Bulgarian Chemical Communications, Volume 52, Special Issue D (pp. 149-154) 2020

Antioxidant activity and chemical composition of crude extracts from different tobaccos and tobacco blends

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Received: February 28, 2020; Revised: March 17, 2020

The aim of this study was to investigate the antioxidant activity by FRAP-method and the chemical composition of crude tobacco extracts obtained with different solvents - 60 % methanol, ethanol and water. Bulgarian varieties of Oriental tobaccos, Virginia tobacco, American and Virginia blends from cigarettes were used. The content of polyphenols, nicotine, carbohydrates and antioxidant activity of the extracts was obtained. The extracts, obtained with 60% methanol were characterized with high content of polyphenols and reduced nicotine and carbohydrates content. They had high antioxidant activity. The lowest levels of antioxidant activity of polyphenols, nicotine and carbohydrates in ethanol extracts were reported. Extraction with water produced insoluble complexes. The yields of polyphenols, nicotine and carbohydrates, as well as the antioxidant activity varied widely. The extracts obtained from Virginia tobacco had a lower content of polyphenols compared to the Oriental tobaccos extracts, but the antioxidant activity was higher. The results showed that the antioxidant activity, except for the content of polyphenols, is probably related to the synergistic or antagonistic effect of the other compounds present in the samples.

Key words: Tobaccos, tobacco blends, tobacco extracts, FRAP-method, chemical composition.

INTRODUCTION

Tobacco (*Nicotina tobaccum* L.) is grown in different parts of the world. The main use of tobacco is for the production of tobacco products for smoking and less for chewing, snuffing, etc. On the other hand, tobacco is a plant containing a huge number of substances. It can be considered as a raw material that is more widely used in addition to its traditional uses [1, 2]. Tobacco as a medicinal plant is knowns since 15th century. After the isolation of nicotine from tobacco leaves in 1828, the medical world became yet more mistrustful of tobacco as a general treatment, because the plant contained the alkaloid nicotine [3].

Currently, the interest in the investigation of phytochemicals in tobacco has increased. The metabolites isolated from leaves, flowers and other parts of the plant include alkaloids, terpenoids, polyphenols, isoprenoids and many other classes of chemicals [1, 4]. More than 15 polyphenols have been identified in tobacco. The major polyphenols in tobacco are chlorogenic acid, neochlorogenic acid, 4-*O*-caffeoylquinic acid, rutin and kaempferol-3-rutinoside [5-7].

Polyphenols are secondary metabolites isolated from plants. They possess a wide variety of activities such as antimicrobial, antioxidant, anti-cancer, anti-inflammatory and wound healing [8]. Polyphenols are recognized as antioxidant and scavenging agents against free radicals related to oxidative damage [9, 10]. The antioxidant compounds are capable of neutralizing free radicals and may play a major role in the prevention of certain diseases such as cancer, cataracts, cerebral pathologies and rheumatoid arthritis properties [11].

The aim of this study was to investigate the antioxidant activity by FRAP-assay and the chemical composition of crude tobacco extracts obtained with different solvents – methanol, ethanol, water and their mixtures.

MATERIALS AND METHODS

Plant material

Bulgarian oriental tobacco varieties Djebel basma 1, Basma 79, Srednogorska yaka, Myumunovo seme and Virginia tobacco Linia 543 were used for analysis. The plants were grown on the experimental fields of the Tobacco and Tobacco Products Institute, Plovdiv, under identical agro-ecological and meteorological conditions. Packs of the Virginia blend and American blend cigarettes were purchased from the local shops in Plovdiv city.

Preparation of extracts

Dry tobacco powder (0.5 g) was extracted with 10 ml of 60% (v/v) MeOH, 100 % ethanol and water for 30 min on a mechanical shaker. The extract was filtered. An aliquot of the obtained extracts was subjected to analysis for content of nicotine, sugars, polyphenols and antioxidant activity by FRAP-assay.

