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Isolation, Chemical and Microbiological Characterization of Essential Oils from Tobacco Dust

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Abstract: The isolation, chemical and microbiological characterization of essential oils originate from tobacco dust where performed. The main source of subjected tobacco dust was technological process of Tobacco Factory Banja Luka. In extracted essential oils 25 components where identified. Simultaneously, in the petrol ether extract where identified 31 component. The main compounds of extracted essential oils are Neophytadiene and Solanone. The main compounds of petrol ether extract are Nicotine, Neophytadiene, Sorbitol and Neodihydrocarveole.

The microbiological activity examination was showed significant antibacterial affection of extracted essential oils and petrol ether extract on *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Key words: tobacco dust, essential oil, petrol ether extract, antibacterial activity, neophytadiene, solanone, nicotine.

Introduction

The contemporary approach to industrial production, establishes harsh economy, quality and environmental assignments for the cigarette manufacturers. Those assignments caused need for the technological solutions which providing manufacturers to good market position as well as to provide them to best possible and environmental friendly use of row materials and energy. So, the modern production imperative is to decrease the waste production and maximum exploitation of unavoidable produced industrial waste.

During the primary process and cigarette making, usually appears two kinds of waste material - tobacco dust and tobacco waste.

The tobacco waste is mainly produced during manipulation and primary process, while the tobacco dust appears primarily in fabrication.

All the parts of tobacco plant represents useful row material for many of industrial branches. Essential oils are very interesting row material obtained from tobacco. They're widely used in cosmetic industry, in tobacco industry for flavoring and sousing of tobacco products and, lately, from these oils are leaded out procedures for the isolation of pharmacologically active substances[4,5,8,9,10]. Today, interest for the natural therapeutic remedies is growing. Very different kinds are increasing and the significant importance of pharmacologically active isoprenoide compounds of tobacco dust for the pharmacology-medicine industry it can bi expected.

There is possibility of essential oil presence in all plant organs: flower, stalk, rut, embryo and seed, and, in the mater of the fact, they are the source of characteristic smell and taste of the plant itself.

From the essential oil contents point of view (primarily in tobacco leaf), few thousands of works and studies related to tobacco, give us various data about this plant.

Some authors were investigated domestic tobacco types[8] and determined the chemical properties of the aromatic complex part which is responsible for the smell characteristics of plants.

Considering the fact that the chemical properties of tobacco waste, domestic tobacco type origin, wasn't investigated, the main goal of our investigation was the chemical characterization of essential oil and tobacco dust extract, as well as the investigation of they're antibacterial activity.

Materials and methods

Subjected tobacco waste material (tobacco dust and tobacco waste), was collected in the primary process of Tobacco factory Banja Luka. From collected material was taken average sample.

Sifting trough sieve, carried out separation of tobacco dust from tobacco waste. That way separated material was stored at room temperature in the dark.

Tobacco dust originate essential oils where isolated by water steam distillation method which is already described[1]. Distillation time was 7 hours.

The pH value is adjusted at pH=3-4, by adding of H_2SO_4dilution into a distillate. Extraction of essential oils from distillate was carried out by chloroform and the adding Na2SO4 dried the chloroform extract. After drying, the chloroform was removed by vacuum distillation. That way taken essential oils where stored at temperature of -18°C, up to GC/MS analysis.

The extraction of tobacco dust was carried out by petrol ether in Soxtlet extractor[2]. The extraction time was 7 hours, while the solvent appears colorless. Obtained extracts where stored under the same conditions as the essential oils.

For qualitative composition analysis and quantitative content analysis of taken samples compounds, the combination of gas chromatography and mass spectrometry methods was used. It was performed bay using of HEWLETT PACARD 5890 Series II apparatus, incorporating HP5MS (5% phenylmetyl-syloxane) middle polar capillary column, 30 m of length and 0.25 mm of diameter with FID detector. The applied film thickness was 0.25 μm.

As the carrier gas was used Helium with 0.8 ml/min of constant flow. The temperature of injector was 250°C with fallowing temperature program:

- 50°C - 130°C, 20°C/min,
- 130°C - 280°C, 9°C/min.

Ionization was performed by the electron clashes of 70 eV energy, and all of eluted compounds specters where recorded by HP5971 Mass Selective Detector.

Identification of essential oils and petrol ether extract compounds where carried out by comparison between the recorded specters and specters of known compounds according to MS library WILEY 275.1.

Microbiology analysis of essential oil and petrol ether extract. Some pharmacological features of tobacco are already observed[4,5,6]. According to this and according to results of GC/MS analysis of essential oils and petrol ether extract, we are decided to investigate the tobacco dust pharmacology effects. For that purpose the determination of antibacterial activity vas used.

For determination of antibacterial activity, the Diffusion Disk method[7] vas used. Standard test microorganisms *(Straphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 i *Pseudomonas aeruginosa* ATCC 27853) for this part of investigation where taken from Bacterial Culture Collection TORLAK.

