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WASTE PRODUCT OF GROWING THE GENUS ECHINACEA (ECHINACEA MOENCH) AS PROMISING SOURCE OF BIOLOGICALLY ACTIVE SUBSTANCES

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The experimental foundation bioconversion of waste product of growing (chaff), purple coneflower (Echinacea purpurea (L.) Moench.) and pale coneflower (Echinacea pallida (Nutt.) Nutt.) was to carried out. The high activity of extracts of coneflower chaff, which had growth-stimulating effect to the test culture, was proved. Found that the highest stimulating activity has the extract of chaff purple coneflowers: aqueous solution at concentrations of 0.01 %, alcohol-0.01 %-0.001 % and with increasing concentration of alcohol in the extracts decreased their activity. For the first time it has been shown that chaff of Echinacea contains specific proteins - lectins, and their activity in the waste product of pale coneflower significantly exceeded then activity in the waste product of purple coneflower. Technological schemes to get of lectins and extracts of biologically active substances from waste growing product (chaff), was worked out for their effective use.

Key words: *growing product (chaff), bioconversion, lectins, biologically active substances, Echinacea purpurea (L.) Moench, Echinacea pallida (Nutt.) Nutt.*

Statement of the problem. Members of the genus Echinacea (Echinacea Moench) - known medicines, fodder and ornamental plants the raw material containing unique combination of bioactive substances. Owing to of that the vegetation mass, roots and rhizomes of Echinacea produce drugs immunostimulatory actions, which are used in medicine, veterinary medicine, extracts were also added to foods, drinks, etc. [9].

In the process of growing coneflower at the end of the growing season after harvesting the seed remains chaff which contains that the remains of stems and inflorescences of Echinacea. The bioconversion of these wastes represents the meaning of, as they can have active substances which may be used in medicine, agriculture, livestock and more. The bioconversion of these wastes products represents the meaning of, as they can have active substances which may be used in medicine, agriculture, livestock and more.

Analysis of major studies and publications which discuss the problem. Nowadays, utilization waste products of pharmaceutical production are very topical problem [10]. The presence of waste medicinal plants biologically active substances broad-spectrum indicates the feasibility of using them in medicine, biotechnology, biochemistry, food technology, cosmetics, perfume industry, agriculture and so on. It is known that the waste medicinal plants contain many bioactive compounds that can effectively isolate and purify [11]. It is established chaff of sea buckthorn is rich in coumarins, complex of

polyphenols, some acid and lipids [1, 5]. In waste from production after preparation of hypericum found hypericin, quercetin, oksykorychni acid [3]. According to research, the preparation of the meal aloe leaves has grows activity during the pre-treatment of crops [1].

Taking note the fact that raw purple coneflower (Echinacea purpurea (L.) Moench.) and pale coneflower (Echinacea pallida (Nutt.) Nutt) contains a wide variety of biologically active components [7, 8], we can assume that growing waste product also contain substances with biological activity. It is known that during seed maturation inflorescences and stems lose water and change their chemical composition, including the ratio of biologically active substances [6]. Therefore, we can predict the biological activity of extracts chaff and vegetative mass (grass) will be different.

The purpose of research and methods. The aim of the study was to investigate the biological activity of extracts of growing waste (chaff) purple coneflower (Echinacea purpurea (L.) Moench.) and pale coneflower (Echinacea pallida (Nutt.) Nutt) and examine the possibility of using them as a source of biologically active substances.

The study was performed by biological tests on seedlings of barley seeds (Hordeum sativum Lessen.) during in 2006-2009. For this barley seeds soaked for 24 hours in water, then laid 20-25 seeds in a petri dish with extracts (4-5 ml of a concentration in each cup) at a temperature of 20-25 ^{0}C.

Control measurements of the length of roots and seedling performed at 24, 48, 72 and 96 hours [2]. Statistical analysis of the data was carried out using computer program Excel.

In order to evaluate the biological activity of extracts we have used two methods of extraction: water and alcohol (ethyl with content to 20% and 40%). Aqueous extracts produced by infusion in distilled water for two hours at room temperature. We used aqueous solutions of the following concentrations: 0.1%, 0.01%, and 0.001%. Alcoholic extracts produced by extraction. The study used 20% and 40% alcohol solutions. In control options using distilled water [2].

Obtaining lectins of waste (chaff) coneflower was conducted by the method of cold ethanol fractionation [4]. Determination of lectins activity in extracts of coneflower chaff was performed activity of red blood cells in the human ABO system by conventional methods [4].

