

Research Article

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Lucia Pirvu*, Isabela Nicorescu, Cristina Hlevca, Bujor Albu, Valentin Nicorescu

Epilobi Hirsuti Herba Extracts Influence the *In Vitro* Activity of Common Antibiotics on Standard Bacteria

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Abstract: *Epilobium* genus has been confirmed as an effective source of natural antimicrobials. However, the influence of *Epilobi hirsuti herba* derived products on usual antibiotics activity has not been studied. In this study, several standardized *Epilobi hirsuti herba extracts* (EHE) were evaluated in order to assess their potential effects on usual antibiotics tested on standard Gram-positive and Gram-negative bacterial strains *in vitro*. The results emphasized that the bacterial strains ranged from sensitive (MIC values between 50–200 µg GAE mL⁻¹) (*S. epidermidis* ATCC 12228) to very resistant (*E. coli* strains), *E. faecalis* ATCC 29212 being practically immune to EHE. In terms of synergistic interaction, Tetracycline and Ampicillin combinations lead to the most important stimulatory effects, the diameters of the inhibition zone being even 60% bigger compared to the antibiotic alone. Synergistic effects between myricetin(galloyl) derivatives and Tetracycline were also revealed on *P. aeruginosa* and *E. coli* strains. Together, it clearly demonstrated not only EHE's own antimicrobial properties, but also their capacity to influence the antimicrobial potency of some common antibiotics. These results could be useful for the area of herbal medicines and as potential candidates in managing microbial resistance, but also for physicians and pharmacists using combined antibacterial therapy.

Keywords: great willowherb, antimicrobial, interaction with antibiotics

1 Introduction

Epilobium species (*E. hirsutum*, *angustifolium*, *E. parviflorum*, *E. rosmarinifolium*, *E. spicatum*, *E. tetragonum*) have been confirmed to have effective antimicrobial [1-3], anti-inflammatory [4-6] and analgesic [7] properties as well as with certain antiproliferative and cancer preventive effects on prostate tissue [8-11]. Furthermore, *Epilobium* species are described as non-toxic [6]. *Epilobium* species' active phytochemicals are polyphenols such as ellagitannins, flavonoids (e.g., anthocyanidins and miricetin and quercetin derivatives) and gallic acid derivatives along with saponins, volatile oils and vitamins [12-14], essential amino acids (e.g., tyrosine, serine, threonine, cysteine, valine, serine, leucine and isoleucine, phenylalanine, proline) [15] and minerals (e.g., K, Mg, P, S, Cu, Co, Zn) [16].

Regarding the antimicrobial activity of *Epilobium* genus, studies [1] on a series of ethanolic extracts from *E. angustifolium*, *E. hirsutum*, *E. palustre*, *E. tetragonum* and *E. rosmarinifolium* supplied from (Boiron) France were tested on Gram-positive (*Staphylococcus aureus* two strains, *Streptococcus pyogenes* ATCC 12345, *Streptococcus sanguis* CDC SS 910, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis*, *Listeria monocytogenes* ATCC 19111) and Gram-negative (*Escherichia coli* ATCC 15221, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 7853, *Shigella flexneri* CDC 9767 IAL 1517 and *Salmonella enteritidis* IAL 1132) bacteria, yeasts (*Candida albicans* two strains, *Candida glabrata* two strains and *Candida krusei*) and fungi (*Microsporum canis*, *Microsporum gypseum* two strains, *Tricophyton rubrum* and *Tricophyton mentagrophytes* four strains) indicated that all extracts acted against studied bacteria in a range of concentrations measuring from 10 to 650 µg mL⁻¹ dry

*Corresponding author: Lucia Pirvu: National Institute for Chemical-Pharmaceutical Research and Development (ICCF), Vitan 112, 031299, Bucharest, Romania, E-mail: lucia.pirvu@yahoo.com
Isabela Nicorescu: Institute of Hygiene and Veterinary Public Health, Campul Mosilor 5, 021201, Bucharest, Romania
Cristina Hlevca, Bujor Albu: National Institute for Chemical-Pharmaceutical Research and Development (ICCF), Vitan 112, 031299, Bucharest, Romania
Valentin Nicorescu: University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Splaiul Independentei 105, 050097, Bucharest, Romania

extract; *E. angustifolium* and *E. rosmarinifolium* acted against yeasts and fungi as well.

Similarly, studies [2] on flowers and leaves of *Epilobium angustifolium* L. collected from Croatia (Mt. Velebit) that were tested on standard ATCC and clinical isolated *Staphylococcus aureus* (including MRSA), *Bacillus subtilis*, *Escherichia coli* (including p-fimbriae positive strain), *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Candida albicans*, *C. tropicalis*, *C. dubliniensis* and *Saccharomyces cerevisiae* confirmed antimicrobial potency of both plant pieces and MIC values ranging from 4.6 ± 0.2 to 18.2 ± 0.8 mg mL⁻¹. Our previous studies [14,17] on different whole and selective *Epilobi hirsuti* extracts using raw material (*herba*) from Romanian Carpathians highlighted the antimicrobial activity of the tested extracts against *Staphylococcus aureus* (standard ATCC and clinical isolated strains) and *E. coli* (standard ATCC strains).

Given the high therapeutic potential of *Epilobium* species, this work was aimed at evaluating potential interactions between some whole and selective, polar and non-polar, standardized *Epilobi hirsuti herba* extracts and several well known antibacterial drugs, and were tested on nine (standard) Gram-positive and Gram-negative bacteria.

This interest is motivated by current worldwide concern regarding the increasing phenomenon of bacterial resistance to antimicrobial drugs in parallel with more and more scientific proofs of the effectiveness of plant compounds in managing microbial infections. Therefore, the effect of cooperation between different plant compounds is well known, as well as that between plant compounds and chemical drugs leading to more complex biological benefits. The more so as there is ample literature data confirming additive and synergistic effects between plant compounds and susceptible antibiotics [18-20].

