

EXTRACTION OF ANTHOCYANINS FROM BLACKBERRY FRUITS (*RUBUS ANATOLICUS*) AND EXAMINATION OF HISTOLOGICAL DYEING PROPERTIES

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Abstract: fruits of blackberry contains high amounts of anthocyanin pigments. Histological stains were obtained from alcoholic extract of blackberry fruit and their tissue staining properties were studied. Positive charges of anthocyanins in the extract of blackberry fruit give their basic dye properties. This allows it to stain the nucleus and basophilic parts of the cytoplasm. At the basis of staining cellular structure stay donor-acceptor interaction. As a result of our scientific work, a general methodology of preparation of high-quality dye from blackberry fruit was developed. This dye can be widely used in cytological, histological and morphological analyses.

Keywords: blackberry, anthocyanin, histological dye, freeze drier, vacuum drier, thin layer chromatography.

Blackberry plant has enough stock in Turkmenistan. Annually it gives 300-500 tons of harvest in Kopetdag mountain. There are 3 types of blackberry in Turkmenistan. Among them, the most common one is the *Rubus anatolicus* and it differs from others by its high anthocyanin concentration [1].

Dyes which are used in scientific and other laboratories for conducting cytological, histological and morphological analyses are synthetic dyes and imported from abroad. Turkmen nation have been used natural dyes which are obtained from plants for textile and food purposes since ancient times. But there is still no significant work on the production of histological dyes from plants. Considering the abundance of types and raw materials from plants our goal is obtaining histological dyes from them. For that reason, we have studied the staining features of blackberry fruit [4].

Materials and methods.

To prepare histological dyes blackberry fruits were collected from central Kopetdag mountain in June-October months. Soxhlet extraction method was used to extract anthocyanins. Methanol, ethanol and etc. were used as a solvent. Anthocyanin yield was different according to the solvent type and quantity. Extraction and anthocyanin analysis were conducted at Biotechnology laboratory at Oguz han Engineering and technology university of Turkmenistan.

For the extraction fresh fruits were grinded. Alcohol as extraction solvent was placed into Soxhlet flask. Extraction temperature was 80-85 °C. 70 g of fresh blackberry fruits were placed into 100x16mm cellulose container and extracted with 500ml of alcohol for 3 hours. At the end of the extraction dark-reddish color solution with pH=4.6 was obtained. This extract was stored in a dark container until the usage (Fig. 1).



Fig. 1. Soxhlet extraction and blackberry extract

Anthocyanine analysis. An obtained 100mg/ml of solution was prepared for UV spectrum analysis, and UV spectrometer scanned in the wavelength range of 300-600 nm. A 70% alcohol solution used as a standard sample. Spectrum analysis performed using a spectrum instrument (SP-UVDM spectrophotometer) [5, 6]. The obtained results have shown in figure (Fig. 2-3).

