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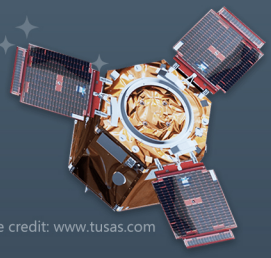

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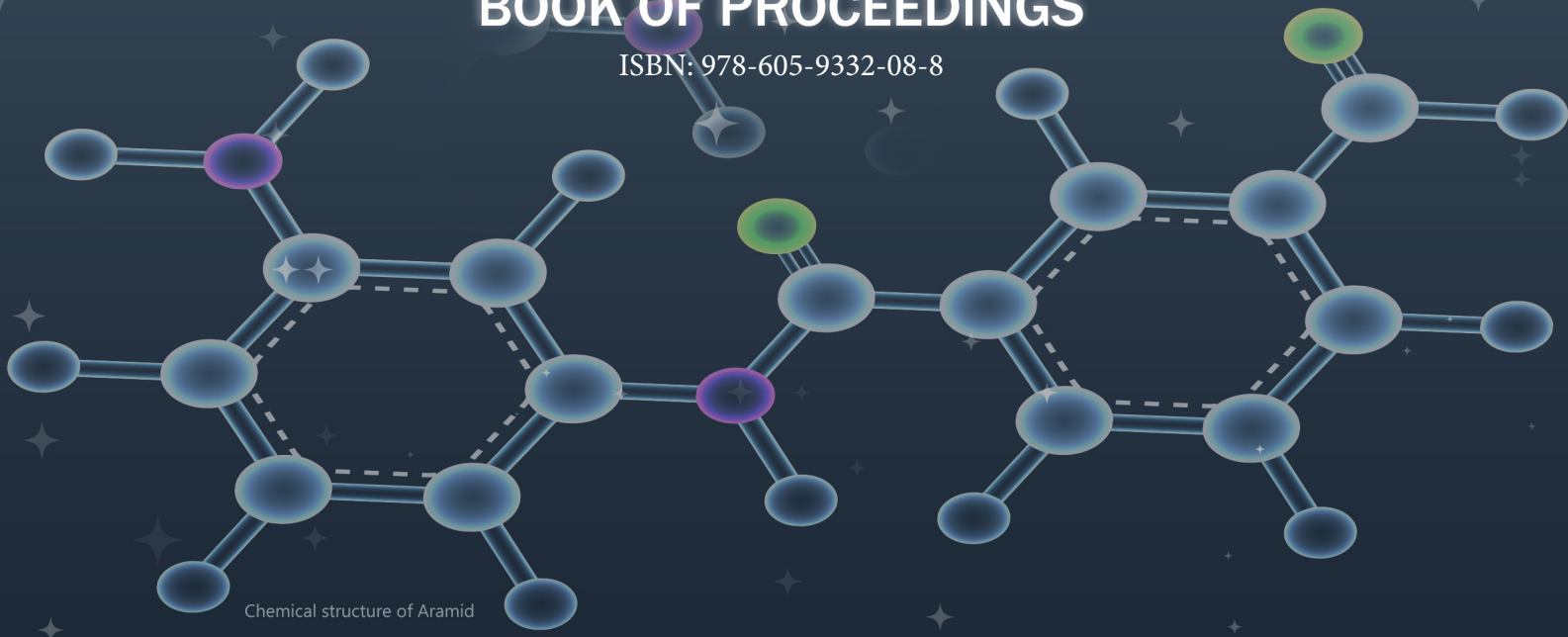

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SAVUNMA SANAYİİ BAŞKANLIĞI

TÜRKHAVACILIK UZAYSANAYİİ · aselsan · TEI · HAVELSAN · roketsan · MKE · boren

Investigation of Polar and Nonpolar Cyclotides Separation from Violet Extract Through Microfluidic Chip

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OBJECTIVES

Cyclotides (CTs) as a cyclic peptide obtained from different groups of plants have been very attractable field of research for scientists because of their specific properties like their natural function as host defense agents.

CTs are bioactive peptides from plants that characterized by their head-to-tail cyclic backbone and knotted arrangement of their three conserved disulfide bonds. Their natural function is thought to be as host defense agents and a single plant can express dozens to hundreds of CTs. CTs stand out as a family of antimicrobial peptides (AMPs) because of their exceptional stability, structural plasticity, unique biochemical target, and Gram-negative selective antimicrobial action. These features together with recent advancements in the methods of production of CTs make them an intriguing prospect from a drug development perspective.

