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DETECTION OF CHOKEBERRY ADULTERATION BY HPTLC-BASED METABOLOMICS

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Introduction

Chokeberry (*Aronia melanocarpa* (Michx.) Elliott) is one of the richest sources of biologically active polyphenols, which has a long history of edible and medicinal use. [1]

The aims of this study are:

i) to investigate of HPTLC profile of berries samples using HPTLC technique based metabolomics approach,
ii) to determine the botanical origin of investigated chokeberry adulterations
iii) to identify the most important botanical markers responsible for classification.

HPTLC method is simple and rapid, and in this study, combination with multivariate techniques for differentiating chokeberry samples and identification of the main metabolites of chokeberry and four common adulterants, is applied.

Experimental

Three chokeberry (*A. melanocarpa* (Michx.) Elliott) fruit samples were purchased from a local market in Valjevo, Kraljevo, and Belgrade, while two samples were collected from a local orchard on mountain Suvobor, near Ljig, and Ralja, Serbia.

Elderberries (*Sambucus nigra* L.), dwarf elderberries (*Sambucus ebulus* L.), and pokeweed berries (*Phytolacca americana* L.) were collected at on mountain Suvobor, Serbia. Black nightshade berries (*Solanum nigrum* L.) were collected near Prijepolje.

Fresh berries were frozen in ultra-low temperature freezer, prior lyophilisation. Prior extraction, the samples were ground into a powder. Berries were extracted with 3% formic acid in methanol. The extracts were centrifuged and supernatants were filtered. The obtained extracts were used for HPTLC analysis.

A camera-connected stereo microscope Olympus SZ61was used for macroscopic analysis. Dried and frozen fruits were observed in total and afterward, seeds were isolated from the pericarp, and observed.

References

[1] N. Ćujić, *Lekovite Sirovine*, **2017**, 37, 57

Results

Morphological characteristics

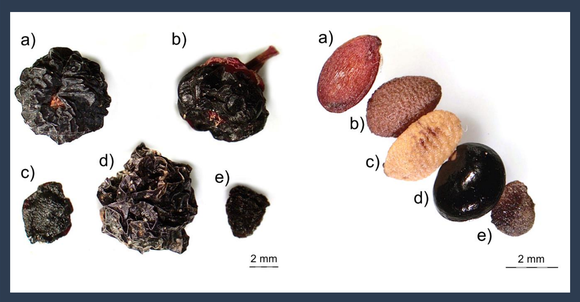

Fig. 1. Dried berries (left) and seeds (right) of chokeberry and its common adulterants. a) chokeberry (*Aronia melanocarpa*), b) pokeweed (*Phytolacca americana*), c) elderberry (*Sambucus nigra*), d) black nightshade (*Solanum nigrum*), e) dwarf elderberry (*Sambucus ebulus*)

When dried and shrunken, these berries have similar shape and are not easily recognizable. Seeds on the other hand, although with a similar size, can be easily recognized by stereo microscope on the bases of the shape and the texture of the seed coat (Fig. 1.).

The crucial determinative feature of chokeberry seed coat is longitudinal striated surface while the seeds of elderberry, dwarf elderberry, and black nightshade are warty. These characteristics could be valuable for identification of intact or roughly ground plant material.

Identification of metabolites

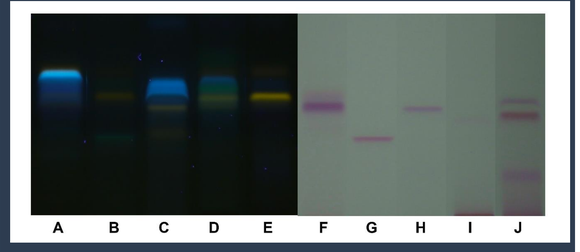

Fig. 2. HPTLC chromatograms of chokeberry and its adulterants. A-E after derivatization with NP/PEG, UV light at 366 nm; F-J without derivatization, under visible light; A,F – chokeberry; B,G – pokeweed; C,H - black nightshade; D,I - dwarf elderberry; E,J – elderberry.

The Rf values, coloration patterns, and UV spectral data of the identified compounds were compared to those of commercially available standard substances (Table 1).

Two major chokeberry anthocyanins, 7 and 9, appeared on the HPTLC plate of the chokeberry extracts. The flavonoids 6 and 11, were visualized as yellow bands after deeping in NP/PEG reagent. For the visualization of caffeoylquinic acid derivatives as light blue bands, UV light at 366 nm was applied after NP/PEG reagent derivatization.

The only band under visible light in *P. americana* (pokeweed) extract was pink band attributed to betanin. A complex anthocyanin, 5 was detected in *S. nigrum* (black nightshade) extract. At the investigated concentration of *S. ebulus* (dwarf elderberry) extract, no clear band under visible light appeared. In *S. nigra* (elderberry) extracts, four cyanidin glycosides were detected, 1, 2, 4, and 8. This anthocyanin pattern has already been described for elderberries.