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Ferric reducing antioxidant power (FRAP-assay)

The method measures the ferric reducing ability in which a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex was reduced to ferrous (Fe^{2+}-TPTZ) form. The FRAP assay was conducted according to the method reported by Benzie and Strain [12]. The FRAP reagent was freshly prepared before analysis by mixing 0.3 M acetate buffer (pH 3.6), 0.01 M 2,4,6-tripyridyl-triazine (TPTZ) in 10 ml 0.04 M HCl and 0.02 M $FeCl_3.6H_2O$ in distilled water in a ratio of 10:1:1. For the assay 0.05 ml extracts and 0.15 ml distilled water were mixed with 1.5 ml FRAP reagent. Absorbance against a blank sample was measured at 593 nm with a UV–Visible spectrophotometer Pharo 300, Merck after 15 min in dark at room temperature. $FeSO_4.7H_2O$ at a concentration between 0.1 M and 1 M was used for calibration. The calibration curve was generated from detection of samples containing known amounts of the standard $FeSO_4.7H_2O$; $R^2 = 0.9969$. The results were expressed as mM Fe^{2+}/g DM.

Determination of polyphenols in tobaccos and tobacco extracts

One hundred milligrams (0.1 g) of tobacco powder was sonicated for 30 min with 5 ml of 60% (v/v) CH_3OH. The extract was filtered under vacuum. The polyphenols were purified by passing the solution through a C18 cartridge according to the validated method described by Dagnon and Edreva [5] and subjected to HPLC analysis.

Determination of nicotine in tobaccos and tobacco extracts

Content of nicotine in tobaccos and tobacco extracts was determined by continuous-flow analysis method according to ISO 15152:2003 [13].

Determination of carbohydrates in tobaccos and tobacco extracts

Content of carbohydrates in tobaccos and tobacco extracts was determined by continuous-flow analysis method according to ISO 15154:2003 [14].

Statistics

All experimental procedures were done in triplicate. The quantitative data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Tobacco samples

Tobacco is a rich source of phytochemicals – alkaloids, polyphenols, terpenes and other [4, 15].

The main components of tobacco – alkaloids as nicotine and carbohydrates, which are related to the quality of tobacco and the polyphenols that are associated with the antioxidant activity of tobacco are presented in Table 1. The tobaccos were selected from low to high content of polyphenols, nicotine and sugars. Oriental tobaccos had higher polyphenols (average 34.23 mg/g) compared to the Virginia tobacco (19.83±1.56 mg/g). The highest content of polyphenols was reported in Myumunovo seme 50.79±3.99 mg/g, and Basma 79 - 35.78±2.81 mg/g. Polyphenols in Djebel basma 1 (27.62±2.17 mg/g) and Srednogorska yaka (22.75±1.79 mg/g) were approximately equal. The content of polyphenols in these varieties is lower than that in Myumunovo seme. Tobacco blends had a lower content of polyphenols (average 14.5 mg/g), which was related to the different qualitative and quantitative composition of the blends tobacco and the presence of additives [6].

At the moment, there is a lot of published data on the content of polyphenols of tobaccos [6, 7, 16]. The total content of polyphenols in Oriental tobacco ranged from 10 mg/g and 30 mg/g, while in Virginia tobacco it may exceed 30 mg/g [5]. Comparing our investigations with previous ones, the data for polyphenols in Oriental tobacco Djebel Basma 1 (24 mg/g) and Virginia tobacco Virginia 454 (15 mg/g) appear to be close [6, 16].

Table 1. Polyphenols, nicotine and sugars in tobaccos and tobacco blends, mg/g

Sample	Varieties	Polyphenols	Nicotine	Carbohydrates
Oriental tobaccos	Djebel basma 1	27.62±2.17	2.0±0.6	193±8
	Basma 79	35.78±2.81	4.3±0.1	161±6
	Srednogorska yaka	22.75±1.79	4.1±0.1	188±8
	Myumunovo seme	50.79±3.99	6.8±0.2	133±5
Virginia tobacco	Linia 543	19.83±1.56	17.2±0.5	128±5
Tobacco blends	Virginia blend	15.57±1.22	16.0±0.05	121±5
	American blend	13.52±1.06	17.5±0.05	108±4

Tobacco, as a source of a polyphenols, can be used for preparation of extracts containing polyphenols [7, 17-20]. Extraction of tobacco and tobacco blends with solvents of different polarity presumes the extraction of different groups of polar substances, which would affect the chemical composition of the extracts and their antioxidant activity [9].

