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Therapeutic Effect of Polysaccharides from *Plantago Species*

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Analgesic and sedative effects of polysaccharides extract separated from alcoholic media of Plantain*, were performed "*in vivo*" on mice, using the writhing and single exploration test. Antimicrobial activity of this extract was investigated "*in vitro*" using the Kirby Bauer method. The analgesic and sedative effects of this fraction without antimicrobial activity were also demonstrated. Physico-chemical analyses of this extract reveal the presence of C, H, P, K, microelements and the presence of polysaccharidic chain which comprises D-xylose, L-arabinose, rhamnose, and D-galacturonic acid.*

Keywords Analgesic; antimicrobial; polysaccharidic extract; sedative

Introduction

The medicinal herbal plants have an important therapeutic potential, already used in past by the folk medicine. Nowadays accumulation of scientific knowledge allows the isolation and the identification of many compounds with interesting therapeutic activities.

A range of pharmacological activities was found in tests with *Plantago major*. The saccharides galacturonic acid, galactose, arabinose, rhamnose, glucose, xylose, as well as a pectic polysaccharide, a galactoarabin and a galactan have been isolated from the leaves.

The polysaccharides have variable amounts of xylose, arabinose, galacturonic acid and glucuronic acid as main components. They swell in contact with water and form mucilage with high viscosity, which increases stool bulk, stimulates peristalsis and facilitates bowel movements. Together these are referred as 'plantaglucid', which have been used to treat ulcers. Plantaglucid reduced the development of peptic

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Table 1. Commercial product used in the antimicrobial tests

Nr. crt	Commercial antibiotic	Active compounds
1	azithromycin	(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethylamino)-*β*-D-*xylo*-hexopyranosyl]oxy}-1-oxa-6-azacyclopentadec-13-yl 2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranoside
2	vibramycin	(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6, 11,12a-octahydrotetracene-2-carboxamide
3	josamycin	2*S*,3*S*,4*R*,6*S*)-6-{[(2*R*,3*S*,4*R*,5*R*,6*S*)-6-{[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-10-hydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-6-yl]oxy}-4-(dimethylamino)-5-hydroxy-2-methyloxan-3-yl]oxy}-4-hydroxy-2, 4-dimethyloxan-3-yl 3-methylbutanoate
4	erythromycin	(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione
5	oxacillin	(2*S*,5*R*,6*R*)-3,3-dimethyl-6-[(5-methyl-3-phenyl-1,2-oxazole-4-carbonyl)amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
6	zinat	6*R*,7*R*)-3-{[(aminocarbonyl)oxy]methyl}-7-{[(2*E*)-2-(2-furyl)-2-(methoxyimino) acetyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
7	gentamicin	(3*R*,4*R*,5*R*)-2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-{[(2*R*,3*R*,6*S*)-3-amino-6-[(1*R*)-1-(methylamino)ethyl]-oxan-2-yl]oxy}-2-hydroxycyclohexyl]oxy}-5-methyl-4-(methylamino)oxane-3,5-diol
8	ampicillin	2*S*,5*R*,6*R*)-6-([(2*R*)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
9	G penicillin	4-Thia-1-azabicyclo (3.2.0)heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-((phenylacetyl)amino)-(2*S*-(2α,5α,6*β*))-
10	bacitracin	(4*R*)-4-[(2*S*)-2-({2-[(1*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-5-yl}formamido)-4-methylpentanamido]-4-{[(1*S*)-1-{[(3*S*,6*R*,9*S*,12*R*,15*S*,18*R*,21*S*)-18-(3-aminopropyl)-12-benzyl-15-(butan-2-yl)-3-(carbamoylmethyl)-6-(carboxymethyl)-9-(1*H*-imidazol-5-ylmethyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclopentacosan-21-yl]carbamoyl}-2-methylbutyl]carbamoyl}butanoic acid

(*Continued*)