Results of studies. The results of the study of to represent in Table 1. Analysis of the data showed that the extract of growing waste (chaff) purple coneflower in all studied concentrations showed significant catalytic activity on root growth. Thus, measuring the length of roots after 24 hours was significantly dominated research options control 7.4% -9.3%. Subsequent measurements (on 48 and 72 hour) length of roots in the experimental variants are mostly higher than control at 7.56%-10.8%, respectively. Just a concentration of 0.01% after 48 hours the length of roots was slightly lower than the control (at 4.08%).

Aqueous extracts of growing waste (chaff) purple coneflower also stimulated growth seedling barley in experiments. Throughout the duration of the experiment the average length seedling most options were higher than the control. For measurements over 24 hours in experimental lengths

significantly greater than control at 1% -43.7%. The highest activity was characterized by the extract at a concentration of 0.1%.

During these measurements most options on 48 and 72 hour length of shoots at concentrations of 0.1% and 0.01% higher than the benchmark of 14.8% -17.7% and 8.7% to 16, 0%, respectively. The highest activity was found at a concentration of 0.01% (options studied in 48 hours was 17.7% greater than control).

1. The influence of water extracts chaff of purple coneflower on roots and seedling of caryopsis barley mm / ±% for control

exposition		**Control**		**Concentration**					
				0,1 %		**0,01 %**		**0,001 %**	
		root	shoots	root	shoots	root	shoots	root	shoots
24 hour	values, mm	27,0 ±0,59	5,65 ±0,75	29,52 ±0,63	8,13 ±0,88	29,43 ±0,74	5,7 ±0,71	29,21 ±0,62	5,9 ±0,71
	± to control	100 %	100 %	+9,3 %	+43,7 %	+9,0 %	+1,0 %	+7,4 %	+4,42 %
48 hour	values, mm	60,42 ±0,83	30,05 ±1,28	64,99 ±0,86	34,51 ±1,36	65,43 ±0,88	35,38 ±1,57	57,95 ±0,84	29,13 ±1,26
	± to control	100 %	100 %	+7,56 %	+14,8 %	+10,8 %	+17,7 %	-4,08 %	-4,49 %
72 hour	values, mm	87,45 ±0,99	52,51 ±1,7	97,80 ±1,07	56,72 ±1,8	89,15 ±1	60,93 ±1,81	91,59 ±1,02	54,69 ±1,75
	± to control	100 %	100 %	+11,8 %	+8,7 %	+1,9 %	+16,0 %	+4,73 %	+4,15 %
96 hour	values, mm	-	67,67 ±1,95	-	67,21 ±1,91	-	77,5 ±2,03	-	64,70 ±1,87
	± to control	-	100 %	-	-0,6 %	-	+14,5 %	-	-4,3 %

Length seedling of caryopsis barley in these options exceeded the control by 14.5%. At the same time at concentrations of 0.1% and 0.001% the length seedling of caryopsis barley was 0.6% and 4.3% lower than the control.

The aim of our research was the study of the biological activity of 20% and 40% ethanol extracts of Echinacea. The studies of influence 20% ethanol extract chaff of purple coneflower on seedlings barley are shown in Table 2.

Found that it showed the growth-stimulating roots of barley for concentrations of 0.01% and 0.001%. The length of roots at the first dimension to 8.10% and 10.18% was higher than the control. At the same time the solution concentration of 0.1% extract inhibited root growth at 9.96%. In further measurements observed a similar pattern: 48 and 72 hour concentration of 0.01% and 0.001% slightly stimulated test system (2.83% and 2.35% - after 48 hours and 9.12 and 16.3% - 72 hours). By concentration of 0.1% extract significantly inhibited root growth at 16.09% in 48 hours and at 9.26% - in 72 hours.

The greatest growth-stimulating effect on root extracts showed after 48 hours at concentrations of 0.01% and 0.001%. Analysis of the results of 20% ethanol extracts of purple coneflower chaff on length seedlings barley showed that they are more actively influenced the growth of shoots of barley compared with the length of the roots. Length shoots in 48 hours in the variants of experiments was significantly greater than their length in control benchmark, and only a concentration of 0.1% less than it was for them to 6.16%. The following measurements show a tendency to increase in length shoots in experimental models: the concentrations of all extracts were more effective for the control to 6.94%, 14.48% and 22.2% - in 72 hours, and 8.07%, 12 15% and 11.67% - after 96 hours of experiment.