Extracts obtained with 60 % methanol

The chemical composition of crude tobacco extracts is presented in Table 2. Full recovery of polyphenols was achieved with 60% methanol [17]. At the same time, the content of nicotine and sugars in tobacco extracts was lower than that in tobaccos. The yield of nicotine varied between 49% and 59%, except for the extract from Djebel basma 1 – 70%. The amount of sugars in tobaccos was proportional to the content of sugars in tobacco extracts. An average yield of 57% was reported.

Table 2. Chemical composition of crude tobacco extracts, obtained with 60 % methanol

Sample	Varieties	Extract, mg/g	Nicotine, mg/g	Carbohydrates, mg/g
Tobaccos	Djebel basma 1	370±10	0.9±0.03	123±5
	Basma 79	360 ±10	2.2±0.07	99±4
	Srednogorska yaka	370±10	2.6±0.08	108±4
	Myumunovo seme	380 ±10	3.6±0.11	65±3
	Linia 543	370 ±10	10.8±0.32	70±3
Tobacco blends	Virginia blend	340 ±10	9.0±0.27	81±3
	American blend	360±10	8.8±0.26	56±2

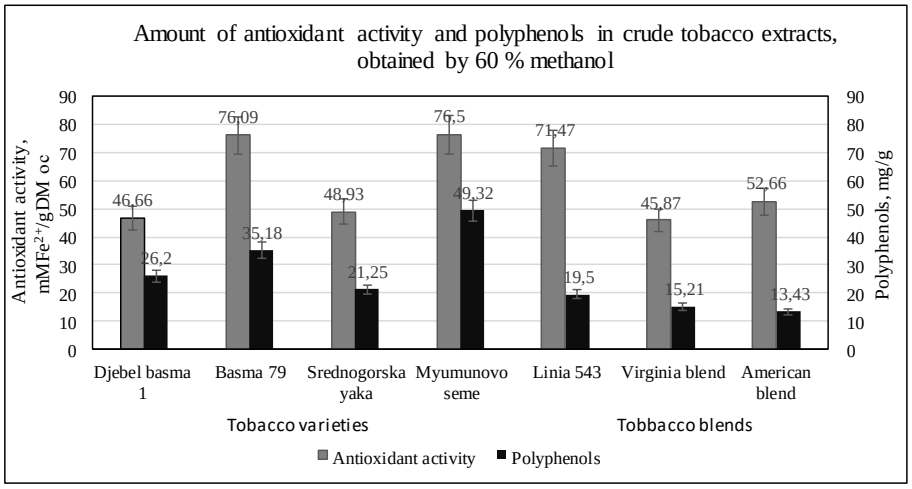

Fig. 1. Antioxidant activity and polyphenols in crude tobacco extracts obtained with 60 % methanol

Antioxidant activity of tobacco extracts can be separated in two groups – extracts with higher antioxidant activity - Myumunovo seme (76.50±7.12 mMFe^{2+}/g DM), Basma 79 (76.09±7.32 mMFe^{2+}/g DM) and Linia 543 (71.47±7.16mMFe^{2+}/g DM) and extracts with lower antioxidant activity - Djebel basma 1 (46.66±4.23 mMFe^{2+}/g DM) and Srednogorska yaka (48.93±4.81 mMFe^{2+}/g DM). The antioxidant activity of tobacco blends ranged from 45.87±4.62 mMFe^{2+}/g DM (Virginia Blend) to 52.66±5.29 mMFe^{2+}/g DM (American Blend) and was close to the antioxidant activity of extracts obtained from Djebel Basma 1 and Srednogorska yaka.