2 Experimental Procedure

2.1 Materials

2.1.1 Plant material description

Fresh mature aerial part (*herba*) of *Epilobium hirsutum* L. (*Onagraceae* family) was harvested in August from the wild, Romanian Carpathians respectively, at about 1000 m altitude (Sinaia region). The plant material was authenticated by the specialist's of National Institute of Chemical-Pharmaceutical R&D (ICCF), Bucharest,

Romania; specimen samples are deposited in ICCF Plant Material Storing Room (EH_AM2013-P8). *Epilobi hirsuti herba* raw material was shade dried and ground to a medium-size plant powder.

2.1.2 Vegetal extracts preparation

The plant powder was (twice) extracted in 70% (v/v) ethanol (500 mL) for 1 hour at 80–85°C. The extracts were mixed and filtered using paper filter Whatman No. 1 resulting in 850 ± 20 mL final ethanolic extract (codified E8).

The filtrate obtained with ethanol (250 mL E8) was concentrated to dryness under reduced pressure using a rotary evaporator (Büchi) and the resulting *sicc* product was passed into 20% (v/v) propylene glycol (PG) solution to provide the exactly 5 mg total phenols (expressed as gallic acid equivalents [GAE]) per 1 mL of extract/sample. The resulting standardized propylene glycol extract is further called great willowherb *whole* extract (codified P8).

Other 250 mL of filtrate obtained with ethanol (E8) were concentrated under reduced pressure and the final *sicc* product was solved into 100 mL of distilled water. The resulting aqueous solution was further (manually) extracted, first with (3 × 100 mL) chloroform and then with (3 × 100 mL) ethyl acetate, 24 hours for each stage of extraction. Aqueous and ethyl acetate fractions were (separately) concentrated at residue and the residues were passed into 20% PG so as to obtain the identical final concentration of 5 mg total phenols (GAE) per 1 mL of sample; the chloroform fraction was also concentrated at *spiss* product then passed into 20% PG by the whole extract (P8) algorithm, thus obtaining the equivalent of the whole extract (P8) as concerning the total non-polar compounds. Three standardized fractions resulted: the aqueous fraction (codified P8aq), ethyl acetate fraction (codified P8ea) and chloroform fraction (codified P8chl), further called *Epilobi hirsuti herba* selective extracts.

The *whole* extract (P8) as well as the three *selective* extracts (P8aq, P8ea and P8chl) were separately divided into 2 mL Eppendorf tubes and stored at -8°C until microbiological studies.

2.1.3 Chemicals, reagents and references

Reagents (*Folin-Ciocalteu* and *Natural Product*), solvents (methanol, ethanol, ethyl acetate, formic acid, acetic acid and chloroform) and reference compounds quercetin (95%), rutin (min. 95%), chlorogenic acid (>95%), caffeic

acid (99%) and gallic acid (95%) were supplied by Sigma-Aldrich and Fluka (Bucharest, Romania).

2.2 Experimental Design

2.2.1 Qualitative (HP)TLC analysis

Studies were performed according to Wagner and Bladt's [21] and Reich and Schibli's [22], general method for polyphenols assessment, and detailed in our previous studies [14,17].

2.2.2 Estimation of Total Phenolics Content

Studies were performed according to a standard method described in *Romanian Pharmacopoeia* [23]. Briefly, three aliquots of 50–100 μL vegetal samples were mixed with 200 μL of Folin-Ciocalteu reagent and accurately finished at 5000 μL volumetric flasks with (5% w/v) sodium carbonate. Flasks were mixed shaken and left in the dark at room temperature for 5 minutes, and then the absorbance at maximum absorption wavelength ($\lambda = 750 \text{ nm}$) measured. Gallic acid standard calibration curve ($r^2 = 0.9989$) was used and the results were expressed as mg gallic acid and equivalent (GAE) per 1 mL of vegetal sample.

2.2.3 Antimicrobial Activity Assay

Test organisms: The tests were carried out on four Gram-positive bacteria, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228 and five Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 29245, *Escherichia coli* ATCC 35218, *E. coli* ATCC 11229, and *E. coli* ATCC 8739.

Test antimicrobials: Reference antibiotics (Biolab Zrt) included microtablets of Ampicillin (AM, 10 μg), Gentamicin (CN, 10 μg), Tetracycline (TE, 30 μg), Sulfamethoxazole/Trimethoprim (SXT, 25 μg), Ciprofloxacin (CIP, 5 μg) and Cefoxitine (FOX, 30 μg).

Inoculum preparation: Nutrient broth (Oxoid CM0001) and Muller-Hinton agar (Oxoid CM0337) were used as culture media. Test microorganisms were stored in freezing conditions and activated by cultivation in Nutrient broth at 37°C for 24 hours. Overnight cultures were diluted with Mueller-Hinton broth until a turbidity equivalent to 0.5 McFarland standard was achieved ($1-2 \times 10^8 \text{ CFU mL}^{-1}$) [24]. The purity of the cultures was

verified by streaking each culture on Nutrient agar supplemented with 5% sheep blood.

Minimum inhibitory concentration (MIC) assay:

The experiments were conducted using the reference method for testing the *in vitro* activity of the antimicrobial agents [25] where MIC is the lowest concentration of an antimicrobial agent that, under defined *in vitro* conditions, prevents the appearance of visible growth of the microorganism after its incubation overnight [25,26]. The MIC assay was carried out using an accurate working scheme (Table 1) as follows: four dilution series were prepared, corresponding to the four vegetal extracts tested, the whole extract (P8) and the three *selective* extracts, aqueous (P8aq), ethyl acetate (P8ea) and chloroform (P8chl) fractions, each of them precisely quantified for total phenol content. The dilutions were made by incorporation of each vegetal extract into Muller-Hinton Agar (MHA); MHA + PG 20% (4.1 mL + 0.9 mL) were used as negative control. Total phenol concentrations in the resulting culture medium ranged in the 25–900 $\mu\text{g GAE mL}^{-1}$ interval in the case of P8, P8aq and P8ea samples and between 1.25–45 $\mu\text{g GAE mL}^{-1}$ in case of P8chl samples.

After the solidification of MHA containing vegetal extracts, the bacterial inoculums (10^4 CFU/spot) were placed on the medium surface [27] and the plates were incubated for 24 hours at 37°C. The results were interpreted

Table 1: Dilution series for MIC assay - working scheme.

