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Therapeutic Effect of Irridoidic Compounds from *Plantago Species*

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The aim of the paper is to present the research study concerning the therapeutic effect of irridoidic extracts from Plantago sp. *"*in vitro*" tests performed on different microorganisms like* Streptoccocus aureus, Pseudomonas sp. *and* Bacillus subtilis *using a diffusive Kirby Bauer method shown the significantly bactericidal effect in comparison with common antibiotics. In the same time, "*in vivo*" tests performed on mice, reveal sedative and analgesic effects which appear after 60 minutes and 30 minutes after irridoidic extract administration.*

Keywords Analgesic; antimicrobial; irridoidic compounds; sedative

Introduction

Plantago sp. leaves have been used as wound healing remedy for centuries in almost all parts of the world. The cases for which this plant was used include diseases related to skin, pain relief and against infections. The leaves of *Plantago major* are used almost worldwide as a diuretic and astringent, and to treat wounds, insect stings, sunburn, skin diseases, eye irritation and inflammation of mouth and throat. In modern phytotherapy they are used to alleviate irritation in catarrh of the upper respiratory tract. Macerates, extracts, syrups and fresh juice are applied for these purposes. The roots are considered astringent and febrifuge, and used in decoction to treat cough. The seeds are considered demulcent, stimulant, diuretic and tonic, and are mainly used as a remedy for dysentery and diarrhea. Aucubin is one of the major iridoid glycosides isolated from *Plantago major*; the content in dried leaves can be up to 1.3%. It showed anti-inflammatory and hepatoprotective activities in tests with mice, spasmolytic properties in rats, and antiviral activity against hepatitis B virus.

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Plantago species contain biologically active compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, irridoids glycosides and terpenoids [1–3]. A large number of biologic activities has been found for plant extracts including anti-inflammatory, analgesic, antioxidant, weak antibiotic immuno modulating agent and antiulocerogenic activity [4,5].

Experimental

Irridoidic fraction was separated from *Plantago sp.* dry materials, according to scheme presented in Figure 1 [6].

The analgesic effect was tested using the writhing and hot plate test during 15; 30; 60 and 120 minute after intraperitoneal (i.p.) administration of the extract. Each time 2 groups with 8 mice each (25–35 g weight) were used, 1 group as witness and the second one as test.