A correlation (R^2=0.7434) between antioxidant activity and content of polyphenols was obtained only in Oriental tobaccos. The extract obtained from Virginia tobacco Linia 543 had half the content of polyphenols compared to Myumunovo seme, but the antioxidant activity was close to that of Myumunovo seme – Fig. 1.

It was reported that the FRAP value strongly correlated with the flavonoids and polyphenols ($R^2 = 0.951$, $R^2 = 0.953$). This is highlighted by the

observation that the total flavonoid contents showed a strong correlation with FRAP values, whereas rutin and chlorogenic acid concentrations showed a weaker correlation with the FRAP values [21].

The differences in the antioxidant activity of the extracts were related to the different qualitative and quantitative composition of the tobacco varieties and accordingly, the different composition of the extracts. In addition, the large amounts of extracts (340±10 mg/g - 380±10 mg/g) indicated the presence of many other substances besides the investigated ones, that affected antioxidant activity.

Tawaha *et al.* (2007) determined the antioxidant activity and polyphenols content of Jordan's plant species, and estimated that a polyphenol content higher than 20 mg/g extract can be considered to be high. On the basis of this result, tobacco extracts, obtained with 60 % methanol must be considered to be promising sources of polyphenols [22].

Extracts obtained with water

Chemical composition of aqueous extracts is presented on Table 3. The amounts of tobacco extracts obtained with water were relatively high - 350±10 mg/g - 360±10 mg/g, and were close to the extracts obtained with 60% methanol (Table 2). This indicated the presence of many substances in the extracts. In aqueous extracts a precipitate was observed. There were differences in the type and amount of precipitate in the different extracts. The content of nicotine (0.1±0.003 mg/g - 4.2±0.13 mg/g) and carbohydrates (0.14±0.01 mg/g - 11.1±0.44 mg/g) varied significantly (Table 3).

The antioxidant activity of the extracts varied widely – Fig. 2. The highest antioxidant activity is manifested by the extract obtained from Basma 79 - 60.38±6.08 mMFe^{2+}/g DM. The lowest activity was recorded in Djebel Basma 1 extract - 3.36±0.33 mMFe^{2+}/g DM, where a large amount of precipitate was reported.

Tawaha *et al*. (2007) established that there was no significant difference between aqueous and methanolic extracts for antioxidant activity or total phenolic content testing 51 species, while there was a difference in tobacco extracts [22].

Table 3. Chemical composition of crude tobacco extracts, obtained with water

Sample	Varieties	Extract, mg/g	Nicotine, mg/g	Carbohydrates, mg/g
Tobaccos	Djebel basma 1	350±10	0.1±0.003	11.1±0.44
	Basma 79	360±10	1.4±0.04	5.2±0.21
	Srednogorska yaka	350±10	2.3±0.07	10.0±0.40
	Myumunovo seme	360±10	2.4±0.07	0.14±0.01
	Linia 543	350±10	4.2±0.13	0.63±0.02
Tobacco blends	Virginia blend	360±10	3.8±0.11	2.8±0.11
	American blend	360±10	3.5±0.10	3.9±0.16