Table 1. Continued

Nr. crt	Commercial antibiotic	Active compounds
11	colistin	*N*-(4-amino-1-(1-(4-amino-1-oxo-1-(3,12,23-tris(2-aminoethyl)-20-(1-hydroxyethyl)-6,9-diisobutyl-2,5,8,11,14,19,22-heptaoxo-1,4,7,10,13,18-hexaazacyclotricosan-15-ylamino)butan-2-ylamino)-3-hydroxybutan-2-ylamino)-1-oxobutan-2-yl)-*N*,5-dimethylheptanamide
12	ampicillin	(2*S*,5*R*,6*R*)-6-([(2*R*)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
13	sulfonamide	2-Aminophenol-4-(N-methyl)sufonamide (competitive inhibitors of the enzyme dihydropteroate synthetase)
14	cefaclor	(6*R*,7*R*)-7-{[(2*R*)-2-amino-2-fenilacetil]amino}-3-cloro-8-oxo-5-tia-1-azabiciclo[4.2.0]oct-2-enă-2-carboxiic acid
15	tetracycline	2-(amino-hydroxy-methylidene)-4-dimethylamino-6,10,11,12a-tetrahydroxy-6-methyl-4,4a,5, 5a-tetrahydrotetracene-1,3,12-trione
16	norfloxacin	1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1*H*-quinoline-3-carboxylic acid
17	metronidazole	2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol
18	cephalexin	(6*R*,7*R*)-7-{[(2*R*)-2-amino-2-phenylacetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
19	nalidixic acid	1-ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxylic acid
20	furazolidone	3-{[(5-nitro-2-furyl)methylene]amino}-1,3-oxazolidin-2-one

Table 2. Sleek parameter at 15 minute

Sleek (15 minute)	Witness	100 mg/kg body	200 mg/kg body	400 mg/kg body	800 mg/kg body
1	10.79	13.75	4.19	6.72	4.97
2	14.6	6.88	12.81	5.31	6.06
3	8.22	11.47	6.13	8	4.97
4	11.12	7.25	11	3.87	8.06
5	4.44	11.43	3.15	6.5	9.25
6	7.84	5.94	9.25	9.94	5.78
7	9.35	4.87	5.59	6.19	7.87
8	8.3	8.72	5.97	9.15	6.41
9	8.72	10.09	7.5	10.96	6.81
10	9.22	6.22	7.62	12.78	8.25
average	9.26	8.662	7.321	7.942	6.843
SD	2.6219	2.9135	2.9990	2.7511	1.4627
p-value		*0.6771*	*0.0182*	*0.3694*	*0.0529*

Table 3. Leap parameter at 15 minute

Leap (15 minute)	Witness	100 mg/kg body	200 mg/kg body	400 mg/kg body	800 mg/kg body
1	102.44	59.03	103	114.44	180
2	28.63	87.13	58.44	142.91	143.41
3	80.71	94.28	91.31	115.28	80.2
4	86.72	54.3	61.65	108.88	119.28
5	68.87	70.38	66.29	42.72	81.85
6	52.86	48.56	101.25	142.34	162
7	46.6	109.15	81.13	92.25	171
8	95.63	120	86.16	90.04	121.12
9	89.66	107.03	75.25	78.72	133.44
10	50.06	62.91	105.56	122.13	180
average	70.218	81.277	83.004	104.971	137.23
SD	24.5882	25.5866	17.3936	30.3507	37.0677
p-value		*0.3285*	*0.1785*	*0.0401*	*0.0021*

ulcers in rats and reduced inflammatory oedema too, without toxic effects even after prolonged administration.

In this article we present the study concerning the analgesic and sedative affects of the polysaccharides extract obtained from *Plantago sp*.

Experimental

Polysaccharidic fraction was separated from *Plantago sp.*, from alcoholic extract [1–2]. **The analgesic effect** was determined using the writhing and hot plate test, for 15; 30; 60; and 120 minutes after intraperitoneal (i.p.) administration for four

Table 4. Sleek parameter at 60 minute

Sleek (60 minute)	Witness	100 mg/kg body	200 mg/kg body	400 mg/kg body	800 mg/kg body
1	8.5	7.59	8.25	9	9.06
2	7.75	7.9	7.44	9.82	5.04
3	9	8.56	7.1	8.53	8.82
4	14.84	6.25	6.5	11.43	11.82
5	10.68	9.06	7.85	16.75	8.25
6	10.59	10.82	11.03	7.59	7.25
7	9.4	9.22	9.87	8.06	7.15
8	14.1	8.47	7.44	7.5	7.97
9	10.16	9.13	14.16	11.6	9.68
10	11.15	12.69	9.78	6.53	8
average	10.617	8.969	8.942	9.681	8.304
SD	2.2915	1.7708	2.3259	2.9852	1.7762
p-value		*0.044*	*0.0610*	*0.2209*	*0.0106*