2. The influence of 20% ethanol extracts of waste growing product (chaff) purple coneflower the length of roots and shoots barley mm / ±% for control

exposition		**Control**		**Concentration**					
				0,1 %		**0,01 %**		**0,001 %**	
		root	shoots	root	shoots	root	shoots	root	shoots
24 hour	values, mm	13,45 ±0,36	-	12,11 ±0,35	-	14,82 ±0,35	-	14,54 ±0,35	-
	± to control	100 %	-	-9,96 %	-	+10,1 %	-	+8,1 %	-
48 hour	values, mm	38,46 ±0,87	25,47 ±0,66	32,27 ±0,84	23,9 ±0,84	39,55 ±0,80	26,37 ±0,59	39,51 ±0,89	26,37 ±0,65
	± to control	100 %	100 %	-16,09 %	-6,16 %	+2,83 %	+3,53 %	+2,35 %	+3,81 %
72 hour	values, mm	57,11 ±1,27	43,5 ±1,84	51,82 ±1,11	46,52 ±1,65	62,32 ±1,22	49,8 ±1,47	66,42 ±1,20	53,16 ±1,58

	± to control	100 %	100 %	-9,26 %	+6,9 %	+9,12 %	+14,4 %	+16,3 %	+22,2 %
96 hour	values, mm	-	66,23 ±3,37	-	71,58 ±3,04	-	74,28 ±2,57	-	73,96 ±3,02
	± to control	-	100 %	-	+8,0 %	-	+12,1 %	-	+11,6 %

Thus, 20% alcohol extract of purple coneflower chaff in all concentrations affect the growth of shoots of barley, indicating the presence of raw materials biologically active substances.

The results of studying the influence of 40% ethanol extracts of purple coneflower chaff the length of the roots of barley are shown in Table 3. Their analysis showed that these extracts are mainly inhibited growth of the root of barley. Note, however, that this activity was not detected at all stages of the measurement.

At the 24th hour of the experiment root length in the variants treated for alcohol extracts concentration 0.01% and 0.001%, was higher than the control at 7.74% and 7.52% respectively. In the embodiment, the concentration of a solution of 0.1% showed significant inhibition of (32.10%).

3. The influence of 40% ethanol extracts of waste growing product (chaff) purple coneflower the length of roots and shoots barley mm / ±% for control

exposition		**Control**		**Concentration**					
				0,1 %		**0,01 %**		**0,001 %**	
		root	shoots	root	shoots	root	shoots	root	shoots
24 hour	values, mm	13,55 ±0,32	-	9,20 ±0,19	-	14,6 ±0,34	-	14,57 ±0,32	-
	± to control	100 %	-	32,10 %	-	+7,74 %	-	+7,52 %	-
48 hour	values, mm	47,29 ±0,78	33,07 ±0,26	29,5 ±0,62	26,4 ±0,15	42,53 ±1,94	32,39 ±0,15	45,4 ±0,87	32,94 ±0,14
	± to control	100 %	100 %	37,61 %	-20,16 %	-10,06 %	-2,05 %	-3,99 %	-0,39 %
72 hour	values, mm	65,86 ±1,25	55,37 ±1,27	45,54 ±1,11	45,8 ±1,31	65,13 ±1,36	51,28 ±1,36	64,35 ±1,27-	50,46 ±1,27

	± to control	100 %	100 %	30,85 %	-17,28 %	-1,10 %	-7,38 %	2,29 %	-8,86 %
96 hour	values, mm	-	82,62 ±0,97	-	78,94 ±1,23	-	89,06 ±1,16	-	88,27 ±1,47
	± to control	-	100 %	-	-4,45 %	-	+7,79 %	-	+6,83 %

In 48 hours the length of roots in the experimental variants for concentration of 0.1% was lower than the control at 37.61%. For concentrations of 0.01% and 0.001%, this figure was lower than the control (-3.36% to 10.06%). At the end of the experiment (after the 72hour) in these variants the length of roots though it was less than the control, but this difference was not reliable and was only 1.10% -2.29%.

The length of barley shoots was less than the control after 48 and 72 hours of the experiment in the all concentration extract. For the concentration of 0.1% showed the greatest growth inhibition, the length shoots was lower than in controls, at 20.16%. All concentrations of extract inhibited growth shoots in 72 hours. Only in the 96 hour of the experiment was marked negligible growth-stimulating activity of extracts on barley shoots (at 6.83% -7.79%).

Thus, 40% ethanol extracts of purple coneflower chaff had the most depressing effect both on root growth and barley shoots.