<i>E. hirsutum</i> extract (mL)	MHA (mL)	Total phenols – P8, P8aq, P8ea extracts ($\mu\text{g GAE mL}^{-1}$)	Total phenols – P8chl extract ($\mu\text{g GAE mL}^{-1}$)
0.900	4.100	900	45
0.800	4.200	800	40
0.700	4.300	700	35
0.600	4.400	600	30
0.500	4.500	500	25
0.450	4.550	450	22.5
0.400	4.600	400	20
0.350	4.650	350	17.5
0.300	4.700	300	15
0.250	4.750	250	12.5
0.200	4.800	200	10
0.150	4.850	150	7.5
0.100	4.900	100	5
0.050	4.950	50	2.5
0.025	4.975	25	1.25

by evaluating the bacterial growth, depending on the vegetal extract concentration.

Synergism assay: In order to evaluate the potential synergistic effects between the four *Epilobi hirsuti* extracts and antibacterial substances tested, the vegetal extracts were incorporated in MHA in concentrations which did not by themselves inhibit the bacterial growth (MIC/2) [28]. Separately, plates with MHA without vegetal extract were prepared and used as controls. The dried surface of the culture medium was inoculated with bacterial suspension ($1-2 \times 10^8$ UFC mL⁻¹) by streaking with a sterile cotton swab and subsequently the antimicrobial micro-tablets were distributed. The plates were incubated for 24 hours at 37°C, and then the antimicrobial effect was evaluated by measuring the inhibition area around the micro-tablets.

Statistical analysis: All assays were conducted in triplicate and the values were expressed as mean \pm (SD).

3 Results and Discussion

3.1 Qualitative and quantitative aspects of *Epilobi hirsuti herba* extracts

Similar to our previous study [14], *Epilobi hirsuti herba* whole extract (Fig. 1, T2 tracks) showed eight major polyphenolic compounds (s1 – s8 spots), myricetin (the five intense red-orange fluorescent zones represented of s2/s2'', s3, s5, s6 and s7 spots) and myricetin-galloyl derivatives (the two indigo fl. zones represented of s1 and s4 spots) as well as two phenolic acids, chlorogenic acid (blue fl. zone overlapped by myricetin derivate – s2/s2' spot) and gallic acid aglycone (dark blue fl. zone – s8 spot).

Epilobi hirsuti herba selective extracts (Fig. 1, T2aq, T2ea and T2chl tracks) revealed the following qualitative aspects: the aqueous fraction (T2aq tracks) showed two major phenolics, chlorogenic acid (s2') and one kaempferol derivate (s2'''); the ethyl acetate fraction (T2ea tracks) showed myricetin and myricetin-galloyl derivatives; the chloroform fraction (T2chl track) did not reveal the type of polyphenols compounds.

With regards to quantitative aspects, *Epilobi hirsuti herba* polar extracts including the whole extract (P8) as well as the two selective extracts, aqueous (P8 aq) and ethyl acetate (P8ea) fractions were made so as to assure identical total phenols content, 5 mg GAE mL⁻¹ sample. As for the selective, non-polar, chloroform (P8chl) fraction, although chloroform is not a solvent in which polyphenols are typically soluble and extracted, our laboratory practice

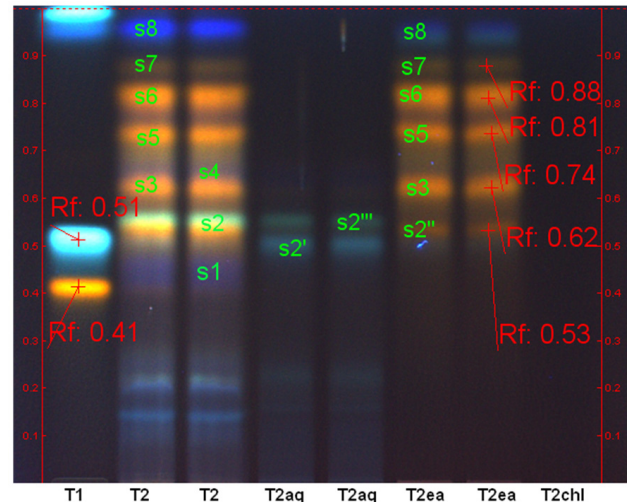


Figure 1: Qualitative (HPTLC) analytical aspects of *Epilobi hirsuti herba* whole and selective extracts. Track T1, rutin, chlorogenic acid and caffeic acid (ref.); Tracks T2, great willowherb whole extract (duplicate); Tracks T2aq, selective aqueous extract (duplicate); Tracks T2ea, selective ethyl acetate extract (duplicate); Track T2chl, selective chloroform extract (single sample).

has revealed that chloroform extracts often contain small amounts of polyphenol compounds. In the particular case of *Epilobi hirsuti herba* extracts, the chloroform fraction (P8chl) showed 0.25 mg total phenols (GAE) mL⁻¹ extract.

Therefore, the four *Epilobi hirsuti herba* extracts (EHE) differed in terms of qualitative content, but were identical in terms of quantitative content and the chloroform fraction was designed to provide the same amount of non-polar compounds as the whole extract does. This allows further assessments of *Epilobi hirsuti herba* polar and non-polar compounds' contribution to antimicrobial activity and synergistic effects in combination with usual antibiotics on standard Gram-positive and Gram-negative microbial strains.

3.2 Evaluation of the minimum inhibitory concentration (MIC)

Foremost it must be noted that during the tests, the nine bacterial strains properly developed on MHA and MHA + PG culture medium, thus demonstrating the lack of influence of propylene glycol solution (PG 20%, v/v) on bacterial growth. MIC values of the four *Epilobi hirsuti herba* extracts tested on the (nine) bacterial strains are shown in Table 2.

The results (Table 2) indicated good antimicrobial activity of the three polar, polyphenolic extracts (whole/

P8, aqueous/P8aq and ethyl acetate/P8ea extracts), which were effective on five microbial strains, *S. epidermidis* ATCC 12228, *P. mirabilis* ATCC 29245, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *S. aureus* ATCC 6538 respectively; MIC values ranged between 50 - 350 µg GAE mL⁻¹. Also, it clearly revealed the lack of activity of *Epilobi hirsuti* extracts on *E. faecalis* ATCC 29212 and the three *E. coli* strains (MIC > 500 µg GAE mL⁻¹). The chloroform fraction (P8chl) proved to be effective mainly on *S. epidermidis* ATCC 12228 and, in a smaller extent, on *S. aureus* ATCC 25923. On the other seven microbial strains, the P8chl extract was basically ineffective.