Table 1. Metabolites identified in chokeberry and its adulterants

No.	Compound	R_F value	Color	Detection
1	Cyanidin 3-O-sambubioside-5-O-glucoside	0.24	purple	visible
2	Cyanidin 3,5-O-diglucoside	0.24	purple	visible
3	Betanin	0.37	pink	visible
4	Cyanidin 3-O-sambubioside	0.50	purple	visible
5	Petunidin 3-O-(p-coumaroyl)-rutinoside-5-O-glucoside	0.53	purple	visible
6	Rutin	0.58	yellow	366 nm
7	Cyanidin 3-O-galactoside	0.56	purple	visible
8	Cyanidin 3-O-glucoside	0.56	purple	visible
9	Cyanidin 3-O-arabinoside	0.60	purple	visible
10	Neochlorogenic acid	0.71	light blue	366 nm
11	Isoquercetin	0.73	yellow	366 nm

Principal component analysis (PCA)

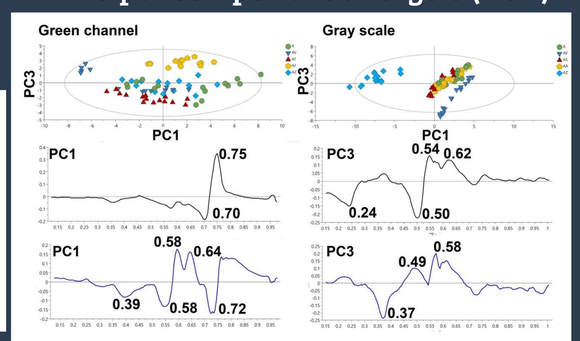

Fig. 3. PCA score (the first raw) and loading plots of pure chokeberry samples (A) or spiked with different adulterants (AS-spiked with black nightshade; AZ-spiked with elderberry; AA-spiked with dwarf elderberry; AV-spiked with pokeweed); left - green channel of the HPTLC image obtained after derivatization with NP/PEG under UV light at 366 nm; right – gray channel of the HPTLC image obtained under visible light.

In order to differentiate pure chokeberry samples (A) or spiked with different adulterants, PCA has been applied to the obtained images of HPTLC chromatograms.

In samples of pure chokeberry together with samples spiked with elderberry and one part of the samples spiked with pokeweed shared the same chemical composition: rutin. PC2 was highly contributed by 6, 10 (and 11), and compounds with RF values of 0.40, and 0.53. Further, PC3 was positively contributed by 6 and compound with RF of 0.64; negatively contributed by 10 (and 11) and compounds with RF of 0.40 and 0.55.

OPLS-DA

Four OPLS-DA models were created containing HPTLC data of chokeberry extracts as one class, and corresponding data of chokeberry extracts spiked with different adulterants as second class.

Variables with the highest VIP score (above 1.5), and the |pcorr| values above 0.5 were considered as important for the separation. According to this criteria, 3 was most influential variable AV-spiked model, and 5 in the model of AS-spiked model. Compounds 1, 2, and 4 were most influential in AA-spiked model. Adulteration of chokeberry with dwarf elderberry resulted in two fused green colored bands at Rf values of 0.60 and 0.64, which were the most influential variables in the corresponding OPLS-DA model (Fig.4). These results are in agreement with previous results in which 3 was identified as the main compound in pokeweed, while 1, cyanidin 3,5-O-diglucoside, and 4 were main metabolites in elderberry identified using HPTLC fingerprint. Further, 5 was recognized as characteristic markers of the black nightshade.

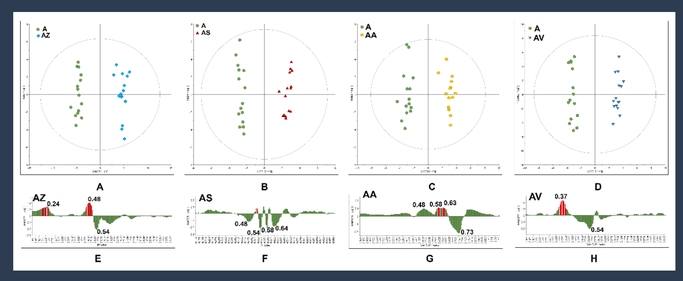

Fig. 4. OPLS-DA score (the first raw) and loading plots of pure chokeberry samples (A) or spiked with different adulterants (AS-spiked with black nightshade; AZ-spiked with elderberry; AA-spiked with dwarf elderberry; AV-spiked with pokeweed)

Conclusion

The HPTLC-based metabolomics approach has demonstrated to be a very reliable technique for the detection of chokeberry adulteration. The utilization of PCA and OPLS-DA multivariate analysis methods on HPTLC data obtained after image analysis, enabled determination of botanical origin of investigated chokeberry adulterations.

Proposed workflow confirmed the potential of planar chromatography in combination with chemometrics to detect adulteration of food-based products.

Acknowledgements

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