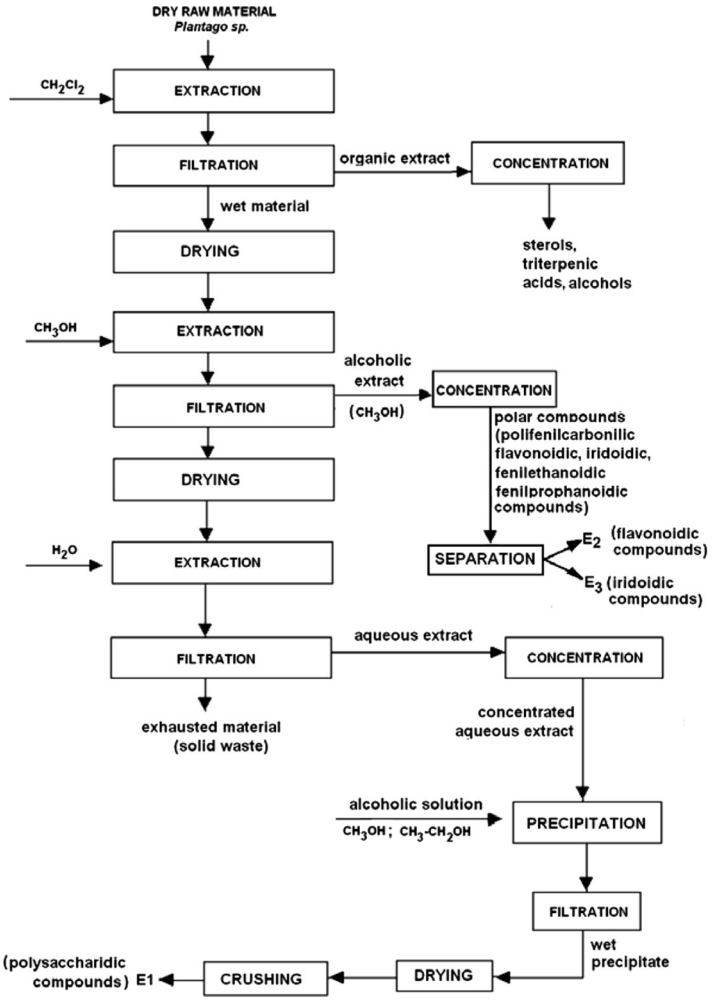

Figure 1. Irridoidic compounds separation scheme.

In the writhing test, each mice was injected i.p. with a solution of 0.75% CH_3COOH (0.15 mL)/10 g weight in order to provoke the writhing. Then, the mice from the test group were injected i.p. with irridoidic extract administrated as a solution in physiological serum (532 mg irridoidic extract dissolved in 10 mL physiological serum) 0.1 mL/10 g mice while for the mice from the witness group physiological serum was used, administrated i.p. 0.1 mL/10 g mice.

In the hot plate tests, each mice was placed on the hot plate at 55°C monitorizing both the sleek and leak parameters. Four times were tested: 15; 30; 60 and 120 minutes.

The sedative effect was tested using the single exploration test which evaluates the motion activity of a 2 lots, with 8 mice each (25–35 g weight). The active compound was i.p. administrated, with a dose of 532 mg *Plantago sp.* extract (irridoidic extract)/kg mice (532 mg was dissolved in 10 mL physiological serum), 0.1 mL/10 g mice. For the witness mice physiological serum was used administrated i.p. 0.1 mL/10 g mice. In the single exploration test a box of 300 × 300 × 400 mm was used, box in which the floor was divided in rectangles of 80 × 75 mm. Each mouse was put in one corner of the box and during 5 minutes the number of rectangles walked by the mouse was counted.

The antimicrobial effect was studied using the Kirby Bauer method for three pathogenic microorganisms: *Pseudomonas aeruginosa, Staphilococus aureus and Bacillus sp*., in comparison with commercial antibiotic. The composition of irridoidic extract established by HPLC method (using a HPLC type Agilent 1100 Series with DAD detector and column type Zorbax Eclipse XDB C18), was the following: 4.94% catalpol; 7.84% aucubin; 18.78% acteozid. Physico – chemical analyses of the solid extracts with irridoidic compounds have been performed by energy dispersive X-ray fluorescence using a spectrometer type PW 4025 MiniPal, atomic emission with inductively coupled plasma using a spectrometer type ICP-AES Varian Liberty 110, CHNS/O analyser type Perkin Elmer Series II, 2400, infrared spectra using a FT-IR spectrophotometer type Spectrum GX Perkin Elmer with accessories: DRIFT (Diffuse Reflectance Infrared Fourier Transform) and ATR (Attenuated Total Reflectance), thermo-gravimetric and differential thermal analysis using a Mettler-Toledo thermogravimetric analyzer type TGA/SDTA851[e] and Differential scanning *calorimeter type* DSC 823[e] Metter Toledo.

Results and Discussion

Analgesic Effect

In these tests, the mice groups treated with irridoidic compounds reveal the statistic significantly average number of writhing at 30 minute and 60 minute (results statistic

Table 1. Results obtaining in the writhing test

Writhing number	Witness 15 min	Irridoid extract 15 min	Witness 30 min	Irridoid extract 30 min	Witness 60 min	PL1 60 min	Irridoid extract 120 min	PL1 120 min
Average	10.5	7.13	16	6.9	18.37	8.75	11.5	13
STD	5.78	4.52	9.84	7.1	6.43	4.2	6.28	5.78
p-value		0.11		0.03		0.002		0.36