3.3 Evaluation of the synergistic effects upon several usual antibiotics

Studies included six common antibiotics massively used for treating infections worldwide; their continuously increasing susceptibility to bacterial resistance is confirmed by official reports of the European Union [29]). Furthermore, each selected substance for the assay belongs to a separate class of antibacterials as follows: Penicillins (Ampicillin/AM), Aminoglycosides (Gentamicin/CN), Tetracyclines (Tetracycline/TE), Potentiated Sulphonamides (Sulfamethoxazole-Trimethoprim/SXT), Fluoroquinolones (Ciprofloxacin/CIP), and Cephalosporins (Cefoxitine/FOX).

As the working principles, for those bacterial strains whose MIC values could not be determined (e.g., *E. faecalis* ATCC 29212) the synergism tests were not performed. For bacteria (e.g., *S. epidermidis* ATCC 12228) or fractions (e.g. P8chl) that showed MIC values lower than 200 µg GAE mL⁻¹ (high-susceptible) or higher than 900 µg GAE mL⁻¹ (low-susceptible), the synergism tests were not performed either; these samples are pointed out as ND (not determined). Significant synergy (stimulator effect) between the antimicrobial substances and *Epilobi hirsuti herba extracts* was considered when the enlargement of the combined inhibition zone of at least 5 mm was recorded [30]. The results are presented in Table 3.

Figs. 2–7 illustrate bacterial response after the treatment with respective vegetal-chemical antimicrobial combination (*Epilobi hirsuti herba* whole or selective extract plus antibiotic), stimulator response compared to the reference sample, relative ratio (%) respectively.

As can be seen in Fig. 2, the Tetracycline (TE) antibiotic acted much more effectively with whole and selective *Epilobi hirsuti* extracts on all tested strains, mainly on *P. aeruginosa* ATCC 27853 when combined with the ethyl acetate (P8ea) fraction previously shown as containing

Table 2: MIC values of *Epilobi hirsuti herba* extracts tested on ATCC bacterial strains.

No.	Bacterial strain	MIC (µg GAE mL ⁻¹)			
		Whole P8	Selective P8ea	extracts P8aq	P8chl*
1	<i>Staphylococcus aureus</i> ATCC 6538	350	250	300	45
2	<i>Staphylococcus aureus</i> ATCC 25923	200	150	100	22.5
3	<i>Enterococcus faecalis</i> ATCC 29212	> 900	> 900	> 900	> 45
4	<i>Pseudomonas aeruginosa</i> ATCC 27853	200	200	250	35
5	<i>Proteus mirabilis</i> ATCC 29245	150	150	100	25
6	<i>Staphylococcus epidermidis</i> ATCC 12228	50	100	50	7.5
7	<i>Escherichia coli</i> ATCC 35218	700	900	> 900	> 45
8	<i>Escherichia coli</i> ATCC 11229	700	700	900	> 45
9	<i>Escherichia coli</i> ATCC 8739	500	700	600	> 45

***Note:** Antimicrobial activity of the non-polar, P8chl fraction can be explained by particular non-polar compounds activity or by non-polar environment that favours antimicrobial activity of other compounds (e.g., polyphenols) thus allowing less augmented quantities of the latest. Given these and also considering the way in which the four extracts were prepared, in the specific case of chloroform fraction (P8chl), the above values (µg GAE mL⁻¹) represent the effective concentration of polyphenols in P8chl at the same dilution series applied for the whole (P8), aqueous (P8aq) and ethyl acetate (P8ea) extracts and less MIC results.

myricetin and myricetin-galloyl derivates; TE efficiency also increased on *E. coli* strains by combining with *Epilobi hirsuti* polar extracts, whole extract (P8), aqueous (P8eq) and ethyl acetate (P8ea) fractions respectively, as well as on *S. aureus* strains when combined with non-polar, chloroform (P8chl) fraction; TE and chloroform (P8chl) fraction also resulted in a increase of efficacy on *P. mirabilis* ATCC 29145.

Ampicillin (AM) antibiotic (Fig. 3) also acted more effective on two of the three *E. coli* strains when combined with *Epilobi hirsuti* polar extracts (P8, P8aq and Pea). Similar to TE, AM gained efficacy against *S. aureus* ATCC 25923 and *P. mirabilis* ATCC 29145, when combined with the non-polar, chloroform (P8chl) fraction.

Ciprofloxacin (CIP) indicated (Fig. 4) an increase of efficiency on *S. aureus* strains when combined with the chloroform (P8chl) fraction, as well as on *P. aeruginosa*

Table 3: Synergistic effects of *Epilobi hirsuti herba* extracts and antimicrobials on standard microbial strains.

