 µL of DMSO per disc.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Broth microdilution test was done to determine the minimum inhibitory concentration (MIC) as described by the CLSI guidelines[26]. Serial dilutions of the leaf extract and the positive control, doxycycline, an antibiotic of the tetracycline family, were prepared with concentrations ranging from 0.0078 to 500 mg mL^{-1} in Broth Heart infusion (BHI) in a 96-well microtiter plate (Sterile, Flat bottom, with lid, Greiner Bio-one, Germany). Each well was inoculated with a 10^7 CFU mL^{-1} bacterial culture. Then, the plate was incubated at 37 °C for 24 h under anaerobic conditions. After that, 5 µL of the clear wells were spread on Muller-Hinton Petri dishes and incubated for another 24 h. The clear well with the lowest concentration represented the MIC inhibiting a visible bacterial growth. The Petri dish with the lowest concentration showing no colonial growth corresponds to the minimum bactericidal concentration (MBC) defined as the concentration that killed 99.9 % of the initial inoculum.

Cytotoxicity Tests

Human Macrophage-like Monocytic Leukemia Cells

Cell Culture

Human macrophage-like monocytic leukemia cell line, THP-1[27], was grown in RPMI 1640 medium with sodium pyruvate (1 mmol.L^{-1}) and Hepes buffer (1000 mmol.L^{-1}) supplemented with L-Glutamine (2 mmol.L^{-1}), 10 % heat-inactivated fetal bovine serum (FBS, Lonza, France), and antibiotics (penicillin 100 mg.mL^{-1} and streptomycin 50 mg.mL^{-1}) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. For THP-1 differentiation into macrophages, phorbol 12-myristate 13-acetate (PMA) was used at 10 ng.mL^{-1} for 72 h. 96-well plates (Sterile, Flat bottom, with lid, Greiner Bio-one, Germany) were seeded with 7.10^4 THP-1 cells/well. The plates were incubated with PMA for 72 h and then exposed to decreasing concentrations obtained from an initial plant extract solution dosed at 300 µg.mL^{-1}. Wells without extract constituted the negative controls. Triton X-100 (1 %), a nonionic detergent, was used as a positive control. The plates were incubated for 24 h and then treated according to the MTT [3-(4, 5-dimethylthiazol-2-phenyl)-2, 5-diphenyltetrazolium bromide] assay[28].

Determination of Cell Viability

Ten microliters of 5 mg.mL^{-1} MTT solution, prepared in PBS and filter-sterilized through a 0.22 µm filter (Whatman), were added to wells containing 100 µL of culture medium. The 96-well plates were incubated for 4 h at 37 °C under a 5% CO_2 atmosphere. Then, 100 µL of 0.04 mol.L^{-1} HCl in isopropanol were added and mixed to dissolve the formazan crystals. The intensity of formazan

was read after 5 min at 595 nm. The percent of cell viability was calculated by the following formula:

Cell viability (%) = Mean OD / control OD × 100

All chemical products used in this work were obtained from Sigma Aldrich unless otherwise specified.

Normal Human Keratinocytes and Mouse Melanoma B16 Cells

Cell Culture

The normal human keratinocytes (HaCaT) cell line was a generous gift from the Institute of Genetic and Development (Rennes, France) and the mouse melanoma B16 cell line (ATCC-CRL 6475) was purchased from the Chemical Engineering Laboratory (LGC Toulouse, France). The two cell lines were cultivated in RPMI 1640 medium (Dutscher) with 2 mmol.L^{-1} L- glutamine (Dutscher) supplemented with 5% fetal bovine serum (FBS), 1% 10000 U.mL^{-1} penicillin, and 10 000 U.mL^{-1} streptomycin solution (Lonza). They were often maintained at 37°C in a humidified atmosphere with 5 % CO_2 and 95 % air.

For the experiments, cells were prepared using standard trypsinization procedure with trypsin/EDTA (170 mg L^{-1} trypsine, 200 mg.mL^{-1} EDTA, LONZA) and seeded in 96-well-bottom plates for viability assays. The cells were incubated for 24 h and then treated with the appropriate extract dissolved in DMSO and diluted in culture medium. The final DMSO concentration that did not exceed 1 % had no influence on cell viability.

