

Total polyphenols and flavonoids in Macedonian Oriental tobacco

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Introduction

Tobacco (*Nicotiana tabacum* L.) can be used as a valuable source of bioactive compounds, such as alkaloids and polyphenols with antioxidant, anti-inflammatory and anti-fungal activity. Polyphenols are important flavoring substances in Oriental tobacco, accounting for approximately 3 % of dry weight and their concentrations are depended on maturity, variety, curing, and the conditions of fermentation process (Dagnon et al., 2003; Perfetti et al., 2008). The major polyphenols in tobacco are chlorogenic acid (3-*O*-caffeoylquinic acid) and its isomers neochlorogenic acid (5-*O*-caffeoylquinic acid) and 4-*O*-caffeoylquinic acid, as well flavonoids such as rutin (quercetin-3-rutinoside) and kaempferol-3-rutinoside (Zou et al., 2021). The research interest in polyphenols as bioactive compounds extracted from tobacco and tobacco waste (scrap, dust, midrib) has been increased over the past years (Banožić, et al, 2020). Despite the numerous studies that have shown a wide range of biological activities of tobacco, the scientific data focusing on modern, rapid and simple extraction methods followed by a clean-up step are still lacking (Ranušová et al., 2021).

The aims of the present work are: (1) to develop an ultrasound-assisted extraction (UAE) followed by clean-up step with Solid phase extraction (SPE) cartridges prior to the analysis of polyphenols, (2) to optimize the methods for analysis of total polyphenols and flavonoids from purified

extract, and (3) to determine the yields of total polyphenols and flavonoids in extract of oriental tobacco samples -.

Materials and methods

Plant material. Fermented and unfermented leaves of oriental tobacco varieties Prilep 66-9/7 and Yaka JK-125/3 was obtained at the Scientific Tobacco Institute Prilep, Republic of North Macedonia.

Chemicals and Reagents. Analytical grade chemicals that were used for extraction and characterization were purchased from Sigma-Aldrich (UK), including Folin and Ciocalteu's phenol reagent, hexahydrate aluminum chloride, ferric chloride, gallic acid and rutin. Ethanol (96% v/v), potassium acetate, sodium carbonate was supplied from Merck (Darmstadt, Germany). Chromabond C18 cartridge was supplied from Macherey-Nagel (Hoerd Cedex, France).

Extraction procedure. One g of tobacco powder (0.250 mm, 7.2 % moisture) was extracted with 30 mL of ethanol-water (60:40, v/v) solution in ultrasonic bath DU-22, 30 to 40 kHz (Clifton, UK) for 30 min and temperature of 30°C. The extract was filtered through PTFE filter 0.45µm. The filtrate (5 mL) was loaded onto a Chromabond C18 cartridge (6 mL, 500 mg), previously conditioned with 10 mL methanol and 10 mL deionized water, respectively. The first 3 mL eluates were discarded, and the following 2 mL eluates were analyzed.

Determination of total polyphenols content (TPC) and total flavonoid content (TFC). The total polyphenols (TPC) and total flavonoid content (TFC) of the purified extracts from unfermented and fermented tobacco leaves was determined by the Folin-Ciocalteu method and aluminum chloride colorimetric method, respectively. The results were calculated according to the calibration curves of the mass fraction gallic acid and rutin. The TPC value was expressed as mg gallic acid equivalents (GAE), and TFC as mg rutin equivalents (RU) per gram of dry matter (DM). All measurements were made in three replicates.

Determination of nicotine in extracts. Quantitative determination of nicotine in tobacco and tobacco products has been performed according CORESTA Recommended Method No. 62, using a 7890B Gas Chromatograph (GC) System equipped with a Flame ionization detector (FID).

Statistical analysis. STATISTICA 8 software (StatSoft, Inc., USA) was applied. The data were analyzed with one-way ANOVA followed by multiple comparisons with Tukey's honest significant difference (HSD) test at the 5 % significance level ($p < 0.05$).

Results and discussion

Tobacco plants, especially those of Oriental tobacco, are rich source of polyphenols and flavonoids. During the fermentation process, phenolic compounds were metabolized by enzyme polyphenol oxidase and their content was lowered. Ultrasound-assisted extraction has been widely used for the extraction of bioactive compounds from tobacco leaves. In available literature data (Docheva, et al, 2014), various extraction parameters (solvent, temperature, time and solvent-solid ratio) have been reported for extraction of polyphenols and flavonoids.