Table 2. Results obtaining in the hote–plate tests with sleek parameter

Time	Martor 15 min	Irridoid extract 15 min	Witness 30 min	Irridoid extract 30 min	Witness 60 min	Irridoid extract 60 min	Witness 120 min	Irridoid extract 120 min
Average	9.10	8.05	10.06	8.86	12.42	12.10	9.09	9.86
STD	2.11	1.97	4.74	3.33	4.94	6.45	1.54	3.09
p-value		0.10		0.32		0.46		0.27

Table 3. Results obtaining in the hote–plate tests with leak parameter

Time	Witness 15 min	Irridoid extract 15 min	Witness 30 min	Irridoid extract 30 min	Witness 60 min	Irridoid extract 60 min	Witness 120 min	Irridoid extract 120 min
Average	62.24	75.30	50.96	79.94	64.49	81.29	121.7	99.39
STD	22.04	31.67	12.91	35.95	28.40	15.54	29.24	24.92
p-value		0.17		0.025		0.08		0.061

significantly, $p < 0.005$). Analgesic effect was installed faster at 30 minute after iridoidic compounds administration, but keeps a short time in comparison with effect at 120 minute (Table 1).

In the hot-plate tests, sleek parameter is not significantly (Table 2) but the leak parameter reveal an apparent analgesic effect which appears at 30 minutes (Table 3) (value with statistic significance), probably due to acteoside content of irridoidic extract [3,7,8].

Sedativ Effect

Locomotor activity as the indicator of sedative effect, decrease significantly at 30 and 60 minutes after administration, but the effect at 60 minutes keep more time (Table 4). From this point of view, the true sedative effect appear at 60 minutes, probably due to aucubine and catalpol content from the extract [8].

Antimicrobial Effect

Effect of irridoidic extract against *Staphylococcus aureus* shown the powerful bactericidal effect (21 mm inhibition diameter) in comparison with azithromycin

Table 4. Results obtaining in the single exploration test

Square number	Witness 15 min	Irridoid extract 15 min	Witness 30 min	Irridoid extract 30 min	Witness 60 min	Irridoid extract 60 min	Witness 120 min	Irridoid extract 120 min
Average	75.88	53.75	91.00	26.50	128.00	31.88	87.63	72.63
STD	47.91	43.25	38.44	46.99	42.37	39.22	49.05	39.52
p-value		0.174		0.005		0.000		0.256

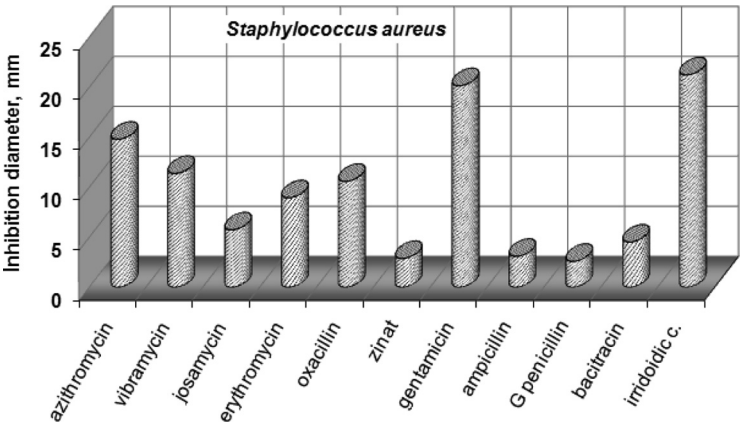

Figure 2. Antimicrobial effect of irridoidic extract against *Staphyloccocus aureus*.

(11 mm inhibition diameter), josamicin (7 mm inhibition diameter), erythromycin (9 mm inhibition diameter), oxacilin (11 mm inhibition diameter), and gentamicin (20 mm inhibition diameter) as it can be seen from Figure 2. In the same time, some common antibiotics like G penicillin, or ampicillin (3 mm inhibition diameter) has no effect against this microorganism.[1]