Antiproliferative Activity

Antiproliferative activity of the leaf extract was evaluated according to the OECD guideline 425 with some modifications[29]. In brief, 100 µL of HaCaT or B16 cells suspension at 2.10^4 cells mL^{-1} were added in each well to be incubated at 37°C the RPMI culture medium in 5 % CO_2 atmosphere for 24 h to form monolayers. Then, the 96-well plates were incubated for 48 h with five different concentrations of the tested compound (in the range 1–200 µg.mL^{-1}). Doxorubicin, an anti-cancer drug, was used as a positive control. Cell viability is measured using the MTT assay as previously described. The absorbance of the developed yellow color, which was directly proportional to the cells viability, was measured by an automated microplate reader (IMARK, BioRad) at 570 nm. The results are presented in percentage where the untreated cells were considered having 100 % of cell viability.

Statistical Methods

Data are expressed as means ± standard deviations of three replicates.

Cell viability at each concentration of the tested compound was compared to that of the untreated control using Student t-test for adequacy. Computations were performed using Microsoft® Excel 2010 (function T.INV.2T to obtain Student two-sided critical values at   5 %, 1 %, and 0.1 % with parameter n=3-1=2).

RESULTS

Chemical Analysis

The yield of the leaves methanol extraction was 14%. The contents of the different types of polyphenols are reported in Table 1. The values of TP, FLAV, TAN, and ANT were

Table 1: Total phenolic (TP), flavonoid (FLAV), tannin (TAN) and anthocyanin (ANT) contents in dry plant material (DPM) and dry plant methanolic extract (DPE) of *L. peploides* leaves.

Polyphenol compounds	TP as mg eq. GAE	FLAV as mg eq. CATE	TAN as mg eq. CATE	ANT as mg eq. C3GE
g^{-1} DPM	122.8 ± 0.1	13.2 ± 0.2	9.2 ± 0.0	32.7 ± 0.2
g^{-1} DPE	17.2 ± 0.2	1.8 ± 0.0	1.3 ± 0.0	± 0.1

Table 2: DPPH radical and NBT superoxide radical scavenging activities, expressed as IC_{50}, for *L. peploides* leaf extract (DPE), gallic acid, quercetin and ascorbic acid being used as references.

	DPE	Gallic acid	Quercetin	Ascorbic acid
DPPH IC_{50} $\mu g.mL^{-1}$	58.0 ± 6.0	6.2 ±0.5	9.0±1.0	ND
NBT IC_{50} $\mu g. mL^{-1}$	30.0 ± 2.8	ND	ND	7.5± 1.0

ND = not determined

only indicative of a state of development of *L. peploides* which, in this study, corresponds to the maximum biological activity of the plant[7]. Indeed, their relative proportions vary as in the cycle of any plant.

Table 2 shows the potential of the methanol leaf extract to scavenge DPPH or NBT free radicals. The IC_{50} values obtained with the extract were higher than those of the reference products, gallic acid, quercetin and ascorbic acid.

Biological Analysis

Antimicrobial Capacities

Table 3 shows the antimicrobial activities of both, the crude extract and doxycycline. The latter was used as reference as it is an antibiotic which is generally prescribed to treat acne vulgaris. The leaf extract was found to be active against all skin microorganisms tested, *P. acnes, S aureus, S. epidermidis, S. enterica, E. coli,* and *C. albicans*, regardless of the strain Gram type. Its highest activity was obtained against *P. acnes* and *S. epidermidis*, with respectively an average inhibition zone of 37 and 28.3 mm and an MIC of 1.9 and 3.9 $\mu g.mL^{-1}$. For these two strains, the MBC was equal to 12.5 $\mu g.mL^{-1}$. However, a higher MIC of 5 $\mu g.mL^{-1}$ was registered against *S. aureus* which was the most sensitive to the extract lethal effect with a MBC = 7.8 $\mu g.mL^{-1}$. A slightly higher MIC was obtained in case of *S. enterica* in comparison to the first three strains. But, the MBC was the same as for *P. acnes* and *S. epidermidis*. Finally, *E. coli* and *C. albicans* were the least sensible to the extract among all the strains tested which were more sensitive to doxycycline than to the extract.