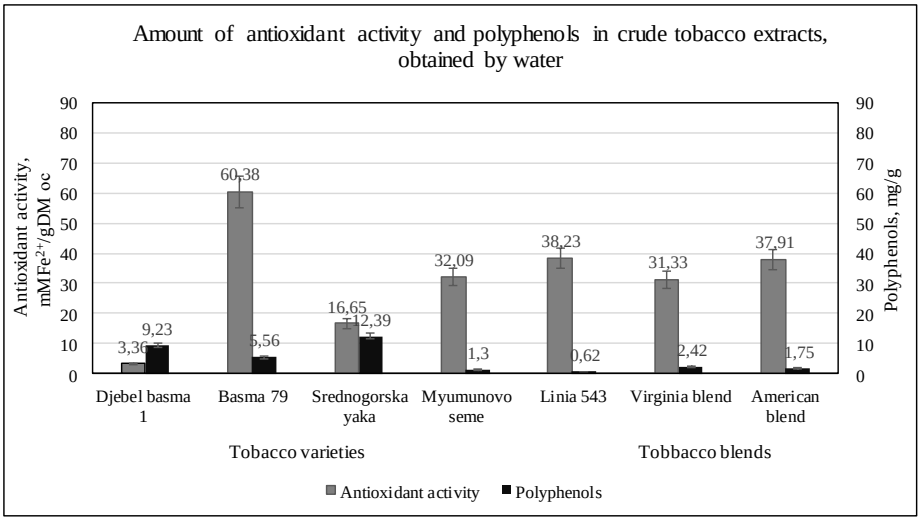

Fig. 2. Antioxidant activity and polyphenols in crude tobacco extracts obtained with water

Extracts obtained with ethanol

By extraction with the least polar solvent 100% ethanol, the most purified extracts were obtained. The average amount of ethanol extracts was 110 mg/g and was about three times lower than with 60 % methanolic and aqueous extracts – Table 2 and Table 3. The content of nicotine in oriental tobacco extracts was lower than 0.35 mg/g, while in the extracts obtained from Virginia tobacco and tobacco blends it was about 1.30 mg/g. A relatively low carbohydrates content of the extracts was reported - an average of 2.23 mg/g – Table 4. The amount of nicotine and carbohydrates in the extracts was proportional to that of tobaccos– Table 1. The polyphenols contents in the tobacco extracts were between 5.09±0.40 mg/g (Myumunovo seme) and 1.35±0.11 mg/g (Linia 543), while in tobacco blends extracts they were equal – 0.96 mg/g, Fig. 3. Polyphenols in ethanolic extracts were 7 and 15 times less than those in tobaccos and in 60 % methanolic extracts and were proportional to those in tobaccos (Table 1).

Tobacco extract obtained from Myumunovo seme, characterized with the high content of polyphenols – 5.09±0.40 mg/g, had the highest antioxidant activity 17.14±1.72 mMFe^{2+}/g DM. The other extracts had approximately the same antioxidant activity – average 9.79 mMFe^{2+}/g DM (Fig. 3). It is important to note that the Virginia tobacco extract Line 543 had twice as low polyphenols content in comparison with the Djebel Basma 1 extract, but has higher antioxidant activity – Fig. 3. The lowest antioxidant activity in Virginia blend and American blend extracts (4.82±0.41 mMFe^{2+}/g DM and 4.25±0.46 mMFe^{2+}/g DM) and the lowest polyphenols content was reported – Fig. 3.

Table 4. Chemical composition of crude tobacco extracts, obtained with ethanol

Sample	Varieties	Extract, mg/g	Nicotine, mg/g	Carbohydrates, mg/g
Tobaccos	Djebel basma 1	100±3	-	3.9±0.16
	Basma 79	100±3	-	2.10±0.08
	Srednogorska yaka	120±3	0.35±0.01	3.30±0.13
	Myumunovo seme	120±3	0.29±0.01	2.26±0.09
	Linia 543	110±3	1.25±0.04	1.55±0.06
Tobacco blends	Virginia blend	100±3	1.31±0.04	1.50±0.06
	American blend	90±3	1.32±0.04	1.01±0.04