Composition of culture medium	Microbial strain / Diameter of the inhibition zone (mm) ± SD						
	<i>S. aureus</i> 6538	<i>S. aureus</i> 25923	<i>P. aeruginosa</i> 27853	<i>P. mirabilis</i> 29245	<i>E. coli</i> 35218	<i>E. coli</i> 11229	<i>E. coli</i> 8739
TE combinations							
MHA+PG (reference)	32 ± 1.00	32 ± 1.32	18 ± 2.64	6 ± 0.00	25 ± 1.32	24 ± 0.86	24 ± 1.00
MHA+P8	32 ± 0.86	32 ± 1.73	24 ± 1.73	ND	30 ± 1.32	28 ± 1.00	29 ± 1.32
MHA+P8aq	38 ± 1.32	ND	22 ± 1.00	ND	ND	33 ± 1.80	32 ± 1.00
MHA+P8ea	33 ± 1.73	ND	28 ± 1.73	ND	34 ± 1.80	28 ± 1.73	31 ± 1.80
MHA+P8chl	40 ± 1.80	39 ± 0.50	21 ± 1.32	8 ± 1.00	ND	ND	ND
AM combinations							
MHA+PG (reference)	44 ± 1.32	31 ± 0.86	6 ± 0.00	19 ± 1.73	6 ± 0.00	13 ± 0.50	15 ± 1.73
MHA+P8	43 ± 1.00	32 ± 1.32	6 ± 0.00	ND	6 ± 0.00	18 ± 1.73	18 ± 0.5
MHA+P8aq	43 ± 1.73	ND	6 ± 0.00	ND	ND	18 ± 1.00	19 ± 1.00
MHA+P8ea	40 ± 1.32	ND	6 ± 0.00	ND	6 ± 0.00	21 ± 1.00	19 ± 0.86
MHA+P8chl	46 ± 1.73	36 ± 1.73	6 ± 0.00	24 ± 1.80	ND	ND	ND
CIP combinations							
MHA+PG (reference)	33 ± 1.00	33 ± 0.5	36 ± 2.02	42 ± 1.80	35 ± 1.73	33 ± 2.59	33 ± 1.00
MHA+P8	35 ± 1.73	33 ± 0.86	41 ± 1.73	ND	36 ± 1.73	34 ± 1.00	40 ± 1.00
MHA+P8aq	36 ± 1.73	ND	40 ± 1.32	ND	ND	36 ± 0.86	36 ± 1.73
MHA+P8ea	34 ± 0.86	ND	44 ± 2.64	ND	37 ± 2.64	36 ± 1.73	36 ± 1.00
MHA+P8chl	44 ± 0.86	38 ± 0.86	42 ± 1.73	40 ± 1.32	ND	ND	ND
CN combinations							
MHA+PG (reference)	24 ± 0.50	25 ± 0.50	30 ± 0.86	22 ± 1.50	20 ± 1.73	21 ± 0.86	21 ± 1.32
MHA+P8	26 ± 1.73	26 ± 1.50	28 ± 1.32	ND	17 ± 1.73	19 ± 1.50	20 ± 1.00
MHA+P8aq	26 ± 1.00	ND	30 ± 1.00	ND	ND	23 ± 0.50	20 ± 0.50
MHA+P8ea	24 ± 0.50	ND	32 ± 1.00	ND	20 ± 1.00	19 ± 2.29	21 ± 1.00
MHA+P8chl	26 ± 1.32	25 ± 0.5	31 ± 1.73	22 ± 1.00	ND	ND	ND
SXT combinations							
MHA+PG (reference)	26 ± 1.32	29 ± 1.32	6 ± 0.00	29 ± 1.32	22 ± 0.86	26 ± 0.86	28 ± 1.73
MHA+P8	26 ± 1.73	31 ± 1.73	6 ± 0.00	ND	21 ± 0.86	28 ± 1.73	32 ± 1.73
MHA+P8aq	34 ± 1.73	ND	6 ± 0.00	ND	ND	37 ± 0.50	36 ± 2.17
MHA+P8ea	26 ± 1.32	ND	6 ± 0.00	ND	22 ± 1.80	30 ± 1.80	34 ± 1.32
MHA+P8chl	32 ± 0.86	30 ± 1.80	6 ± 0.00	34 ± 1.32	ND	ND	ND
FOX combinations							
MHA+PG (reference)	29 ± 0.50	31 ± 1.32	6 ± 0.00	21 ± 1.80	26 ± 2.17	24 ± 1.00	25 ± 1.32
MHA+P8	29 ± 1.73	32 ± 1.00	6 ± 0.00	ND	24 ± 0.86	26 ± 0.86	29 ± 1.73
MHA+P8aq	30 ± 1.32	ND	6 ± 0.00	ND	ND	25 ± 1.00	30 ± 1.00
MHA+P8ea	30 ± 1.32	ND	6 ± 0.00	ND	26 ± 1.00	25 ± 1.32	30 ± 1.32
MHA+P8chl	38 ± 1.00	36 ± 0.86	6 ± 0.00	23 ± 1.00	ND	ND	ND

Where: ND = Not determined

ATCC 27853 by combining with all *Epilobi hirsuti* extracts, but especially with selective ethyl acetate (P8ea) fraction.

Gentamicin (CN) in combination with tested extracts (Fig. 5) indicated weak stimulatory effects and only on

S. aureus ATCC 6538, *E. coli* ATCC 11229 and *P. aeruginosa* ATCC 27853.

Sulfamethoxazole/Trimethoprim (SXT) combinations with polar extracts (Fig. 6) indicated an increase of

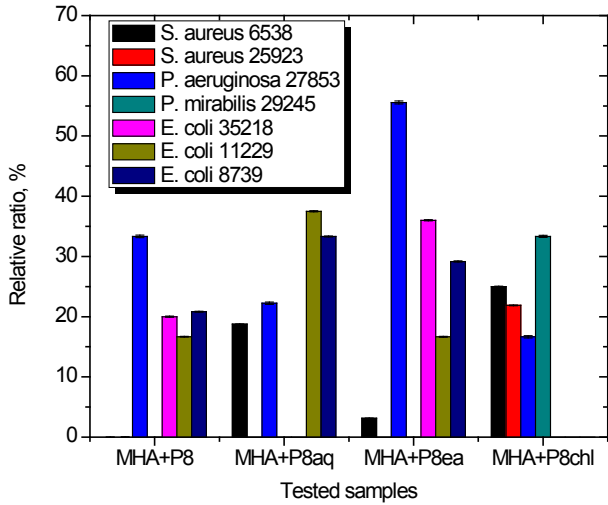


Figure 2: TE combinations.

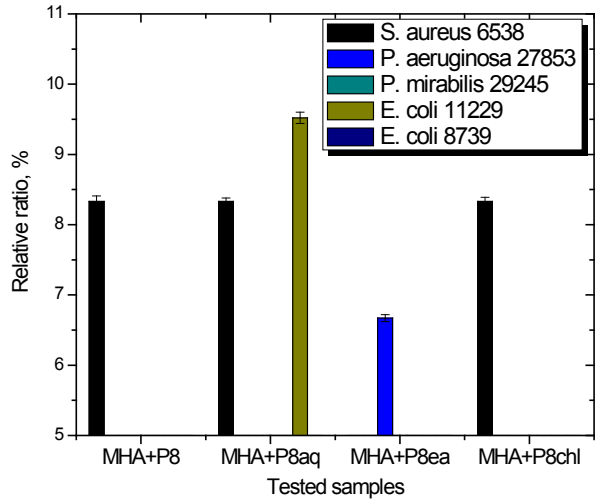


Figure 5: CN combinations.