Table 5. Leap parameter at 60 minute

Leap (60 minute)	Witness	100 mg/kg body	200 mg/kg body	400 mg/kg body	800 mg/kg body
1	82.19	83.19	180	146.1	180
2	180	99.03	168.56	140.81	105.61
3	88.15	136.79	163.22	86.56	88.62
4	133	77.31	136.03	143.66	180
5	180	108.56	131.22	156.93	138.1
6	167.94	170	150.94	111	111.72
7	180	168	120.78	150.75	168.81
8	180	98.74	110.5	110.66	100.56
9	171.06	148.26	168.03	126.5	180
10	173.53	115.78	167.65	94.53	120.44
average	153.587	120.566	149.693	126.75	137.386
SD	38.7341	33.4981	23.6118	24.7324	36.7056
p-value		*0.0389*	*0.0579*	*0.0866*	*0.2553*

doses of extract: 100; 200; 400; and 800 mg/kg body, (dose administration solution of 1%; 2%; 4%; and 8% polysaccharidic extract in physiological serum). For each time 2 groups with 8 mice each (25–35 g weight); 1 group as witness and 1 group as test were used.

The sedative test was realised using the single exploration test which evaluates the motion activity of 2 lots, with 8 mice each (25–35 g weight). The active compound was i.p administrated, with a dose of 532 mg Plantago extract (polysacharidic extract)/kg mice (532 mg dissolved in 10 mL physiological serum, 0.1 mL/10 g mice); as a witness 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 (L × l × h) was used. The floor of

Table 6. Sleek parameter at 120 minute

Sleek (120 minute)	Witness	100 mg/kg body	200 mg/kg body	400 mg/kg body	800 mg/kg body
1	10.18	10.53	11.69	11.47	11.91
2	6.71	11.25	8.6	7.66	9.63
3	7.28	11.37	9	10.15	5.97
4	7.91	11.12	8.59	9.5	7.43
5	7.28	10.5	10.9	7.97	9.03
6	12.94	6.72	11.13	5.78	12.32
7	11.78	9.56	11.68	9.28	8.4
8	7.91	9.94	10.18	13.81	10.56
9	8.91	19.44	11.75	7.81	12
10	8.78	9.4	9	4.59	7.9
average	8.968	10.983	10.252	8.802	9.515
STD	2.060	3.2658	1.3405	2.6788	2.1572
p-value		*0.0581*	*0.0579*	*0.4391*	*0.2846*

Table 7. Leap parameter at 120 minute

Leap (120 minute)	Witness	100 mg/kg body	200 mg/kg body	400 mg/kg body	800 mg/kg body
1	143.44	94.43	48.63	110.78	98.44
2	60.8	112.72	141.75	127.94	146.66
3	67.81	144.59	74.04	128.41	107.22
4	137.47	167.63	101.38	85.88	156.85
5	180	126	94.47	97.15	180
6	143.06	75.34	89.06	98.95	120.63
7	114.47	147	84.44	175.5	99.4
8	180	125.8	88.55	152.06	113.32
9	144.94	122.22	120.32	171.69	136.47
10	144.12	67.5	93.47	137.16	173.47
average	131.611	118.323	93.611	128.552	133.246
STD	40.4159	31.8096	25.0079	31.0755	30.0775
p-value		*0.2123*	*0.0346*	*0.0063*	*0.3677*

this box was divided into rectangles of 80×75 mm dimension. Each mouse was put in one corner of the box and during 5 minutes the rectangles walked by the mice were counted.

Antimicrobial effect was performed using the Kirby Bauer method on two pathogenic microorganisms: *Staphylococcus aureus and Escherichia colli* testing for comparison also some commercial antibacterial products listed in Table 1.

Physico – chemical analyses of the solid extracts with polysaccharidic compounds were performed by the energy dispersive X-ray fluorescence technique 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

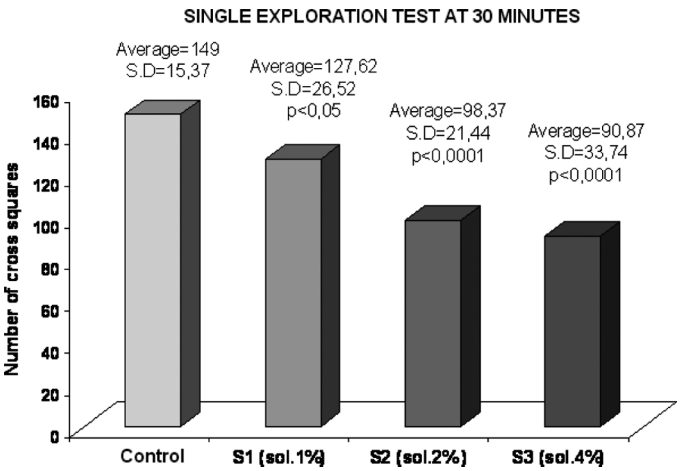

Figure 1. The simple exploration test applied 30 minute after administration of polysaccharidic solutions S1(1%); S2(2%); S3(4%).

