Simple and fast identification of atropine and atropine-like molecules in some medicinal plant extracts by molecularly imprinted polymers

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ABSTRACT

In this study, the extracts of four plants with alkaloid and one plant without alkaloid were used. *Datura innoxia* Miller, *Withania somnifera* (L.) Dunal, *Physalis alkekengi*, *Lactuca serriola* L. Plants with alkaloids and *Salvia sclarea* L. without alkaloids that were collected from Tehran and other plants were collected from Firouz-Abad in Fars Province in 2019. The lowest concentration of atropine, which was detected in interaction with molecularly imprinted polymers (MIP), was 0.1 µg/ml and it was raised with increasing observed fluorescent light concentration. Also atropine-molecularly imprinted polymers (atropine-MIP) with concentrations of 10 µg/ml and 100 µg/ml of tropic acid and tropine, which are atropine-like substances, produced fluorescent radiation.

Keywords: Atropine, alkaloid, molecularly imprinted polymer (MIP)

INTRODUCTION

Alkaloids, a group of natural products that have a great impact on medical, economic and social life of human. Many alkaloids are used as therapeutic agents due to their effects on the mammalian body system and other organisms [1]. Tropane alkaloids are a group of alkaloid compounds found in *Solanaceae* plant family. The most important plant

genera with tropane alkaloids are *Mandragora, Atropa, Hyoscyamus, Datura, Scopolia and Duboisia*. Medicines made from the alkaloids of these toxic plants are used as sedative, anesthetic, antispasmodic, mydriatic and so on [2]. Atropine is an example of tropane alkaloids composed of the compounds (S) -hyoscyamine and (R) -hyoscyamine. Atropine stimulates the central nervous system and causes hallucinations, agitation and disorientation [3]. The importance of alkaloids and especially atropine in different aspects of human life, is the determination of alkaloids in medicinal plants.

The reported methods for the determination of alkaloids include high-performance liquid chromatography (HPLC) [4,5], ion chromatography [6], fluorimetry [7,8], electrochemical chromatography [9] and gas chromatography [10]. Many of these methods have disadvantages such as long time to complete the detection and high cost. Molecular imprinting polymerization is a polymerization method for synthesis of MIPs that include cavities to identify specific compounds. These cavities are made artificially using molecular templates. The cavities in the MIP are similar to the template. Therefore, MIP can be used to detect the presence of template molecules. Template molecule cavities are formed during the polymerization process, then template molecules are removed from the polymer network and cavities (recognition sites). These recognition sites are template-like in size, shape, and function [11,12,13,14].

MIPs have advantages such as good physical strength, excellent stability, easy to synthesize, easily degradable in temperature changes and resistance to pressure [15,16]. The simplicity and low cost of MIP production and its high detection rate make it appropriate method to be used in studies such as the medicinal plants breeding where the number of plant sample is high for evaluation, in such case samples could be screened with MIP according to the desired compound and then only valuable sample be selected for further studies.

In the present work, we used atropine-MIPs to identify atropine and atropine-like compounds in a number of plant extracts. The designed MIPs were intended to be highly sensitive to the target compounds. Different parameters that influenced on the manufacturing process and properties of MIPs were also studied.

MATERIALS AND METHODS

Chemicals and Instrumentation

Ammonium solution, Poly methyl methacrylate (PMMA), bismuth nitrate pentahydrate and 2,2-azobisisobutyronirtile (AIBN) were purchased from Sigma–Aldrich (USA). Ethylene glycol dimethylacrylate (EGDMA), tetraethoxysilane (TEOS), hydrofluoric acid and hydrogen peroxide were purchased from Merck (Germany). Methacrylic acid (MAA) and methanol were purchased from Lobachemie (India). Glacial acetic acid and sulfuric acid were purchased from Reagent company (USA). Potassium iodide was purchased from ChemCentre (Australia). The morphology of the different kinds of synthesized MIP and NIP particles was analyzed by Scanning Electron Microscope (SEM) (TESCAN).

Plant material

The plant samples were *Datura innoxia Miller, Withania somnifera (L.) Dunal, Physalis alkekengi and Lactuca serriola L.* They were collected from Firouz-Abad in Fars Province, in 2019. These plants contained alkaloids, but *Salvia sclarea* L. was used as an alkaloid-free plant. *Salvia sclarea* L. was collected from the Pasteur Institute of Iran Botanical Garden.