To accomplish this aim, as part of a separation, detection and research of anti-cancer properties CTs study, we investigate the separation of cyclotides in violets into polar and non-polar groups by microfluidic chips.

INTRODUCTION

Cyclotides (CTs) are bioactive peptides from plants that are characterized by their head-to-tail cyclic backbone and knotted arrangement of their three conserved disulfide bonds. CT sequences range typically between 28 and 37 amino acid residues. Their natural function is thought to be as host defense agents and a single plant can express dozens to hundreds of CTs.

The field may be traced to the discovery of kalata B1 in 1970 based on its use in an African folk medicine. Several other macrocyclic peptides were discovered in the late 1990s[1-3], and with the elucidation of the structure of kalata B1 in 1995 [4], the name Cyclotides was introduced in 1999 [5]. In 2011, it became clear that CTs are ribosomally synthesized. The exceptional stability of kalata B1 was clarified in 2004 and the idea that it could be used as a framework in a drug design because of this stability emerged shortly subsequently in 2006 [6].

Now there is increasing proof that CTs are expressed and distributed more widespread in various orders of the plant kingdom. The majority of CTs has been isolated from Rubiaceae [7,8], or Violaceae [9] plant species.
Extraction of the CTs is one of the key steps in their screening. If extraction procedure can be done in high efficiency and purity, it will lead to more successful screening and then anticancer activity.

CTs are capable to be engineered to bind and inhibit specific cancer targets. In addition, some CTs are toxic to cancer cells, though not much is known about their mechanisms of action.

The current techniques for screening of CTs in plants comprises of the steps including extraction, purification, HPLC hydrophobic elution property and mass range. After identifying of a CTs bearing plant, Isolation and characterization are the next steps which will be done by hydrophobic chromatography and mass spectrometry techniques, respectively. All of these methods are expensive and time consuming.

The long-term goal of natural products for researchers is to discover new compounds with biological activities for potential solutions for disease treatments. Cyclic peptide discovery is one

of the most promising fields and one cyclotide candidate is currently under clinical development for multiple sclerosis [10,11]. The objective of current study is to develop a simple and rapid technique to screen of CTs bearing plants. After finding CTs bearing plants, our chance will be increased to discover novel CTs with interesting biological activity against a target human disease.

EXPERIMENTAL SECTION

Materials and Methods

Production of the microfluidic chip

The microfluidic (MF) chip template was prepared using the photolithography method and then, PDMS layer was prepared using the soft lithography method. The mixture of PDMS and crosslinker (9:1) was prepared by stirring up for 3 minutes and added to molds and vacuumed in the desiccator for 20 minutes [12]. It was then left to stand at 50 °C overnight and then the microfluidic chip was prepared by peeling off the PDMS layer. On a PDMS layer, there are three microchannels that connected along the channel by holes. Microchannel had three inputs and three outputs. Each channel diameter for the two side channels was approximately 100 µm(width) * 40 µm(depth) and for the middle channel 60 µm(width) * 40 µm(depth).(Figure 1)

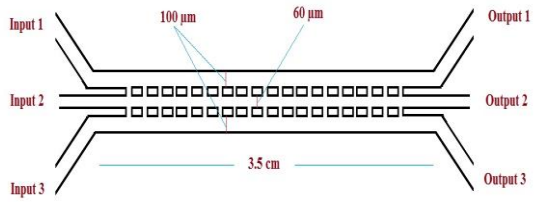

Figure 1. Microfluidic Chip for Separation.

Preparation of Violet Extract

To obtain the violet extract, we first allowed the violet leaves to dry. Later, the dried leaves were got into powder. Then the powdered leaves are mixed with 50% methanol and subjected to ultrasound for 1 hour. The solution was filtered and was dried, then the extract was collected as a powder (Figure 1).

On-chip experiments In the chip we designed, was injected HEPES buffer (PH=8.1) from the first inlet, 50 % methanol + 50 % chloroform + violet extract from the second inlet, and 80 % methanol + 0.1 % acetic acid was injected from the third inlet. Flow rate was set 3 µl / min for 1st and 2nd phases (phase 1: HEPES buffer ; phase 2: 80% methanol + 0.1% acetic acid) and 6 µl / min for 3rd phase (50% methanol + 50% chloroform + violet extract). Then were collected samples from the three outlets. Collected samples were analyzed using HPLC.