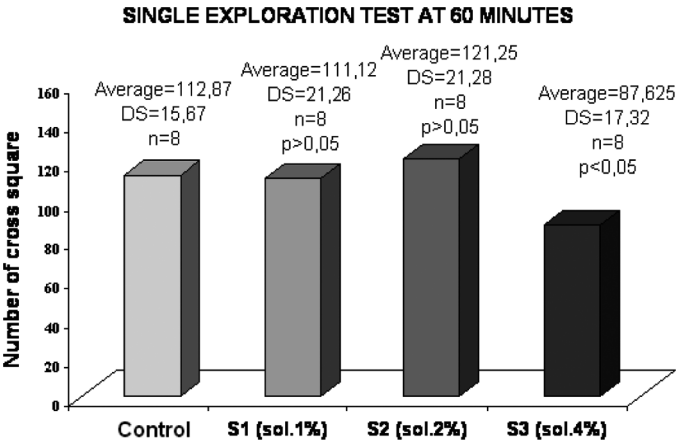

Figure 2. The simple exploration test applied 60 minute after administration of polysaccharidic solutions S1(1%); S2(2%); S3(4%).

type Spectrum GX Perkin Elmer with accessories: DRIFT (Diffuse Reflectance Infrared Fourier Transform) and ATR (Attenuated Total Reflectance), thermogravimetric 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

Results obtained at 15 minutes for sleek parameter, (sleek parameter is a measure of pain perception) was not statistically significant for a dose of 100 and 400 mg/kg body (Table 2). For a dose of 200 and 800 mg/kg body, results obtained are statistically significant, in the meaning of decreasing the level of pain perception.

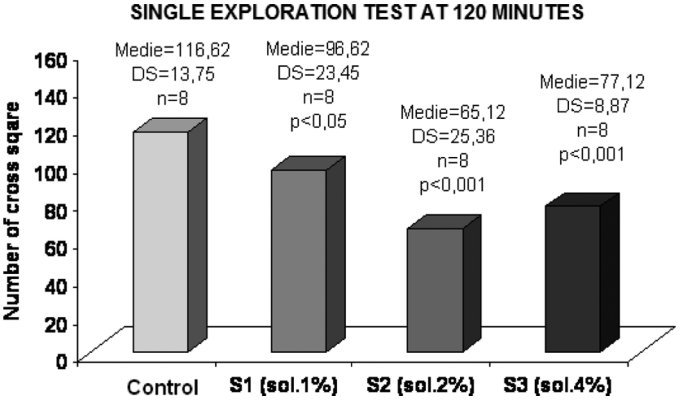

Figure 3. The simple exploration test applied 120 minute after administration of polysaccharidic solutions S1(1%); S2(2%); S3(4%).

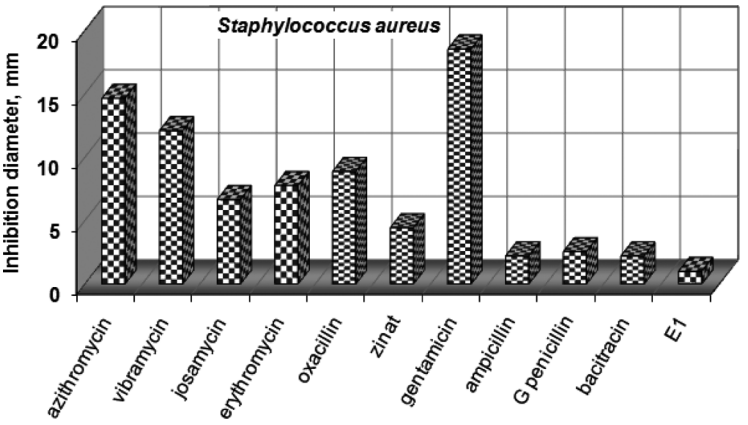

Figure 4. Effect of polysaccharidic extract (50% aqueous solution) against *Staphylococcus aureus*. *E1 = polysaccharidic extract*.