For *Pseudomonas aeruginosa* the extract shown the high bactericidal effect (22 mm inhibition diameter) in comparison with some antibiotics used: colistin, cefaclor and tetraciclin (10 mm inhibition diameter) (Fig. 3). At the same time the majority of common antibiotic (sulfonamide, norfloxacin, metronidazole, cephalexin, nalidixic acid and furazolidon) has no effect against *Pseudomonas sp.* In the case of *Bacillus sp.* (Fig. 4) irridoidic extract reveal the same high bactericidal effect (20 mm inhibition diameter) like norfloxacin, in comparison with the common antibiotics: colistin, ampicillin (9 mm inhibition diameter), sulfonamide (15 mm inhibition diameter), metronidazole, cephalexin (9 mm inhibition diameter), nalidixic acid (14 mm inhibition diameter). Some products, like cefaclor, tetracicline, furazolidone, have no effect against *Bacillus sp*.

Qualitative analysis performed with fluorescent X-ray (Fig. 5) reveal the presence of Ca, K, S, Mg, Mn, Fe, Na in the irridoidic extract. Quantitative analysis performed on atomic emission in inductively coupled plasma indicate a high content of K (37.2%) and a moderate content of C, N, H and other macroelements or microelements (Table 5).

Infrared spectra shown the irridoidic compound presence, due to peaks at 1050.31 cm^{-1} from aucubin; 1635.78 cm^{-1} from α, β unsaturated carboxyl of iridoid glucosides and 2360.52 cm^{-1} from iridoid glucosides type melampyroside, mussaenoside, gardoside methyl ester (Table 6).

Thermal analysis indicate the stability of bioproduct in the range (25–60)°C (Table 7); after 60°C, the compounds were decomposed, in the first step by release

[1]For the active substance from these antibiotic see the article N. Radu *et al. Therapeutic effect of polysacharides from Plantago sp.* in this number.

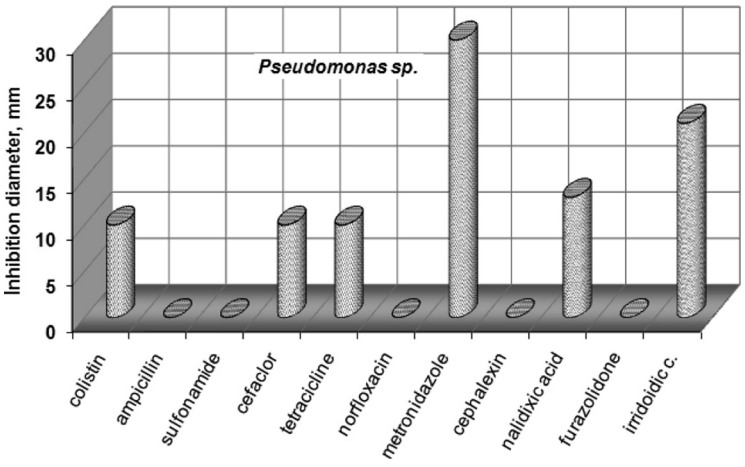

Figure 3. Antimicrobial effect of irridoidic extract against *Pseudomonas aeruginosa*.

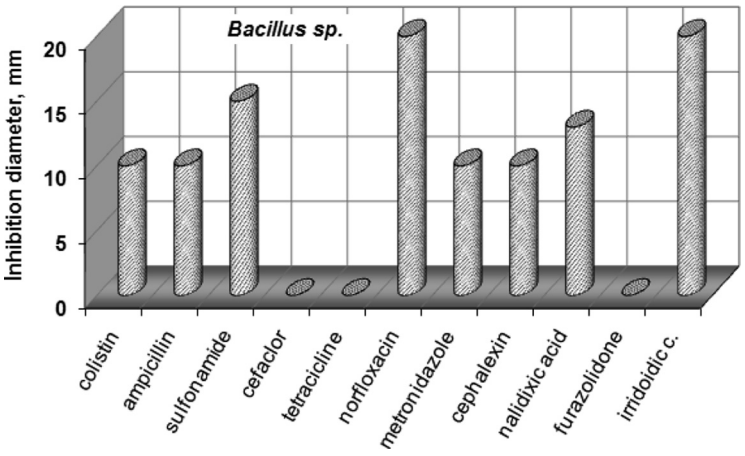

Figure 4. Antimicrobial effect of irridoidic extract against *Bacillus sp*.