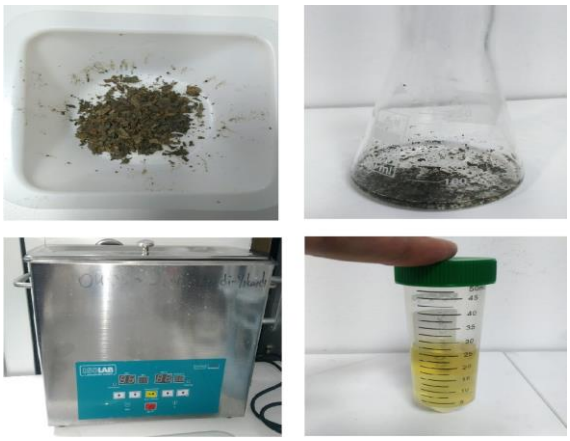

Figure 2. Preparation of Violet Extract

RESULTS AND DISCUSSION

Figure 3 show the HPLC result of samples collected from three outlets of microfluidic chip and the extract. Figure 3 A show the HPLC analysis result for the extract. As showed in Figure 3 A there are 5 peaks for the extract sample that show the presence of five cyclotides (vigno 1, 2, 3, 4 and 5). As shown in figure 3 B, C and D different peaks were seen for 3 outlets of microchip. Only one peak was seen for second channel (50 % methanol + 50 % chloroform + violet extract) that contains violet extract in inlet solution. Results show that only one cyclotide are present in second channel outlet and other 4 cyclotides were transferred to other 2 channels. Figure 3 B shows the HPLC results for sample obtained from outlets of channel 1 (HEPES buffer PH=8.1). As shown in Figure 3 B there are 3 different peaks were seen in outlets of 1st channel that one is same with peak was seen in 2nd channel. Figure 3 D show the HPLC result for 3rd channel (80% methanol + 0.1% acetic acid). As shown in Figure 3 D there are 2 peaks for outlet 3 that show 2 cyclotide transferred from channel 2 to channel 3. This 2 peptides are the same with 2 of 3 peptide were seen in channel 1. There are not distinct result for two of the peptides in any of the three channels. Probably use of highly diluted sample for separation process caused to decrease the peaks height for these peaks belongs to two of the cyclotides that cannot be separated from noises.

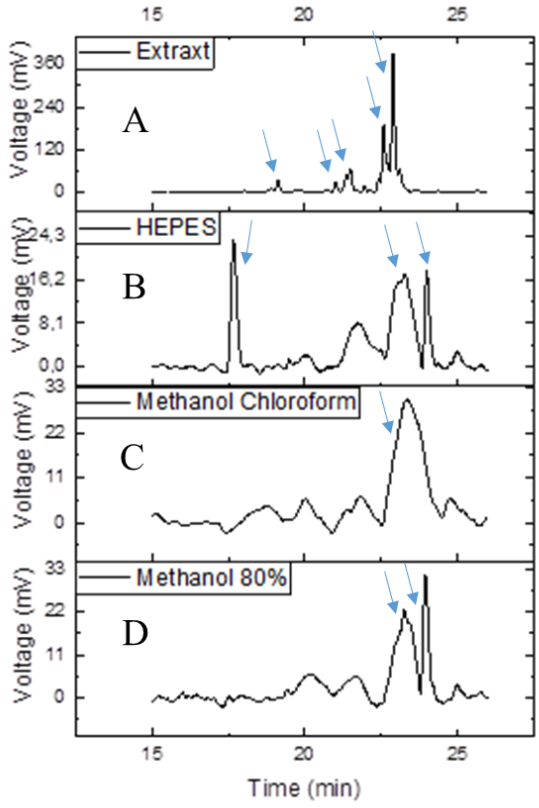

Figure 3. HPLC analysis results for violet extract and samples obtained from separation process by using 3 channel microfluidic chip: A) Violet extract, B) 1st channel, C) 2nd channel, D) 3rd channel.

CONCLUSION

In this work separation of cyclotides present in the violet extract were done by using of 3 channel microfluidic chip. The results show that the designed system has a good applicability for the separation of cyclotides.

FUTURES STUDIES

Separated cyclotides (CTs) will be detected by the electrochemical sensing method (PalmSence4). For this purpose, Monoclonal antibodies will have immobilized onto the modified gold surface of DropSence to electrochemical detection. Finally, the anticancer activity of extracted CTs on cancer cells will be studied.

ACKNOWLEDGMENTS

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