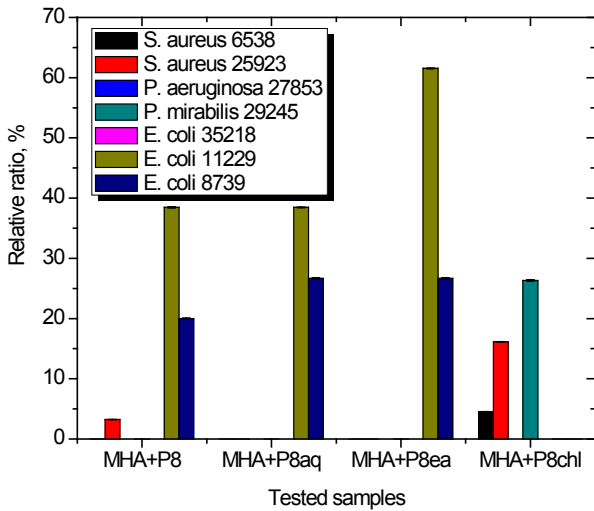


Figure 3: AM combinations.

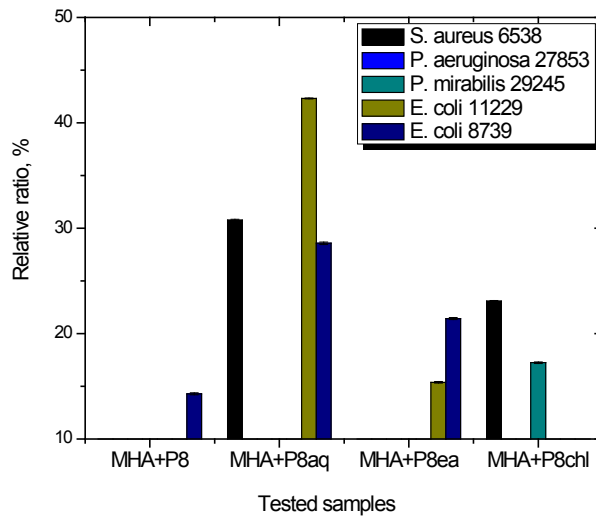


Figure 6: SXT combinations.

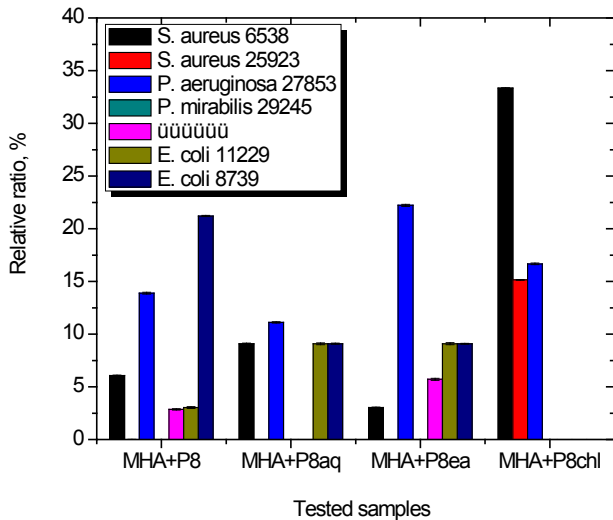


Figure 4: CIP combinations.

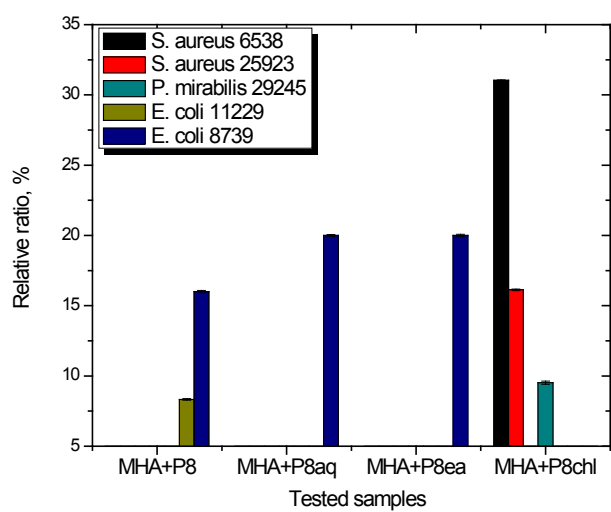
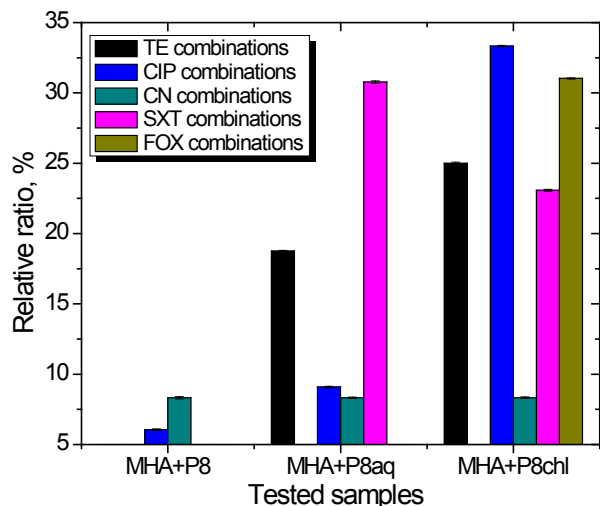
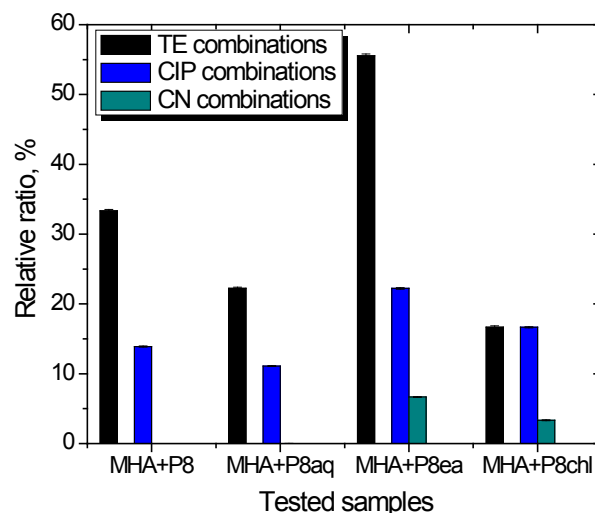
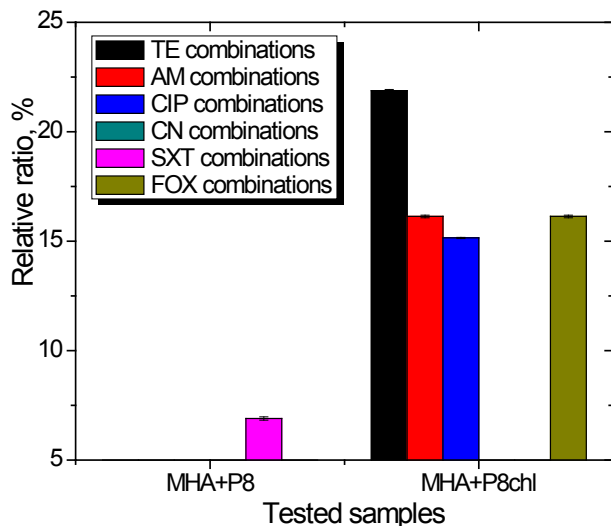
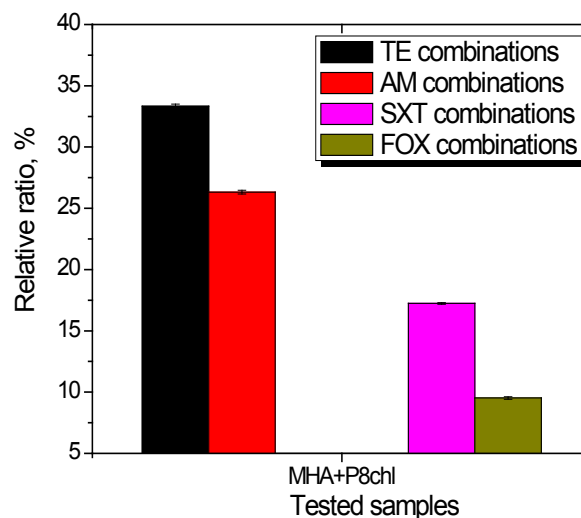


