

ISOLATION, CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF ESSENTIAL OILS FROM TOBACCO WASTE

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Abstract

For the purpose of this investigation, the isolation, chemical and microbiological characterization of essential oils originate from tobacco waste were performed. The subjected tobacco waste was collected during the primary processing and cigarette making process in Tobacco Factory Banja Luka. In extracted essential oils 26 components were identified, as well in petrol ether extract (26 compounds).

The major identified compounds of essential oil are Neophytadiene, Phtalic acid, Palmitic acid, Miristic acid and Epoxy labdenol.

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 waste, essential oil, petrol ether extract, antibacterial activity, nicotine, neophytadiene.*

Introduction

The contemporary approach to industrial production, establishes harsh economy, quality and environmental assignments for the cigarette manufacturers. Therefore, manufacturers find out technological solutions that provide them to good market position by the best possible and environmental friendly raw material and energy usage. Consequently, decrease of industrial waste production and maximum exploitation of avoidable refuses, appears to be an imperative of modern industrial production.

Tobacco waste originates mainly from processing and manipulation process. It's processing begins by the separation of foreign materials. After the separation,

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refined particles are sifting through different sieves. That way arises three fractions of tobacco waste.⁶

These kinds of tobacco waste are used in cigarettes manufacturing, for production of reconstituted tobacco and as the raw material for obtaining some chemicals (nicotinic acid for example).

Because of its usability in different kinds of industrial productions, essential oil appears to be very interesting raw material obtained from tobacco waste.

Survey of accessible literature showed that the essential oil chemical composition of tobacco waste originates from domestic tobacco types was not examined. For that reason, the isolation, chemical and microbiological characterization of essential oil and tobacco extract, sourced from domestic tobacco waste, was the goal of this investigation.

Experimental

Subjected tobacco waste material was collected in the primary process of Tobacco factory Banja Luka. From collected material was taken average sample. Average sample was stored in darkness, at the room temperature up to further analysis. The essential oil isolation was carried out by the water steam distillation method.⁴ Distillation time was 7 hours.

After the distillation the pH value is adjusted at pH=3-4, by adding of H₂SO₄ dilution into a distillate. Extraction of essential oils from distillate was carried out by chloroform. The adding Na₂SO₄ dried the chloroform extract. After drying, the chloroform was removed by vacuum distillation. That way taken essential oils were stored at temperature of -18°C, up to GC/MS analysis.

The extraction of tobacco waste was carried out by petrol ether in Soxhlet extractor.⁴ The extraction time was 7 hours, while the solvent appears colorless. Obtained extracts were stored under the same conditions as the essential oils.

For qualitative and quantitative content analysis of taken samples compounds, the combination of gas chromatography and mass spectrometry methods was used. It was performed by using of HEWLETT PACARD 5890 Series II apparatus, incorporating HP5MS (5% phenylmethylsiloxane) 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 following temperature program:

1. 50°C - 130°C, 20°C/min,
2. 130°C - 280°C, 9°C/min.

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

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

Analysis of essential oil and petrol ether extract antibacterial activity

For determination of antibacterial activity, the Diffusion Disk method¹ was used applied at the standard test microorganisms (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 i *Pseudomonas aeruginosa* ATCC 27853). Subjected microorganisms, for this part of investigation, where taken from Bacterial Culture Collection TORLAK.

Test microorganisms where seeded at the Mueller-Hinton (TORLAK) substrate. The 50 μ L of essential oils ethanol solution (concentration – 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

Content of essential oil in examined tobacco waste samples is 0,011% (w/w). Essential oil, extracted from tobacco waste, is light yellow colored and have sharp and exhaustive odor.

Isolated petrol ether extract of tobacco waste is bright brown colored thick liquid with no clearly defined scent and its yield is 4,83% (w/w).

The composition of tobacco waste essential oil and content of essential oil compounds are revealed in table 1.

Table 1. - Identified compounds of tobacco wastes essential oil and petrol ether extract

COMPOUND	Essential Oil		Petrol Ether Extract	
	Rt(min)	%*	Rt(min)	%*
Nicotine	16.60	0.53	7.93	24.06
Solanone	16.90	2.33	8.05	0.59
Geranyl acetone	18.25	0.73		
Norsolanadione	18.82	3.52	9.66	0.26
Hexadecane	20.28	0.20		
Heptadecane	21.57	0.66		
2,6,10,14 -tetramethyl pentadecane	21.65	0.27		
Miristil aldehyd	21.78	0.96		
Miristic acid	22.47	5.32		
Oktadecane	22.83	1.57		
2,6,10,14 -tetramethyl hexadecane	22.96	0.77		
Neophytadiene	23.45	19.31	13.93	8.79
6,10,14 -trimethyl-2- pentadecane	23.52	3.95		