Cytotoxicity Test

In order to estimate the safety of the methanol extract, its cytotoxicity was evaluated by the MTT assay measuring the viability of macrophage-like cells, THP-1, treated with the extract at various concentrations (Fig.1). Statistically, the extract had a slight significant promotion on the cellular development for the lowest concentration (9.37$\mu g.mL^{-1}$), corresponding likely to a hormetic effect. For the two following values (18.75 $\mu g.mL^{-1}$and 37.5$\mu g.mL^{-1}$), the extract did not have a significant effect on the viability of THP-1 cells in contrast to the Triton X-100, which caused a 95.2 % of cell death. For the three

highest concentrations tested, a partial inhibition of cell growth was observed (Fig.1).

Antiproliferative Activity

The antitumor reference, doxorubicin, was toxic against the HaCaT cell line (normal human keratinocytes) with an IC_{50} of 0.04 ± 0.01 $\mu g.mL^{-1}$, whereas the *L. peploides* extract was not active against this cell line with an $IC_{50}>$ 200 $\mu g.mL^{-1}$ (Table 4). On the other hand, the crude extract showed a stronger cytotoxic activity against B16 cancer cell lines with an IC_{50} of 5.5 ± 2.3 $\mu g.mL^{-1}$ in comparison to doxorubicin which displayed an IC_{50} of 16 ± 1.2 $\mu g.mL^{-1}$.

DISCUSSION

The antimicrobial activity of *L. peploides*[7] and other *Ludwigia* species have been previously reported[30,5,31,32,33,34]; however, the antioxidant, anti-acne, and anti-proliferative properties of *L. peploides* have never been studied.

Measures of the antioxidant activity of *L. peploides* leaf extract suggest that the latter had effective scavenging activities against free radical (IC_{50} = 58.0 ± 6.0 $\mu g.mL^{-1}$) and superoxide anion (IC_{50}= 30 ± 2.8 $\mu g.mL^{-1}$). Khoudali et al. (2014)[35] indicated similar values for methanolic extracts of *Chamaerops humilis* leaves (24.5 $\mu g.mL^{-1}$) and for α-tocopherol (26 $\mu g.mL^{-1}$). However, the *L. peploides* extract had a lower antioxidant activity than extracts of known medicinal plants which have registered stronger scavenging activities such as *Cerasus avium* L.[36], with an IC_{50} of 17.4 μg mL^{-1}, and extracts of young fruit rinds of *G. mangostana* L., with an IC_{50} of 5.6 μg mL^{-1} [37].

Because of their scavenging ability due to their hydroxyl groups, phenolic compounds are often responsible for the antioxidant activity. The principal types of polyphenols, TP, FLAV, TAN, and ANT, were detected in *L. peploides* leaf extract. Our results were similar to those obtained by Yakob et al. (2012)[34] for the methanolic leaf extract of *Ludwigia octovalvis*. However, the phenolic content of 112 traditional Chinese medicinal plants reported by Cai et al. (2004)[38] ranged from 2.2 to 503 mg eq. GAE g-1 of DPM with average value of 38.7 mg eq. GAE g-1 of DPM, indicates that *L. peploides* (122.8 ± 0.1 mg eq. GAE g^{-1} DPM) cannot be classified among plants rich in polyphenol content.

Our study revealed that *L. peoploides* displayed a potent activity against *P. acnes* (Table 3), better than those mentioned by Pothitirat et al. (2009)[37] for *Garcinia mangostana* young fruit extract, used in Thai traditional medicines, (MIC= 15.63 $\mu g.mL^{-1}$ and MBC = 31.25 $\mu g.mL^{-1}$). Among a great number of plant extracts, Sinha et al. (2014)[39] reported the lowest MIC values against *P.*

Table 3: Antimicrobial activities of the *L. peploides* leaf extract against skin microbial strains. Results are expressed according to the disc diffusion method and measures of the MIC and the MBC, where doxycycline (Dcl) was used as the reference.