Until recently pale coneflower has been studied not as perfect as purple coneflower, but its study is considerable scientific interest. With this in mind, we have been researching the presence of biologically active substances. This effect extracts chaff has been studied on growth shoots and roots of barley (Table. 4).

4. The influence aqueous extracts of waste growing product (chaff) of pale coneflower the length of roots and shoots barley mm / ±% for control

exposition		**Control**		**Concentration**					
				0,1 %		**0,01 %**		**0,001 %**	
		root	shoots	root	shoots	root	shoots	root	shoots
24 hour	values, mm	22,09 ±0,85	11,85 ±0,77	22,54 ±0,86	12,7 ±0,73	23,0 ±1,03	12,41 ±0,85	23,28 ±0,91	11,51 ±0,67
	± to control	100 %	100 %	+2,03 %	+7,17 %	+4,1 %	4,72 %	5,3 %	-2,87 %

48 hour	values, mm	50,69 ±1,85	32,16 ±0,945	47,59 ±1,98	30,94 ±2,18	51,79 ±1,83	30,9 ±2,0	51,91 ±1,96	30,88 ±2,03
	± to control	100 %	100 %	-6,12 %	-3,8 %	2,1 %	-3,77 %	2,4 %	-3,99 %
72 hour	values, mm	72,02 ±2,86	51,40 ±2,86	72,73 ±2,74	50,75 ±2,90	71,34 ±2,86	51,76 ±3,12	67,57 ±2,81	48,49 ±3,14
	± to control	100 %	100 %	+0,9 %	-1,27 %	-0,95 %	+0,7 %	-6,18 %	-5,67 %
96 hour	values, mm	-	60,07 ±5,33	-	60,82 ±4,24	-	58,63 ±7,47	-	65,3 ±4,57
	± to control	-	100 %	-	+1,2 %	-	-2,4 %	-	+8,7 %

Studies indicate that extracts chaff of pale coneflower revealed no significant growth-stimulating effect on the length of the roots of barley. So, after 24 hours the length of roots is not significantly higher than control values (2% -5%). In these measurements are mostly extracts inhibited root growths of barley - so their length after 72 hours in the overwhelming majority of research options was less than the control at the 5% -7%.

Similar patterns seen in the course of research length of shoots. In the first 24 hours at concentrations of 0.1% and 0.01% observed a slight longer length shoots in the experimental samples compared with controls. This difference was reliable and was 4% -7%.

During the next day in extracts length was mainly lower than in controls (average 4%). A similar pattern was observed in 72 hours - the length shoots in extracts of 1% -5% was lower than the control.

After 96 hours difference length of shoots compared with control amounted: to extracts 0.1% of higher by 1.2%, in 0.001% - 8.7% (significant difference), and the concentration of 0.01% - lower on 3%. Thus, a maximum dilution manifests stimulation growth.

Chaff extracts of pale coneflower for concentrations of 0.1% -0.001% weakly stimulated growth shoots, but ultimately a concentration of 0.001% was deemed to deserve attention. Obviously, to obtain the effect of stimulation (the effect is desired) to act by reducing the concentration of active ingredients.

Determination of activity lectins in extracts of Echinacea components of the chaff, which was carried out by testing on agglutination of human red blood cells are shown in Table 5. Analysis of the

data showed that the highest agglutination activity was characterized extracts stems of Echinacea pale (16, 0-20, 0 units.), While the activity of extracts receptacle and bracts was 0,5-4,4 units.

5. The activity of lectins in components of chaff Echinacea (units)

Property research	Purple coneflower	Pale coneflower
Stems	4,5–6,5	16,0–20,0
Receptacle	0,0	2,5–4,0
bracts	0,0	0,0–0,5

As for lectin activity in parts component of chaff purple coneflower, it was found only in extracts of stems (4,5-6,5 units.). As for lectin activity in components of chaff of purple coneflower, it was found only in extracts of stems (4,5-6,5 m.). Under laboratory conditions, we have developed technological schemes to extract of lectins and biologically active substances from waste cultivation (chaff) which allow using them effectively (Fig. 1, 2).