Test microorganisms were seeded at the Mueler-Hinton (TORLAK) substrate. The 50μL of essential oils ethanol solution (concentration Ð 100 mg of oils/1 ml of solvent) was applied at paper disks (CTX/50) 6 mm of diameter. Simultaneously, 100 mg of petrol ether extract where dissolved in to the 1 mL of petrol ether. After 18 hour of incubation, the inhibition zone diameter of clear growth was measured at the lower side of plate (measuring instrument according to TORLAK). The result is expressed in millimeters.

Results and Discussion

Essential oils content of tobacco dust, expressed in weight percents is 0.015 % (w/w). Isolated essential oils are bright yellow colored and have intensive and sharp odor.

The composition and content tobacco dust essential oils and petrol ether extract are presented in the Table 1.

Table 1. Identified Compounds Of Tobacco Dust Essential Oil And Petrol Ether Extract

		Etarsko ulje		Petroletarski ekstrakt	
	JEDINJENJE	Rt(min)	%*	Rt(min)	%*
1.	Nicotine	16.60	3.37	7.93	15.61
2.	Solanone	16.90	7.11	8.06	0.39
3.	β-damaskenone	17.23	0.47		
4.	Geranyl acetone	18.24	0.59		
5.	Norsolanadione	18.82	0.92	9.67	0.22
6.	Pentadecane	18.89	1.43		
7.	Miristil aldehyde	21.77	0.59		
8.	Miristic acid	22.37	2.45		
9.	Oktadecane	22.82	0.79		
10.	α -terpineol	23.24	0.49		
11.	Neophytadiene	23.43	40.52	13.93	3.51
12.	6,10,14 -trimetyl -2-pentadecanone	23.50	2.42		
13.	Dibutil phalate	23.91	1.81		
14.	Nonadecane	24.33	0.86		
15.	Penta-O-acetyl-D-xsylitol	24.41	4.25		
16.	Farnesyl acetone	24.75	2.70	14.87	0.20
17.	Palmitinic acid	25.54	1.62		
18.	Phatic acid	25.62	7.01	22.63	2.43
19.	Eicosane	26.30	0.94	22.19	2.59
20.	Thunbergol	27.94	2.13		
21.	Bicyklogermacrene	28.77	1.17		
22.	Heneicosane	28.98	0.88	29.10	1.20
23.	Epoxylabdenol (I)	29.26	0.46	17.83	0.23
24.	Phytol	29.46	1.00	16.91	0.51
25.	Pentacosane	35.58	3.90		
26.	3-oxo-alpha-ionol			11.75	0.35
27.	Panthenol			13.57	0.67
28.	Sorbitol			14.64	6.18
29.	Heptacosane			16.24	1.04
30.	Dotrioacontane			16.44	1,32
31.	Dehydroaromadendrane			16.65	0.65
32.	Norambreinolid			16.76	0.99
33.	8-ethyl-2-methylthioindilizine			17.07	0.98
34.	1-methyl-2-(3-methyl-2buten-1-yl)			18.34	6.73
35.	O-dimethylaminobenzaldehyde			19.19	2.36
36.	Undecane			20.33	2.64
37.	Tetradecane			20.49	0.60
38.	Di-(2-ethylhexyl)phtalate			20.98	3.11
39.	2,6,10,18,22-Tetrakosohexaene			23.51	0.39
40.	Linoleic acid			24.07	0.52
41.	Nonacosane			24.23	2.55
42.	Oktacosane			25.19	1.64
43.	Pentacosane			25.55	0.72
44.	Hexatriacontane			26.52	2.92
45.	Heptadecane			27.16	7.53
46.	Pentatriacontane			28.57	2.80

In essential oils of tobacco dust, 25 compounds where identified. The most important isolated flavour compounds are Neophytadiene (40.52%), from the group of cyclic isoprenoids and Solanone (7.11%), from the group of nor-cerembroides. Other significantly important compounds are following: Nicotine (3.37%), from the

group of alkaloids, β-damascenone (0.47%), from group of norÐcarotenoides, Epoxylabdenol (I) (0.46%), Thunbergol (2.13%) and Norsolanadione (0.92%), from group of cembranoides. In essential oil of tobacco dust, the carbovyclic monoterpenoide, α-terpineole (0.49%) and diterpene, Phytol (1.00%), where detected. From the group of more complex noracyclic therpens the Geranyl acetone (0.59%) and Farnesyl acetone (2.70%) where detected.

The n-alcane mixture (16.64%), Phtalic acid (7.01%), Miristic acid (2.45%), Palmitic acid (1.62%) and Miristic acid aldehyde (0.59) where detected also.

Isolated petrol ether extract is bright brown colored, without obviously expressed odor and have got dens mass.