Concerning the results obtained at 15 minutes for leap parameter only those obtained for a dose of 400 and 800 mg/kg body were statistically significant (Table 3). Results obtained at 60 minutes were significant (Table 4 and Table 5) for a dose of 100 mg/kg body for both sleek and leap parameter. At 120 minutes after polysaccharidic extract administration, the results obtained for a dose of 200 mg/kg and 400 mg/kg body were statistically significant for leap parameter. Regarding sleek parameter, all results obtained after 120 minute were not significant (Table 6 and Table 7).

Sedative Effect

The sedative effect appears 30 minutes after administration of each dose of polysaccharidic extract. All results were statistically significant (Fig. 1) except for 60 minutes (Fig. 2). The fact that the sedative effect was also determined 120 minutes (for all

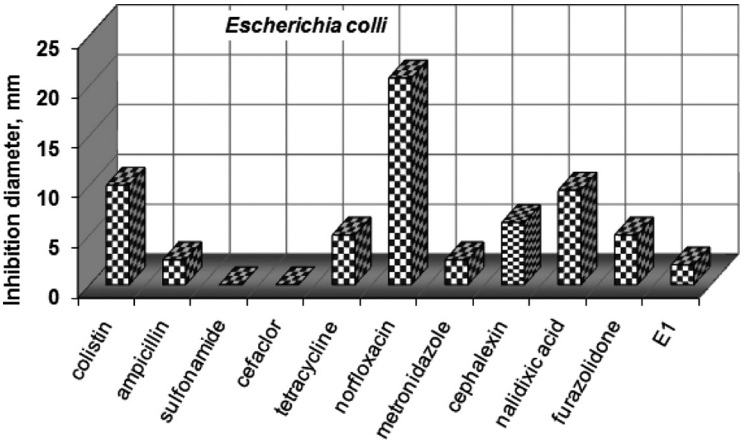

Figure 5. Effect of polysaccharidic extract (50% *E1 = polysaccharidic extract* solution) against *Escherichia colli*.

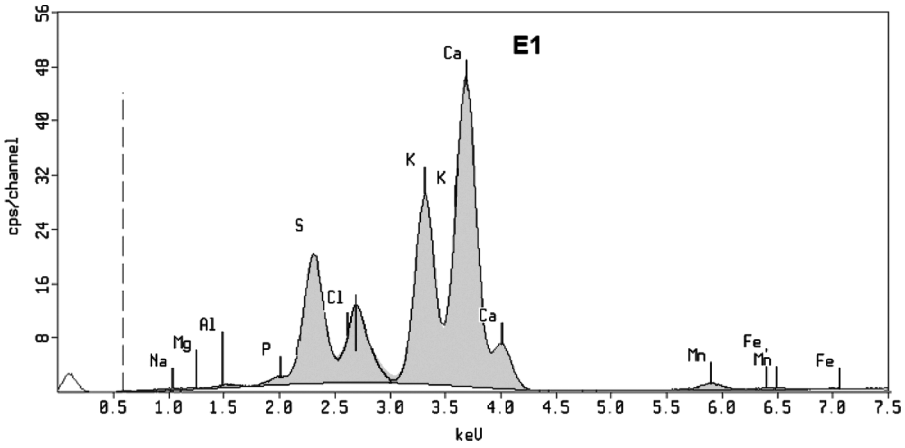

Figure 6. Qualitative analysis of irridoidic extract derived from *Plantago sp.*

doses used in the experiment) after the administration, can be explained by the presence of other compounds with sedative effect in polysaccharide extract (Fig. 3). Again a dose-effect relationship is observed. The sedative effect strength is in agreement with the active fraction dose (100; 200; 400 mg/kg weight).

Antimicrobial Effect

The tests performed with impregnated dishes in 50% polysaccharidic solution, indicate no antibacterial effect against some bacterial strains like *Staphylococcus aureus* (Fig. 4). The level of inhibition development was tested for all commercial antibiotics (Fig. 5). In the case of *Escherichia colli*, polysaccharidic compounds reveal a higher effect in comparison with common antibiotics like sulfonamide and cefaclor (0 mm inhibition diameter). The effect of this extract is similar to that of ampicillin and metronidazole (2.5 mm inhibition diameter).