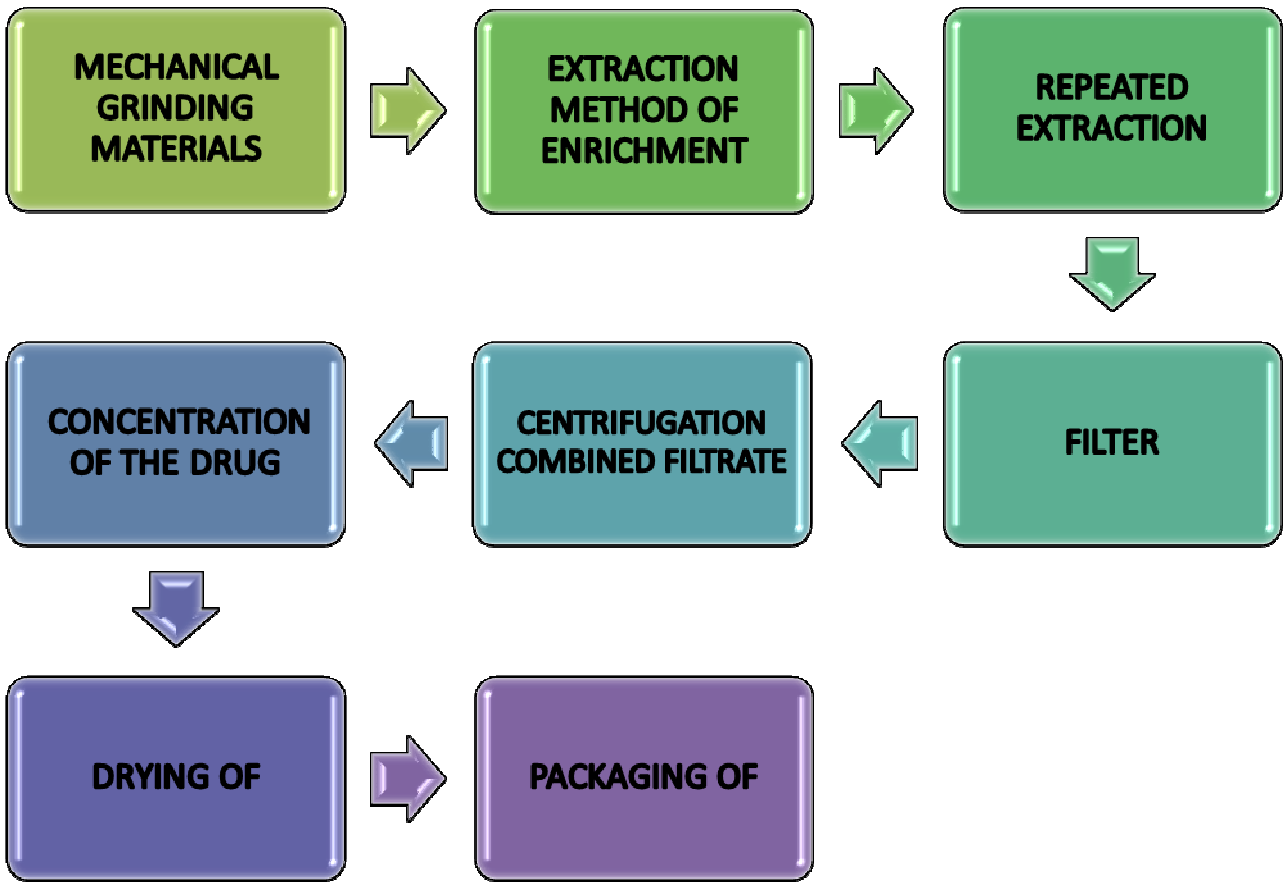

Figure 1. Technological scheme to extract bioactive substances from waste growing Echinacea (chaff)

Technology to extract of biologically active compounds (Fig. 1) is based on the properties of individual solvents that translate them into soluble forms and highlight them with raw materials. To

increase the concentration of the leachate extraction is carried out by the method of enrichment, that is, the same solvent extracted three party materials. Remains of nutrients from the second and the third party to extract a small number of pure solvent, followed by filtration and pressing. The combined filtrate was centrifuged, concentrated by vacuum evaporation and are dried by lyophilization.