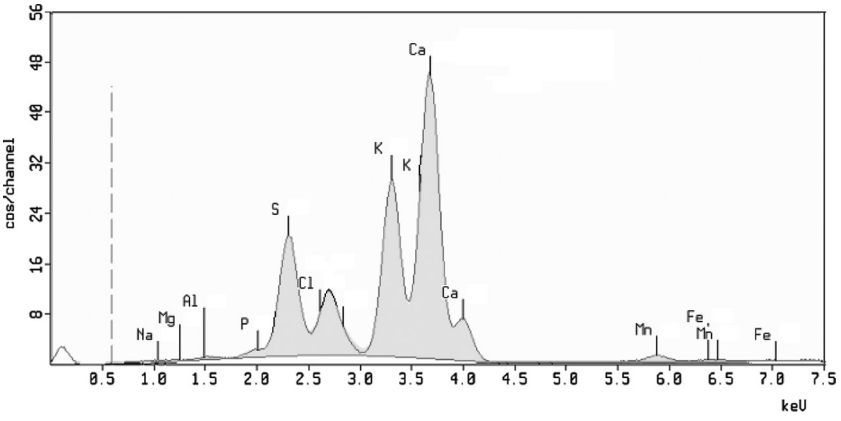

Figure 5. Qualitative analysis of iridoidic extract derived from *Plantago sp*.

Table 5. Elemental analysis of the irridoidic extract obtained from *Plantago sp.*

Element	%	Element	%
C	4.03	Mn	0.007
H	0.88	Fe	0.007
N	0.75	Mg	0.054
S	0.17	Na	0.089
Ca	0.10	B	0.108
K	37.2	Zn	0.02
P	0.00	Cu	0.025

Table 6. Assigned bands from infrared spectra performed on irridoidic extract

Wavenumber, cm^{-1}	Assignment and comment
1050.31	ν C–H bending and ring pickering from aucubin [9]
1080.13	ν C–C–C bending 4′O – acetylloganic acid 2 [9]
1241.43	ν C–O; ethers aromatic
1385.46	δCH_3; δCH_2
1635.78	ν C=C aromatic; enol eter group conjugated with COOMe; α, β unsaturated carboxyl from iridoid glucosides: 6′O – acetylloganic acid; 7′O – acetylloganic acid and gardoside methyl ester [9])
2360.52	iridoid glucosides type melampyroside, mussaenoside, gardoside methyl ester [9]
2940.97	ν C–H methyl

Table 7. Thermal analysis of irridoidic compound

Temperature range, °C	Thermic effect	Loss, mass %	Comments
25–100	61°C; $\Delta H > 0$	1.35	Deshidratation
100–200		1.39	Organic decomposition
200–300	276°C; $\Delta H > 0$	4.99	
300–600	466°C; $\Delta H > 0$		
	490°C; $\Delta H > 0$	4.35	
600–1000	782°C; $\Delta H < 0$		
	928°C; $\Delta H < 0$	47.55	
Total loss (25–1000°C)		59.63	

the inter or intramolecular water and after that by organic material decomposition up to 928°C, when almost all biomaterials are transformed in CO_x and H_2O (59.63% loss weight).

Conclusions

Research regarding therapeutic effects of solid extract from *Plantago sp*. with irridoidic, compounds, performing "*in vitro*" and "*in vivo*" reveal the following:

- samples with irridoid compounds have an apparent analgesic effect which appears after 30 minute and a sedative effect after 60 minute after i.p. administration of irridoidic extract, at the dose of 532 mg extract/kg body.
- the irridoidic extract reveal a significantly bactericide effect on different bacterial strains type *Staphyloccocus aureus, Pseudomonas sp. and Bacillus sp.*

Qualitative and quantitative analyses (elemental analysis, infrared spectra, thermal analysis) revealed the presence of C, H, N, S, P, K in the solid extracts as well as the presence of irridoidic compounds and the thermolability of this extract.

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