Physico-Chemical Analyses

Qualitative analysis performed in the first step by fluorescent X-ray (Fig. 6) reveals the presence of Ca, K, Al, Mg, Na, Mn, Fe elements in the polysaccharidic extract.

Table 8. Elemental analysis of polysaccharidic extract obtained from *Plantago sp.*

Element	%	Element	%
C	23.19	K	6.54
H	3.75	P	0.38
N	0.66	Mn	0.087
S	3.21	Fe	0.013
Ca	8.95	Zn	0.026
Al	0.018	P	0.38

Table 9. Assigned bands from infrared spectra performed with polysacharidic extract

Wavenumber, cm^{-1}	Assignment and Comment
	E1
601.28	δC−H
657.16	δO−H
1023.72	νC−OH
1093.25	νC−O−C glycosidic vibration
1140.47	(identified in Galacturonic acid) [1]
1199.22	Ring vibration overlapped with stretching vibration (fingerprint region specific for pectic polysaccharides)
1601.67	νCOO-free carboxylate (pectine ester and carboxylate groups)

Quantitative analysis performed by the atomic emission spectroscopy in inductively coupled plasma indicates a high content of C, a moderate one of N, H, S and traces of macroelements or microelements (Table 8).

Infrared spectra show the presence of polysaccharidic compound (Table 9) by the bands in the range of $1023.72 \div 1199.22\,cm^{-1}$, which are specific to the fingerprint region of pectic polysaccharides. The peaks at $1601.67\,cm^{-1}$ and $1749.81\,cm^{-1}$, which are due to pectine ester, carboxylate groups and cyclopentanone specific bands [3–4], confirm the presence of polysaccharidic compounds in the extract.

Thermal analysis indicates the stability of bioproduct in the range (25–94)°C. Above 94°C, the compound decomposes. In the first step it takes place by release of inter or intramolecular water and after that by organic material decomposition,

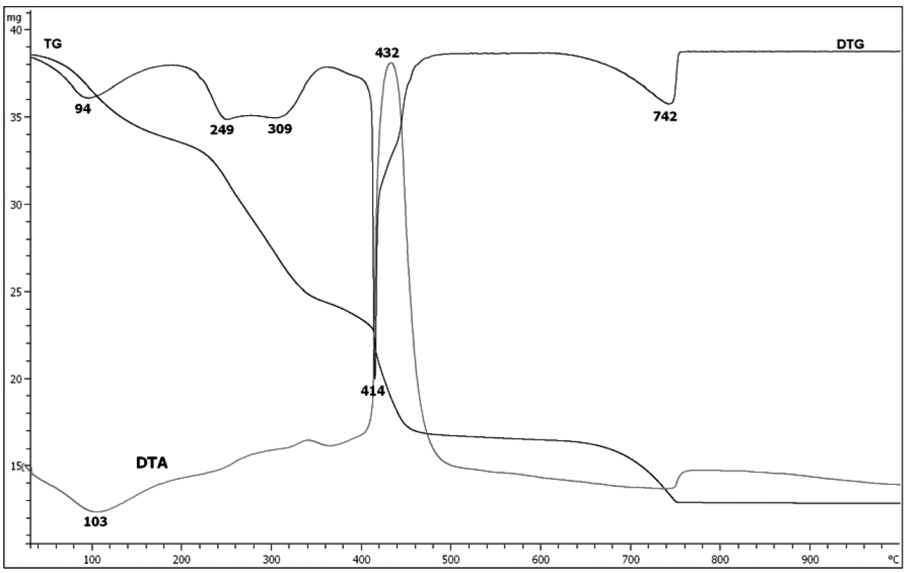

Figure 7. Thermal analysis of polysaccharidic compound.

taking place up to 742°C, when almost all biomaterials are transformed into CO_x, H_2O and other compounds with metallic cation (66.81% loss weight) associated with change of the crystallization system (Fig. 7).

Conclusion

Polysaccharidic fraction shows a sedative effect after 30 minutes of extract administration. After 60 minutes, this effect disappears. Sedative effect observed at 120 minutes implied the existence of other compounds [3–4] in the polysaccharidic extract with sedative effect, probably due to insufficient purification. The analgesic effect strength depends on the used dose (100; 200; 400; 400 mg/kg weight). Qualitative and quantitative analyses reveal the presence of C, H, P and some other microelements in the extract as well as the presence of K. The infrared spectrum analysis shows the presence of polysaccharidic chains in the extract.

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