Preparation of plant extract

Extracts by molecularly imprinted polymers

Extraction was performed by percolation method. 5 g of the powder of each plant was transferred into a separatory funnel and 5 ml of 80 % ethanol was added. The mixture was kept for 24 h in ambient temperature and then dropwise extracted. This process was repeated five times for each plant, and the extracts were pooled. The ethanol was evaporated from the extracts with rotary evaporator and the extract was without solvent. Finally three concentrations (0.1, 1 and 10 µg/ml) of each plant extract were prepared.

Preparation of silica colloidal crystal templates

Silica colloidal microspheres were synthesized by the Stober method [17]. TEOS (8.7 g) and ethanol (180 ml) were mixed, stirred with a magnetic beater in water bath at 300 rpm, and then slowly added 10 ml of ammonia water (25 %) and 9.4 g of deionized water for reacting overnight at ambient temperature. The obtained monodisperse silica particles were washed and centrifuged 3 times with anhydrous ethanol. Then, the obtained particles were mixed with anhydrous ethanol (about 1 %). Glass slides were well-cleaned by rinsing with 1 % hydrofluoric acid, H_2SO_4/H_2O_2 and deionized water mixture (1:1, v/v). Next, the monodisperse silica particles mixed with ethanol were poured onto glass slides. To

form colloidal crystal, the glass slides were dried at room temperature for 1 h. Finally, silica colloidal crystal templates were created on one side of the glass slide.

Synthesis of atropine-MIPs

The MIP was synthesized by 69.4 mg atropine hydrochloride (0.1 mmol), 18.90 ml EGDMA (0.1 mmol), 42.4 ml MAA (0.5 mmol) and 60 ml methanol were mixed overnight. Next, 2.0 mg AIBN (1.2 mmol) was added and exposed to nitrogen gas for 2 min. Then the glass slides with colloidal crystal templates were combined with PMMA slides and the above-mentioned precursor mixture was poured between them. The solution penetrated the space between the slides by capillary force. When the colloidal crystal template became transparent, photopolymerization was performed in an ice bath for 3 h under a UV light at 365 nm. The structure was frozen in a 3D network polymers. After that, the slides were then immersed for 1 h in 1 % hydrofluoric acid to be separated. The PMMA slides were rinsed 4 times (2 h/t) with acetic acid / methanol mixture (99.1, v/v) to eliminate atropine and additives. Next, the imprinted hydrogel was washed successively with deionized water and phosphate buffer (pH 7.6, 10 mmol/l). Thus, the atropine-MIP was ready for analyte determination. Non-imprinted photonic hydrogeals (NIPs) were

used as control and prepared using the same procedure and conditions only without atropine molecules. Experiment of plant extract with atropine-MIPs was performed by adding the extract of each collected plant at specific concentrations was poured onto atropine-MIPs and observed in UV light at 365 nm.

Identification of alkaloid test with Dragendorff solution

Dragendorff reagent is a chemical reagent to check for alkaloids. The Dragendoff solution was prepared by mixing substrates (1) and (2).

Mix 0.8 g bismuth nitrate pentahydrate, 40 ml distilled water and 10 ml glacial acetic acid.

Mix 8 g of potassium iodide and 20 ml of distilled water.

2 ml of Dragendorff was added to 5 ml of each of the extracts of the 5 plants and the precipitate that was formed by the presence of alkaloid compounds, was investigated [18].

RESULTS

Sensing ability of atropine-MIPs in real biological samples

Different amounts of alkaloids exist in different plants. Therefore, the amount of atropine and atropine-like compounds in different plants is different. *Datura innoxia* Miller, *Withania somnifera* (L.) Dunal, *Physalis alkekengi* and *Lactuca serriola* L.