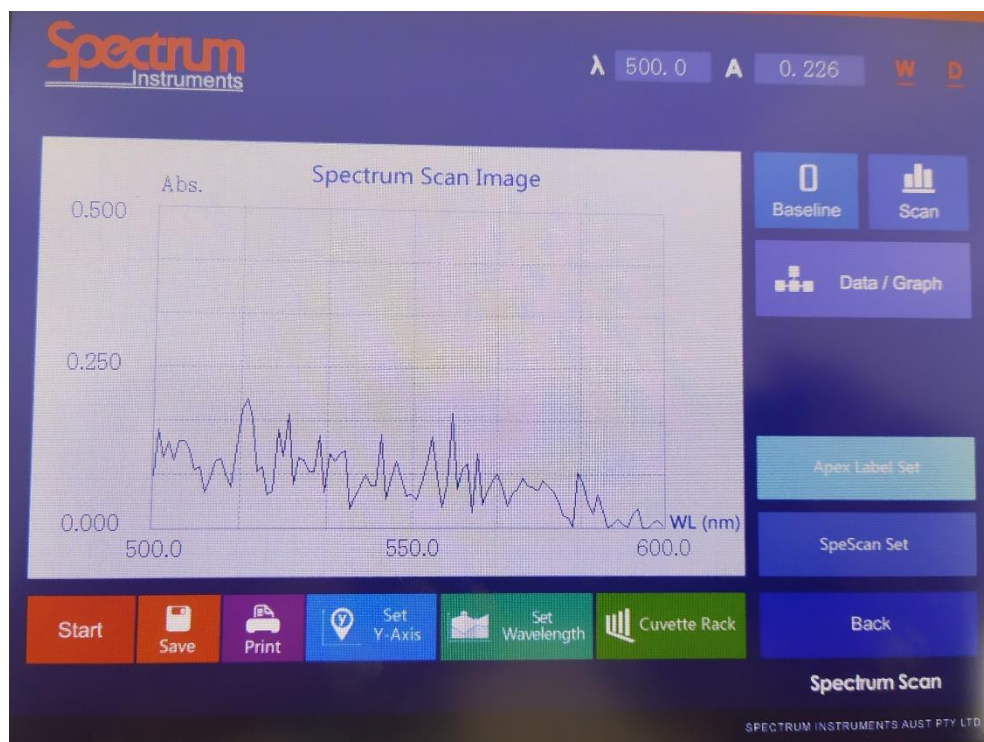


Fig. 2. Spectrum scan image of extraction

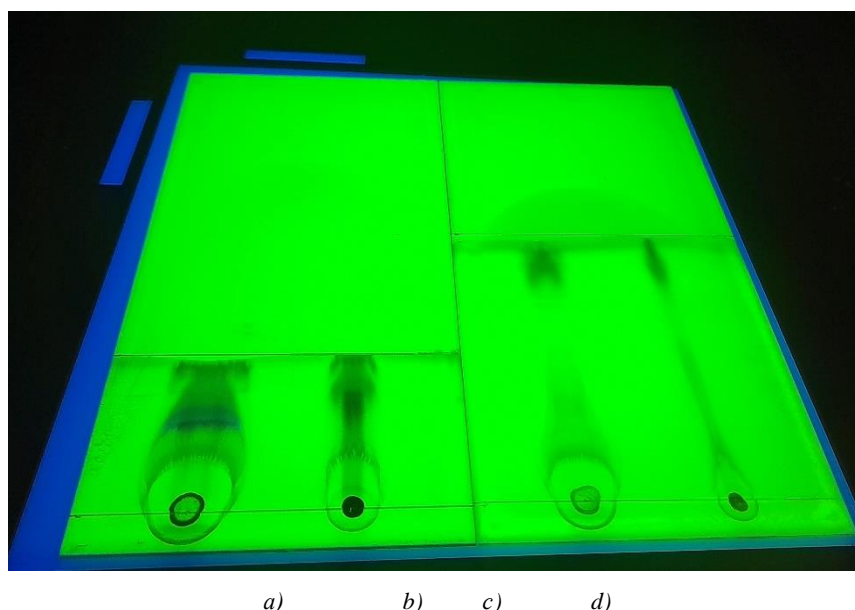


Fig. 3. TLC (thin layer chromatography) analyses: a) – sample of blackberry juice; b) – 5 sm path of blackberry extraction; c) – sample of blackberry juice; d) – 10 sm path of blackberry extraction

Result

Experiment was done to analyze the staining properties of histological dye that was obtained from blackberry fruits at department of cytology, histology and embryology of State Medical university of Turkmenistan named after Myrat Garryev. Histologic dye stained the nucleus of various tissues, also basophilic parts of cytoplasm to the pink color. This dye can be used instead of eosin synthetic dye (Fig. 4).

The main staining component of dye which prepared from blackberry fruits is quercetin. When hydrolyzed it degrades to cyanidin and glycoside. We have done quality analysis of anthocyanins by using ammonium and sodium hydrocarbonate. Copper sulphate (II) was added to prepared dye solution as an acceptor for heterocyclic oxygen of chromatic cycle of anthocyanins [2, 3].

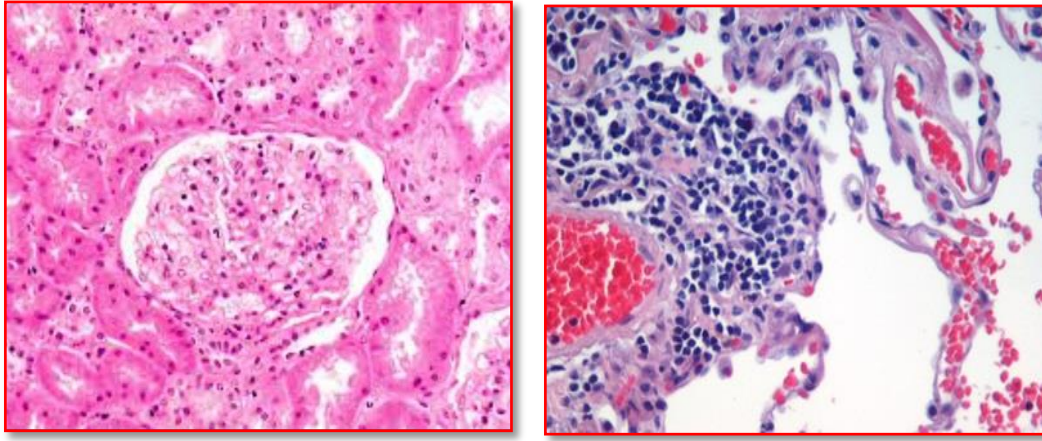


Fig. 4. Microscopic appearance of cells stained by histologic dye from blackberry extract. X400

Conclusion

As you can see from the result histological dye prepared from blackberry fruits can be used at the cytology, morphology and histology laboratories to stain various tissues. Prepared histological dye from native plants referred to be used as a raw material instead of synthetic dyes.

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