	Pentadecic acid	23.78	0.87		
	Nonadecane	24.34	1.25	27.15	7.44
	Farnesyl acetone	24.77	3.70		
	Phtalic acid	25.69	15.99	20.98	1.08
	Palmitic acid	25.78	8.08		
	Eicosane	26.33	1.39	28.57	3.29
	Thunbergol	27.99	2.56		
	Norambrenolid	28.64	2.75		
	Heneicosane	29.01	0.88		
	Epokxylabdenol (I)	29.32	1.51	17.83	0.26
	Phytol	29.53	1.26	16.90	0.47
	Epoxyabdenol (II)	29.69	6.29		
	Epoxyabdenol (III)	32.17	0.57		
	3-oxo- α -ionol			11.76	0.50
	Glyceryltriacetate			14.31	3.00
	Sorbitol			14.64	8.50
	Phenmetrazine			16.65	0.88
	8-ethyl-2-methylthioindolizine			17.08	0.99
	Neodihydrocarveol			18.33	6.99
	O-dimethylaminobenzaldehyde			19.19	0.50
	Tetracosane			19.96	5.47
	Butyloctylphtalate			20.92	0.25
	Di-(2-etylhexyl)phtalate			20.98	0.91
	Heptacosane			22.19	2.23
	2,6,10,14,18,22-Tetracosohexaene			23.51	0.58
	Nonacosane			24.23	2.51
	Oktacosane			25.19	2.04
	Hexatriacontane			26.52	3.16
	Benzenamyne			28.02	2.01
	Triacontane			29.10	1.26

In essential oil of tobacco waste, 26 compounds were identified. The vital compounds are Neophytadiene (19,31%), from the group of acyclic isoprenoids and Phtalic acid (15,99%). It is distinctive content of three kinds of epoxilabdenole from labdenoides compounds group: Epoxyabdenol (I) (1,51%), Epoxyabdenol (II) (6,29%), Epoxyabdenol (III) (0,57%). Nicotine (0,53%), from the group of alkaloids, Solanone (2,33%) and Nor-solanadione(3,52%) from the group of nor-cembranoides, are also present. From the group of nor-acyclic terpens, Geranyl acetone (0,73%) and Farnesyl acetone (3,70%) where detected as well as the diterpene Phytol (0.51%) and cembranoide Thunbergol (2,56%). Essential oil of tobacco waste also incorporates Norambrenolide (2,75%), Palmitic acid (8,08%), Pentadecanoic acid (0,87%), Miristic acid (5,32%) and mixture of *n*-alcanes (10,94%).

In petrol ether extract, 26 compounds were identified. These compounds are following: Neophytadiene (8,79%), from the group of acyclic isoprenoids, Nicotine (24,06%) from group of alkaloids. High Sorbitol content (8,50%) from the group of polyhydroxyl alcohols and Neodihydrocarveol (6,73%) also were detected as well as the diterpene Phytol (0,47%), Solanone (0,59%) and Norsolanadione (0,26%) from the group of nor-cembranoides. Epoxyabdenol (I) (0,26) from group of labdenoides, certain quantity of aldehyds, *n*-alcanes mixture (20,54%) with Nonadecane (7,44%) as the major representative compound, also were detected as well as the Fenmetrazene (0,88%) and Glyceriltriacetate (3,00%).

Tobacco waste essential oil and petrol ether extract compare to CO₂ extract of first class domestic tobacco types⁵ (Jaka, Otlja, Prilep), have coincidental chemical composition caused by large number of common compounds.

Tobacco wastes essential oil mainly consists of terpene structure volatile components weakly soluble in water. Petrol ether extract, however, consists of middle and difficult volatile compounds of various structures.

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

Results of microbiology examination are shown in the Table 2.

Table 2. Anti Microbial Activity Zone Diameter (mm) Of Essential Oil And Petrol Ether Extract Taken From Tobacco Waste

Agens	Eseential Oil (mm)	Petrol Ether Extract (mm)
<i>Escherichia coli</i>	10,5	7,0
<i>Staphylococcus aureus</i>	9,0	6,5
<i>Pseudomonas aeruginosa</i>	13,0	6,5

According to data of Table 2, tobacco wastes essential oil has obvious antibacterial affection to all test bacteria types. The antibacterial affection of essential oil is obviously stronger then the affection 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 following:

1. Quantity of essential oil founded in tobacco waste is 0.011% (w/w);
2. The main components of essential oil isolated from tobacco waste are Neophytadiene (19,31%), Phtalic acid (15,99%), Palmitic acid (8,08%) and Miristic acid (5,32%);
3. Quantity of petrol ether extract founded in tobacco waste is 4,83% (w/w);
4. The main components of essential oil isolated from tobacco waste are Nicotine

- (24,06%), Sorbitol (8,50%), Neophytadiene (8,79%), Neodihydrocarveol (6,99%), Nonadecane (7,44%), and Eicosane (3,29%);
5. Common compounds of essential oil and petrol ether extract isolated from tobacco waste are Nicotine, Solanone, Norsolanadione, Neophytadiene, Nonadecane, Phtalic acid, Eicosane, Epoxyabdenol (I) and Phytol;
 6. Both, the essential oil and petrol ether extract isolated from tobacco waste, have anti microbial activity related to all examined microorganisms – *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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