In petrol ether extract of tobacco dust, 31 compounds where identified. The most important isolated compounds are following: Nicotine (15.61%); from the group of alkaloids, Solanone (0.39%) and Norsolanadione (0.22%); from the group of norcerembroides; 3-oxo-alpha-ionol (0.67%), from group of carbocyclic seskquiterpene; Panthenol (0.67%) and Neophytadiene (3.51%) from group of acyclic isoprenoides. It vas detected high content of Sorbitol probable originate from tobacco additives. From the group of noracyclic therpens the Farnesyl acetone (0.20%) were detected as well as the Norambreinolid (0.99%) and Epoxylabdenol (I) (0.23%) from group of labdenoides. Composition of tobacco dust petrol ether extract also includes the diterpene Phytol (0.51%), Di-(2-etoxyhexil) phtalat (3.11%), 8-etil-2methylindolizine (0.98%), Neodihydrocarveol (6.73%), mixture of *n*-alcane (20.41%) and Heptadecane (7.53%), Phtalic acid (2.43%) and Linoleic acid (0.52%).

The essential oil composition of tobacco dust is obviously similar to essential oil composition of domestic tobacco types (Yaka, Otlja, Prilep)[8]. The comparison of tobacco dust petrol ether extract and domestic tobacco types CO_2[8] extracts cause the similar conclusion. Similarity of their composition is the consequence of mutual compounds content.

The present composition coincidence of essential oil and petrol ether extract of tobacco dust is noticeable only in the short number of components. Mentioned condition is, probable, consequence of the fact that essential oil mainly consisting of volatile terpene like compounds which have week water solubility or haven't it at all. The petrol ether extract, however, consist of middle or weekly volatile compounds of various structure. Main part of it's structure consisting of *n*-alcanes, esters of Phtaleic acid, Linoleic acid, while the terpen structure compounds content is much lower then in essential oil. Components of isoprenoid structure common to petrol ether extract and essential oil are Neophytadiene and Epoxylabdenol I. That fact had consequence on main compounds combination of essential oils and petrol ether extract. So, as the main components of all essential oils (without exception) appears Neophytadiene and Solanone, which is in accordance to data[8] related to composition of essential oil and extracts taken by different ways from different tobacco types.

Antibacterial activity of the isolated essential oils and petrol ether extracts was performed *in vitro* conditions by diffusion disk method.

Results of microbiology investigation are shown in the Table 2.

Table 2. Anti microbial Activity Diameter Of Tobacco Dust Essential Oil And Petrol Ether Extract (mm)

Bacterija Agens	Essential oil (mm)	Petrol ethar extrakt (mm)
Escherichia coli	9,0	6,5
Staphylococcus aureus	8,0	7,5
Pseudomonas aeruginosa	10,0	7,5

According to data of table 2, tobacco dust essential oil has obvious antibacterial affection to all bacteria types used for investigation and its activity is larger then the antibacterial activity of petrol ether extract.

Conclusion

The qualitative and quantitative composition and anti microbial activity of essential oil and petrol ether extracts where investigated and the conclusions can be reported are fallowing:

- Essential oil quantity founded in tobacco dust is 0.015% (w/w);
- The main components of tobacco dust essential oil are Neophytadiene (40.52%), Solanone (7.11%), Phtaleic acid (7.01%) and Pentaacetylxilitol (4.25%);
- Petrol ether extract quantity founded in tobacco dust is 6.31% (w/w);
- The main components of tobacco dust essential oil are Nicotine (15.61%), Heptadecane (7.53%), Neodihydrocarveol (6.73%), Sorbitol (6.18%) and Neophytadiene (3.51%);
- Mutual compounds of essential oil and petrol ether extract are Nicotine, Solanone, Norsolanadione, Neophytadiene, Farnesyl acetone, Phtaleic acid, Eicosane, Heneicosane, Epoxylabdenol (I) and Phytol;
- Both, the essential oil and petrol ether extract are characterized by the anti microbial activity related to examined microorganisms, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

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IZOLOVANJE , HEMIJSKA I MIKROBIOLOŠKA KARAKTERIZACIJA ETARSKIH ULJA IZ PRAŠINE DUVANA

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Rezime

Izvršeno je izolovanje, hemijska i mikrobiološka karakterizacija etarskih ulja iz prašine duvana dobijene pri tehnološkom procesu u Fabrici duvana Banja Luka. Iz etarskog ulja duvanske prašine identifikovano je 25 komponenti, a iz petroletarskog ekstrakta 31 komponenta. Osnovne komponente u etarskom ulju su neofitadien i solanon a u petroletarskom ekstraktu nikotin, neofitadien, sorbitol i neodihidrokarveol. Utvrđena je i antibakterijska aktivnost izolovanih etarskih ulja i petroletarskih ekstrakata u odnosu na *Escherichia coli, Staphylococcus aureus* i *Pseudomonas aeruginosa.*