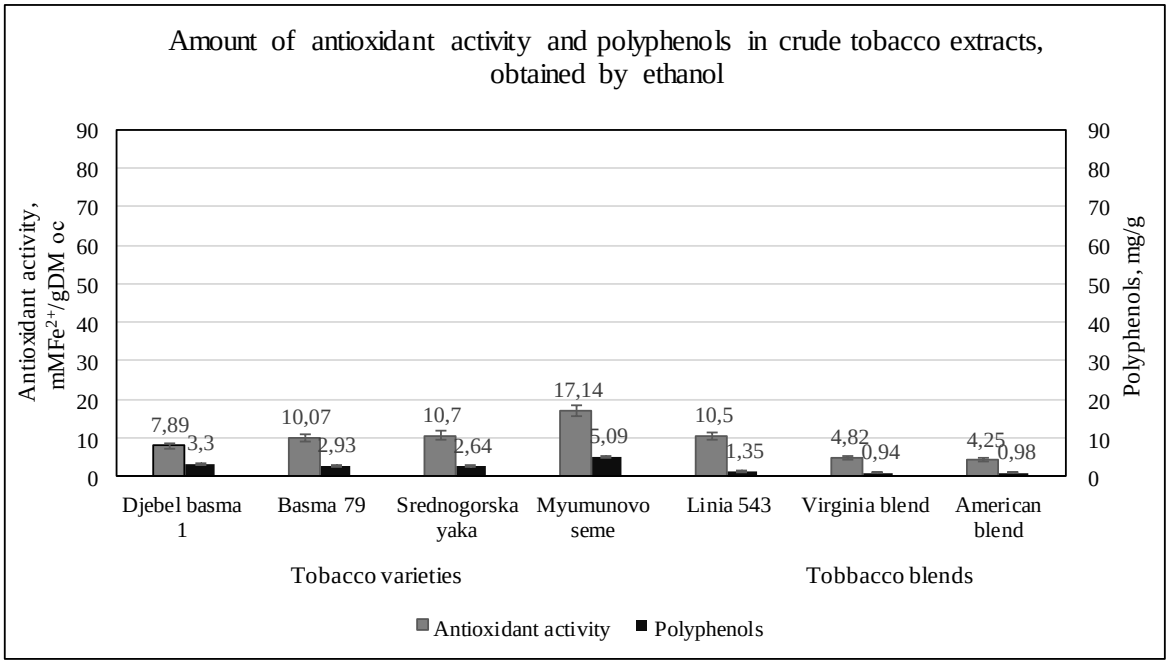

Fig. 3. Antioxidant activity and polyphenols in crude tobacco extracts obtained with ethanol

The average antioxidant activity of ethanol extracts was 6.4 times lower than that of 60 % methanolic extracts – Fig. 1. Thus, ethanol was the most inappropriate solvent for producing tobacco extracts with high antioxidant activity, but the extracts were of highest purity. The reducing ability of the extract obtained from Indian medicinal herb known as wedelia (*Sphagneticola trilobata sp.*) was in the range of 0.172 to 0.630 Mm Fe^{2+}/g and was lower than that of tobacco extracts [23].

CONCLUSION

Extracts from Bulgarian oriental tobacco varieties Djebel basma 1, Basma 79, Srednogorska yaka, Myumunovo seme, Virginia tobacco Linia 543, Virginia blend cigarettes and American blend cigarettes with different solvents were obtained. The extracts obtained by extraction with 60% methanol had the highest antioxidant activity, followed by aqueous extracts, an exception being the extract obtained from Djebel Basma 1. Ethanol extracts exhibited the lowest activity by FRAP-assay, but they were of the highest purity. The content of polyphenols, nicotine and carbohydrates in the extracts was lower than that in tobaccos. The extracts obtained from Virginia tobacco Linia 543 had lower content of polyphenols in comparison with the extracts obtained from Oriental tobaccos, but the antioxidant activity was close to or higher than that of extracts from Oriental tobaccos. The extracts obtained from Oriental tobaccos Basma 79, Myumunovo seme and Virginia tobacco Line 543 obtained with all tested solvents exhibited relatively high antioxidant activity. The differences in the antioxidant activity of the extracts were associated with the different qualitative and quantitative composition of the tobacco varieties and the different composition of the extracts, respectively. Tobacco blends extracts had lower antioxidant activity than tobaccos. The results showed that the antioxidant activity, except for the content of polyphenols, is probably related to the synergistic or antagonistic effect of the other compounds present in the samples.

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