| Bacterial strain	Inhibition diameter (mm)		MIC µg mL^{-1}		MBC µg mL^{-1}		MBC/MIC	
	Extract	Dcl	Extract	Dcl	Extract	Dcl	Extract	Dcl
P. acnes	37.0 ± 0.0	49.7 ± 0.0	1.9	0.12	12.5	0.97	6.6	8.1
S. aureus	18.0 ± 1.5	32.0 ± 0.1	5.0	0.24	7.8	3.90	1.6	16.3
S. epidermidis	24.7 ± 0.5	37.8 ± 0.0	3.9	0.48	12.5	1.90	3.2	4.0
S. enterica	28.3 ±2.0	44.0 ± 0.1	7.8	1.90	12.5	1.90	1.6	1.0
E. coli	27.0 ± 1.9	32.0 ± 0.2	12.5	> 500	ND	ND	ND	ND
C. albicans	28.0 ± 0.0	36.5 ± 0.1	25	0.48	ND	250	ND	521.0

ND = not determined
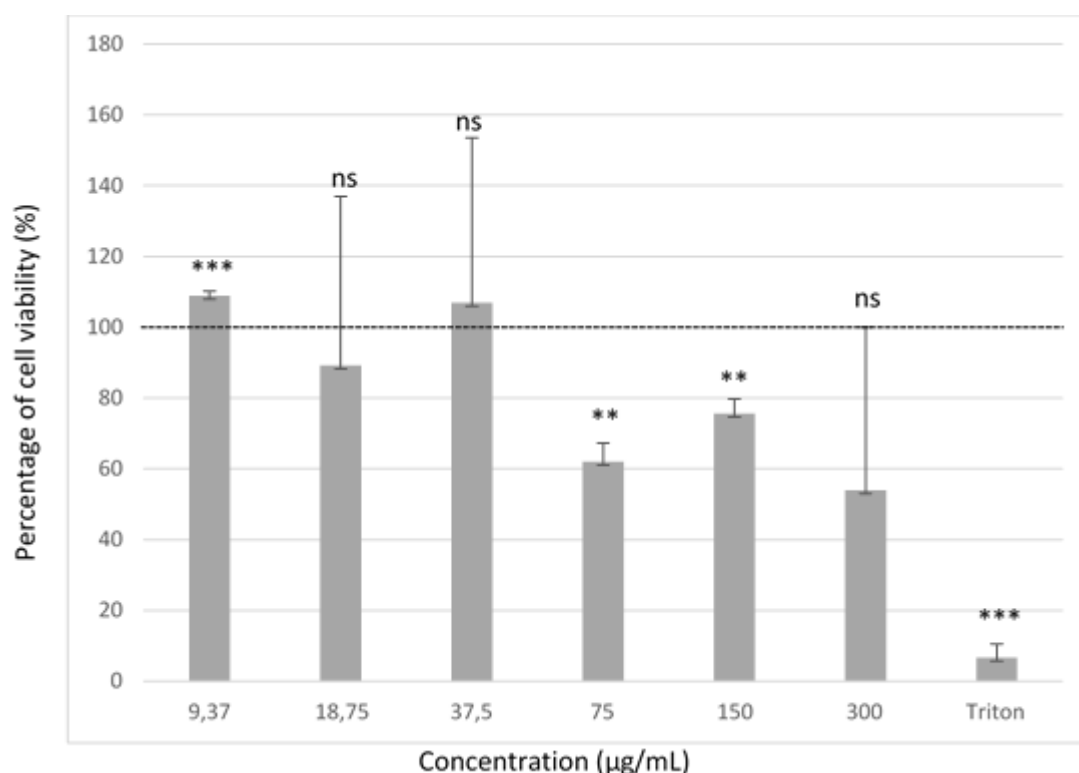
Figure 1: Evaluation of *L. peploides* leaf extract cytotoxicity on THP-1 cells by MTT assay. ns P>0.05; **P <0.01; ***P <0.001 for comparison with 100 % viability threshold (dashed line). Error bars are defined according to 95 % confidence interval upper limit.