Figure 7: FOX combinations.

Figure 8: *S. aureus* ATCC 6538 response.Figure 10: *P. aeruginosa* ATCC 27853 response.Figure 9: *S. aureus* ATCC 25923 response.Figure 11: *P. mirabilis* ATCC 29245 response.

efficiency on two of the three *E. coli* strains, mainly the aqueous (P8ea) fraction previously shown as containing two phenolics, chlorogenic acid and kaempferol glycoside.

Cefoxitine (FOX) combinations (Fig. 7) lead to weak to moderate stimulatory effects on *S. aureus* and *E. coli* strains also by combining with non-polar and, respectively, polar extracts. SXT and FOX antibiotics, both of them gained efficacy against *P. mirabilis* ATCC 29245 by combining with non-polar, chloroform (P8chl) fraction.

In terms of the bacterial sensitivity (Figs. 8–14), for *S. aureus* ATCC 6538 (Fig. 8), the most significant synergistic effects were recorded for the combination of the non-polar, chloroform fraction (P8chl) with CIP, FOX, TE and SXT. Compared to reference sample (MHA + PG),

there were obtained rises of inhibition zones measuring from 6 to 11 mm, meaning 19 to 33% stimulatory effects.

For *S. aureus* ATCC 25923 (Fig. 9), there were synergistic interactions for the combination of the non-polar, chloroform (P8chl) fraction with TE, AM, FOX and CIP, the diameter of the inhibition zone increasing by 5 to 7 mm, quantified as 15 to 22% stimulatory effects.

P. aeruginosa ATCC 27853 (Fig. 10) indicated high sensitivity to TE and CIP combinations with all *Epilobi hirsuti herba* extracts, but especially with the selective, ethyl acetate (P8ea) fraction, leading to increases of the diameter of the inhibition zone by 22% (in case of CIP) and by 55% (in case of TE), compared to reference sample.

P. mirabilis ATCC 29245 (Fig. 11) showed an increase of sensitivity by combining TE, AM, SXT and FOX with

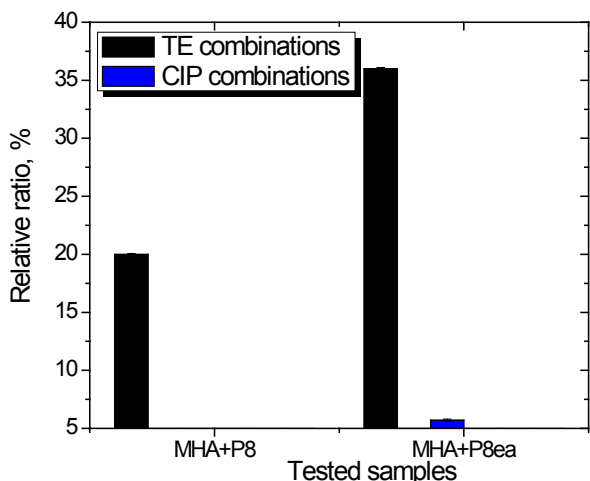


Figure 12: *E. coli* ATCC 35218 response.

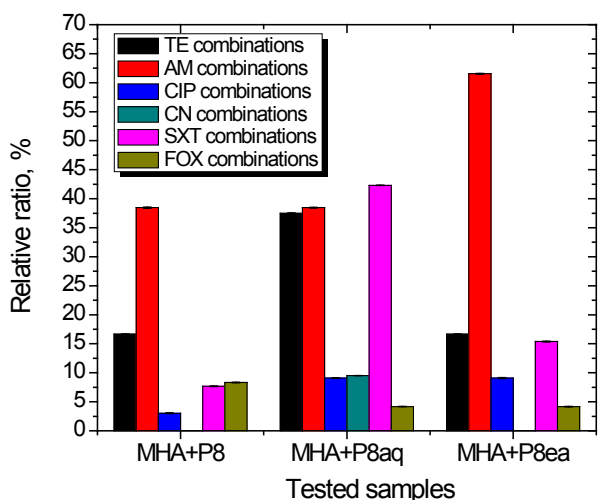


Figure 13: *E. coli* ATCC 11229 response.