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were extracted as alkaloid containing plants. *Salvia sclarea* L. extract was also prepared as an alkaloid free plant. The presence or absence of alkaloids in plant samples were determined using Dragendorff. The combination of Dragendorff reagent with *Datura innoxia* Miller, *Withania somnifera* (L.) Dunal, *Physalis alkekengi* and *Lactuca serriola* L. extracts were caused a red sediment that showed alkaloid was present in these plants but such sediment was not produced in case of *Salvia sclarea* L. extract. The combination of extracts of alkaloid plants with atropine-MIPs produced fluorescent radiation under the UV light at 365 nm. Fluorescent irradiation observed at different concentrations of these plants showed a difference at different concentrations of the extracts. The extracts of these four plants had different fluorescent intensities and this difference was clearly visible. The highest fluorescent intensity was related to *Lactuca serriola* L. and *Datura innoxia Miller* extract, respectively. The combination of *Salvia sclarea* L. extract with MIP atropine was not fluorescent under UV light. By combining the extracts of other plants (which had alkaloid compounds) with NIP, no fluorescent radiation was observed under UV light, indicating that atropine-MIP was working properly.

According to the response of the extract of these *Withania somnifera* and *Physalis alkekengi* to the atropine-MIP (Figure 1), it could be concluded that the tropine in the extract of these plants was attached to the MIP or, more correctly, the tropine or compounds that are part of the atropine structure within the MIP cavities. They were linked to cause fluorescent radiation. The atropine-like alkaloid found in *Lactuca serriola* L. is hyoscyamine [20]. According to the combination of the extract of *Lactuca serriola* L. extract with atropine-MIP (Figure 2), it can be concluded that the hyoscyamine presented in the extract of this plant was binded to MIP. The combination of *Datura innoxia Miller* extract with atropine-MIP (Figure 3) at UV light at 365 nm showed fluorescent and was increased with increased concentration of the extract.