In our study, we applied the extraction conditions reported by Banožić et al., (2019), which are optimized for different tobacco matrices.

The extract yield from different tobacco samples varied slightly. The higher yield was extracted from leaves of Prilep 66-9/7 (0.602 g/g DM) compared to Yaka JK-125/3 (0.581 g/g DM). The variations in the yield of extracts from those tobacco varieties might be attributed to the availability of different extractable phenolic compounds, depending on the plant chemical composition, soil and agro-climatic conditions.

The results showed that the TPC and TFC values in the extract of unfermented tobacco Yaka JK 125/3 were 23.62 ± 0.05 mg GAE/g and 12.91 ± 0.01 mg RE/g respectively. The contents of TPC and TFC in unfermented tobacco Prilep 66-9/7 were 19.34 ± 0.06 mg GAE/g and 10.89 ± 0.01 mg RE/g, respectively. The TPC and TFC values of fermented tobacco were significantly lower than those of unfermented tobacco i.e. 14.06 ± 0.02 mg

GAE/g in Yaka JK 125/3 and 9.19 ± 0.04 mg GAE/g in Prilep 66-9/7. The contents of TFC in tobacco Prilep 66-9/7 was 5.01 ± 0.02 mg RE/g and 7.02 ± 0.01 mg RE/g in Yaka JK 125/3. The optimized extraction and clean-up conditions showed the presence of phenolic compounds in all tobacco samples. On the other side, the nicotine content was non-detectable in analyzed tobacco samples.

Conclusion

This work reveals the differences of phenolic contents among unfermented and fermented leaves of Macedonian Oriental tobacco varieties and provides comprehensive information about phenolic distribution and potential utilization of tobacco varieties in pharmaceutical industry. The results highlight the importance of the clean-up step for purifying the extract from phenolic acids and nicotine and promoting tobacco and its waste as source of high value compounds.

References

- Banožić, M., Babić, J., Jokić, S. 2020. Recent advances in extraction of bioactive compounds from tobacco industrial waste—a review. *Ind. Crops Prod.* 144, 112009, 1–11. <https://doi.org/10.1016/j.indcrop.2019.112009>.
- Banožić, M., Banjari, I., Jakovljević, M., Šubarić, D., Tomas, S., Babić, J., Jokić, S. 2019. Optimization of ultrasound-assisted extraction of some bioactive compounds from tobacco waste. *Molecules* 24, 1611, 1–14. <https://doi.org/10.3390/molecules24081611>.
- Dagnon, S., Edreva, A. 2003. Application of pattern recognition method for color assessment of oriental tobacco based on HPLC of polyphenols. *Beitr. Tabakforsch. Int.* 20, 355–359. <https://doi.org/10.2478/cttr-2013-0750>.
- Docheva, M., Dagnon, S., Statkova-Abeghe, S. 2014. Flavonoid content and radical scavenging potential of extracts prepared from tobacco cultivars and waste. *Nat. Prod. Res.* 1–7. <https://doi.org/10.1080/14786419.2014.902947>.
- Perfetti, A.T. Rodgman, A. 2008. *The chemical components of tobacco and tobacco smoke.* Boca Raton, FL: CRC Press, Taylor & Francis Group. ISBN 1420078836, 9781420078831.
- Ranušová, P., Matušiková, I., Nemecek, P. 2021. Optimization of plant extract purification procedure for rapid screening analysis of sixteen phenolics by liquid chromatography. *Separations*, 8(2), 13, 1–12. <https://doi.org/10.3390/separations8020013>.
- Zou, X., Amrit, B.K., Abu-Izneid, T., Aziz, A., Devnath, P., Rauf, A., Mitra, S., Emran, T.B., Adil, A.H. Mujawah, A.H., Lorenzo, J.M., Mubarak, M.S., Wilairatana, P., Suleria, H.S. 2021. Current advances of functional phytochemicals in *Nicotiana* plant and related potential value of tobacco processing waste: A review. *Biomed. Pharmacother.* 143 112191, 1–13. <https://doi.org/10.1016/j.biopha.2021.112191>.