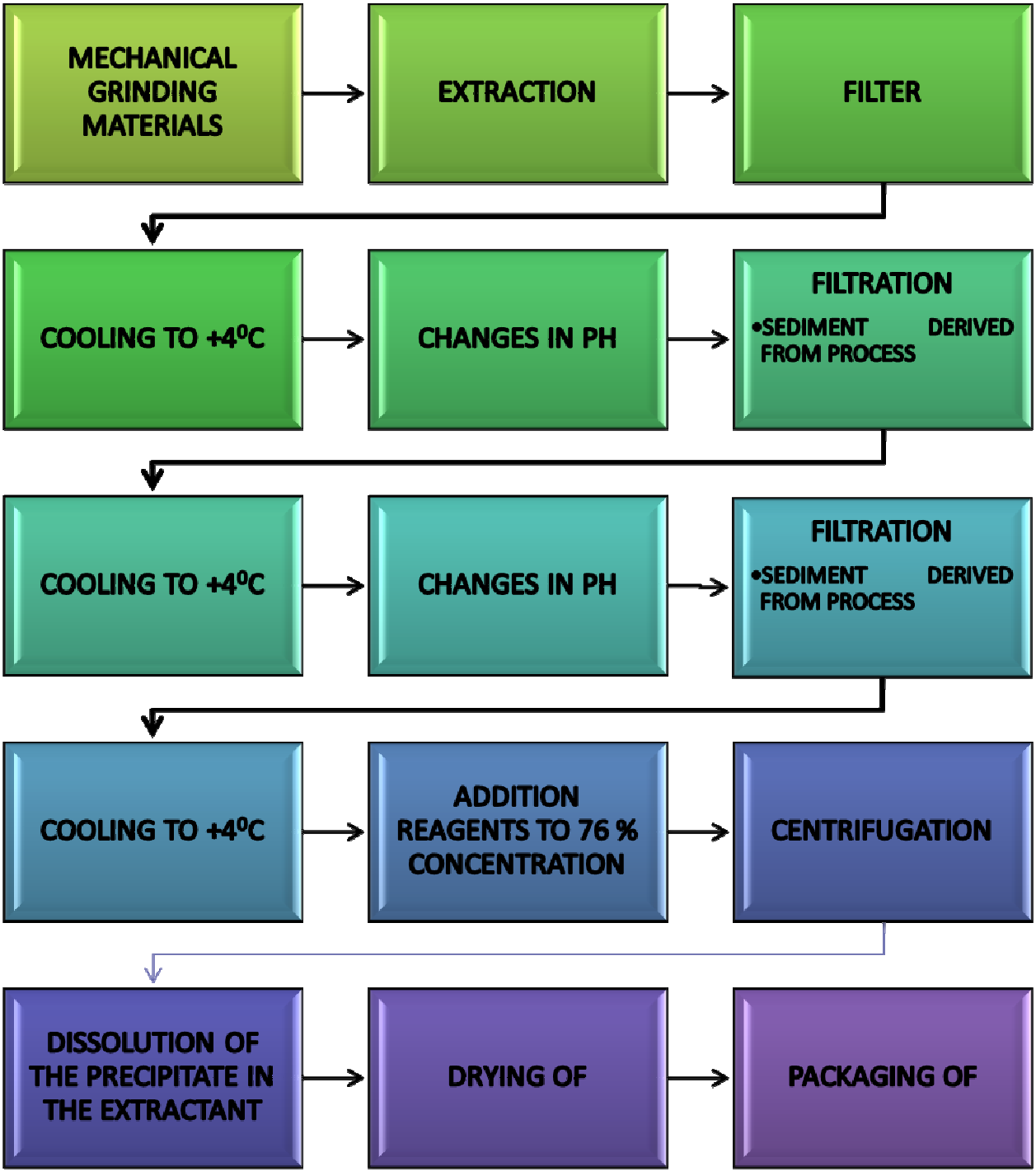

Figure 2. Technological scheme to extract of lectins from Echinacea chaff

Technology of lectins from growing waste Echinacea (Fig. 2) is based on the properties of some organic solvents translates lections to insoluble.

Conclusions:

1. By testing roots and shots barley found that extract waste growing product (chaff) of purple coneflower and pale coneflower contain biologically active substances.

2. Aqueous extracts of waste growing product (chaff) of purple coneflower mainly stimulated the growth of roots and shoots of barley. The highest catalytic activity was characterized by the extract at a concentration of 0.01%.

3. 20% alcohol extract of purple coneflower showed growth-stimulating activity of the test system at concentrations of 0.01% -0.001% (from 2.83% to 16.3% compared to controls, while 40% of extracts from these concentrations showed poor catalytic activity only in the early stages of research.

4. Aqueous extracts of waste growing product (chaff) of pale coneflower after 24 hours for all concentrations slightly stimulated root growth of barley, and for the next measurements showed inhibition of growth.

5. Found that industrial waste (chaff) Echinacea contains specific proteins - lectins. In extracts of Echinacea pale their activity ranged from 0.5 to 20.0 units, and Echinacea purpurea did not exceed 6.5 units.

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