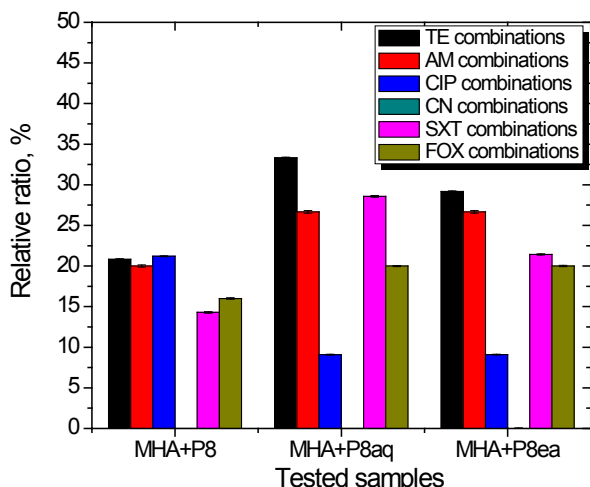


Figure 14: *E. coli* ATCC 8739 response.

the *Epilobi hirsuti* non-polar fraction (P8chl) resulting in the increase of the inhibition zones from 17 to 26%, also compared to reference sample.

In case of the *E. coli* ATCC 35218 strain (Fig. 12), a synergistic activity between TE and two polar extracts, the whole extract (P8) and selective ethyl acetate (P8ea) fraction has been noticed; the diameter of the inhibition zone, antimicrobial effect respectively, increased by 20 to 36%.

E. coli ATCC 11229 strain (Fig. 13) significantly reacted when combined AM with *Epilobi hirsuti* polar extracts; the whole extract P8 (by 38%), the selective aqueous P8aq fraction (by 38%) and ethyl acetate P8ea (by 61%) fraction. Synergistic effects were also recorded by combining the aqueous P8aq fraction with TE (by 37%) and SXT (by 42%) antibiotics. *E. coli* ATCC 8739 strain (Fig. 14) reacted when TE was combined with *Epilobi hirsuti herba* polar extracts; the whole extract (P8) lead to the increase of the inhibition zone by 21%, the selective aqueous (P8aq) fraction by 33% and ethyl acetate (P8ea) fraction by 29%. Significant increases of the inhibition zone diameters were highlighted for the combination of the selective aqueous (P8aq) fraction and selective ethyl acetate (P8ea) fraction with SXT (by 28% and 21%, respectively) and FOX (by 20% for both fractions) as well as by combining *Epilobi hirsuti herba* whole extract (P8) with CIP (by 21%), all compared to reference sample.

Collectively, our results suggest synergistic effects between myricetin and/or myricetin-galloyl derivates and Tetracycline, on *P. aeruginosa* and *E. coli* strains. Similarly, an increased efficacy of Amoxicillin, Ampicillin and Cefoxitine in combination with myricetin has been previously reported [20].

4 Conclusions

Epilobium hirsutum L. is a valuable phytomedicine worldwide renowned for its benefits on skin and prostate tissues thus resulting in numerous plant derived products, herbal medicines, cosmetics and hygiene products as well. The most frequent commercial applications of *Epilobium* sp. refer to topical curative (anti-irritant, anti-inflammatory and anti-oxidant) products. As for internal use, the extracts from *Epilobium* sp. can be found as different formulations (capsules, softgel capsules), single or in combination with other plant-derived products, primarily for prostate treatment. Romanian folk medicine and corresponding commercial products recommend *Epilobi hirsuti* plant derived products for treating urinary,

liver, gastric and intestinal disorders too. Given the high interest for *Epilobi hirsuti* plant derived products in managing urinary, but also various digestive system disorders, studying *Epilobi hirsuti herba* extracts capacity to influence the *in vitro* activity of some usual antibiotics is of high importance.

In this way, our studies indicated that, in terms of the susceptibility to *Epilobi hirsuti herba* extracts (EHE), the nine bacterial strains ranged from sensitive (*S. epidermidis* ATCC 12228) to very resistant (*E. coli* strains), *E. faecalis* ATCC 29212 being practically immune to *Epilobium* active compounds. Thus, if the polar extracts, including whole extract (P8), and aqueous (P8aq) and ethyl acetate (P8ea) fractions were very effective on *S. epidermidis* ATCC 12228, *P. mirabilis* ATCC 29245, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 strains (MIC values from 50 to 200 µg GAE mL⁻¹ sample), the non-polar, chloroform (P8chl) fraction, was effective mainly on *S. epidermidis* ATCC 12228.

In terms of the synergistic interaction with some common antimicrobials (antibiotics), Tetracycline (TE) established the biggest number of interactions (eleven) and with all tested *Epilobi hirsuti herba* whole and selective extracts, this property being highlighted by the enlargement of the inhibition zones diameter by 19 to 55% compared to the antibiotic alone. Comparatively, Ciprofloxacin (CIP) established six synergistic interactions with all tested extracts with the exception of selective aqueous fractions, their intensity ranging from 14% to 33% compared to the antibiotic alone. Sulfamethoxazole/Trimethoprim (SXT) established six synergistic interactions with selective fractions only, their intensity ranging from 17% to 42%. Ampicillin (AM) established five synergistic interactions with all *Epilobi hirsuti herba* whole and selective extracts, their intensity ranging from 16% to 61%. Cefoxitine (FOX) established four synergistic interactions with selective fractions only, their intensity ranging from 16% to 31%, while Gentamicin (CN) revealed only weak stimulator effects in combination with both, polar and non-polar *Epilobi hirsuti herba* extracts.

Together, these results demonstrate not only *Epilobi hirsuti herba* extracts own antimicrobial properties, but also the capacity to influence the antimicrobial potency of some common antibiotics.

These results could be useful for the area of herbal medicines and as potential candidates in managing microbial resistance, but also for physicians and pharmacists using combined antibacterial therapy.

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