acnes between 4 and 50 µg.mL^{-1} for *Punica granatum*, *Morus alba*, *Amanoa anomala* and *Mahonia aquifolium*. However, Nand *et al.* (2012)[40] reported that the MIC values of the methanolic extract from *Camellia sinensis* plant, belonging to *Theaceae* family, were 1250 µg.mL^{-1} against *P. acnes* and *S. aureus* and 650 µg.mL^{-1} against *S. epidermidis*. The MICs were significantly higher than those obtained with *L. peploides* (Table 3).
According to Aloni-Grinstein *et al.* (2015)[41], a compound can be considered bactericidal when its MBC/MIC ratio is lower than four and only bacteriostatic when this ratio is higher than four. In this context, the *L. peoploides* leaf extract can be considered as bacteriostatic against *P. acnes* and bactericidal against *S. aureus, S. epidermidis,* and *S. enterica*, while doxycycline is bacteriostatic against *P. acnes* and *S. aureus,* and bactericidal against *S. epidermidis* and *S. enterica*. The *E. coli* and *C. albicans*

strains used can be considered as tolerant. Since the MBC was too high to be determined (*E. coli*) or 32 times higher than the MIC (*C. albicans*).
All the MIC and MBC values recorded for *P. acnes*, *S. aureus*, *S. epidermidis,* and *S. enteric* were lower than 37.5 µg mL^{-1} (Table 1), the value above that an inhibition of THP-1 cell growth (Figure 1) could render the extract unusable. For *E. coli* and *C. albicans*, only the MIC values were lower than this limit.
L. peploides leaf extract provided also interesting antiproliferative activity against B16 cancer cell lines (Table 4). In fact, our results (IC$_{50}$ = 5.5 µg.mL^{-1}) obtained with an unpurified extract were better than those of Yang *et al.*(2000)[42]. These authors reported IC$_{50}$ values ranging between 9.7 and 57.5 µg.mL^{-1} on several human cancer cell lines with (–)-epigallocatechin-3-gallate, a purified polyphenol present in green and black tea, which inhibited

Table 4: Antitproliferative activity of *L. peploides* leaf extract expressed as IC_{50} ($\mu g.mL^{-1}$) on HaCaT and B16 cell lines using MTT assay. Doxorubicin was used as the reference.

Cell lines	HaCaT	B16
L. peploides IC_{50} $\mu g.mL^{-1}$	>200	5.5 ± 2.3
Doxorubicin IC_{50} $\mu g.mL^{-1}$	0.04 ± 0.01	16 ± 1.2

many cell lines. The same molecule has displayed an IC_{50} ranging from 17.6 to 35.2 $\mu g.mL^{-1}$ against prostate cancer cells[43].

The anti-acne and anti-carcinogenic activities of plant extracts, notably of green and black teas[42,44,15], are often associated to their antioxidant potential since reactive oxygen species are in part responsible for these diseases. This correlation does not seem so obvious for *L. peploides* extracts. Only a statistical study conducted from May to October, period covering the life cycle of *L. peploides* from its post-winter development to its senescence stage, could determine eventual relationships between polyphenol contents, anti-oxidant, anti-acne, and antiproliferative properties of leaf extracts.

The cytotoxicity test showed that the extract concentration (37.5 $\mu g\ mL^{-1}$), beyond which the viability of macrophage-like cells THP-1 was inferior to 100 %, was clearly compatible with the MIC and MBC values against the principal microbial strains responsible for the acne disease and with the IC_{50} for the B16 cancer cell lines. However, according to Saric et *al.*[15], the bioactivity of plant compounds depends mainly on their chemical structure and their bioavailability that sometimes leads to the combination of plant extracts with conventional antibiotics or anti-tumor drugs[16].

CONCLUSION

Although plants have been used therapeutically for millennia, in a more or less empirical way, biomedical research is becoming more and more interested in them by scientifically highlighting their different capacities, determining the active molecules responsible and using them. This renewed interest comes from the fact that medicinal plants represent an inexhaustible source of natural bioactive compounds, with structural diversity and reduced side effects.

It is in this context that our work has been developed on the study of *L. peploides*. Leaves of this species have showed encouraging anti-acne and antiproliferative properties that should be studied more thoroughly to be able to consider *L. peploides* as a new source of compounds which are better than the current drugs that have led to the emergence of resistance among many microbial strains and cancerous cell lines. The rapid propagation of *L. peploides* and its high growth rate could be easily compatible with an annual exploitation of the biomass of *L. peploides* for therapeutic or cosmetic purposes.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.This article does not contain any studies with human participants or animals performed by any of the authors.

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