In general, it can be concluded that atropine-MIP responds well to atropine and atropine-like compounds, so atropine-MIPs can be used to identify atropine and atropine-like compounds. The atropine-MIP reaction with atropine and atropine-like compounds could be detected under the UV light at 365 nm, and the fluorescent radiation was observed.
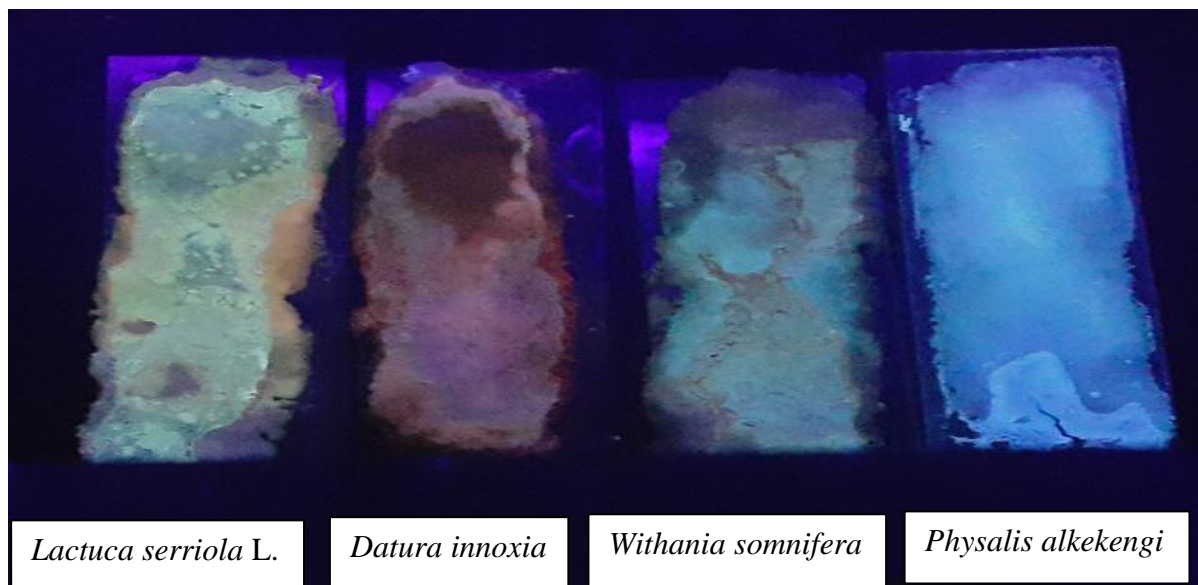
Figure 1. MIP slides combined with the highest concentration (10 µg/ml) of four extracts of *Datura innoxia Miller*, *Withania somnifera* (L.) Dunal, *Physalis alkekengi* and *Lactuca serriola* L. under UV light.
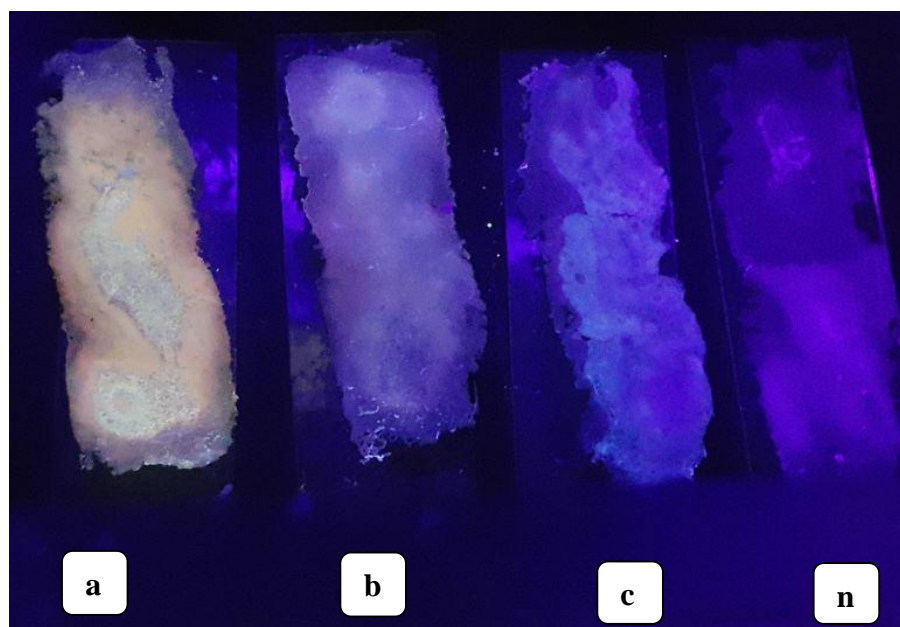
Figure 2. MIP slides combined with three concentrations of *Lactuca serriola* L. extract under UV light. (a) 10 µg/ml, (b) 1 µg/ml, (c) 0.1 µg/ml, (n) NIP combined with the highest concentration of *Lactuca serriola* L.
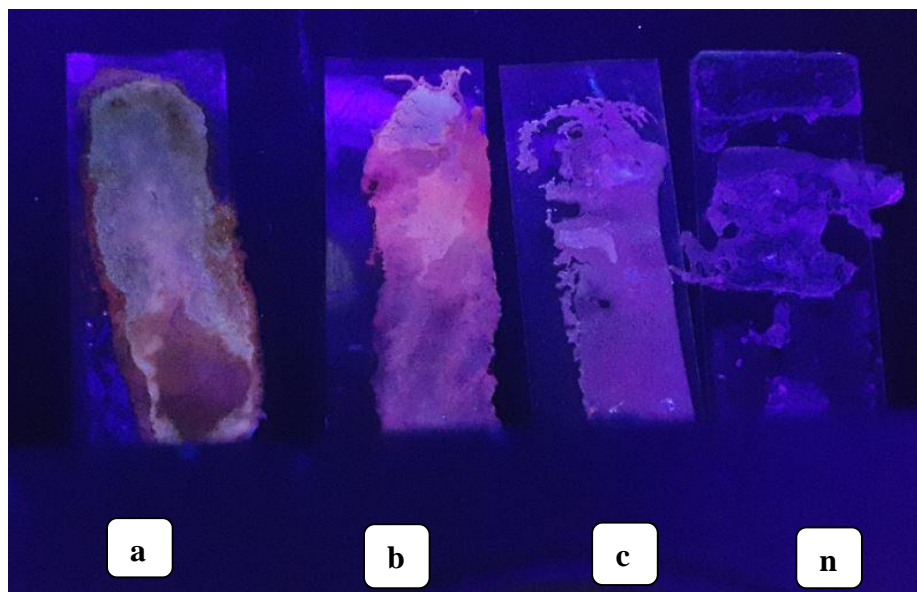
Figure 3. MIP slides combined with three concentrations of *Datura innoxia Miller* extract under UV light. (a) 10 µg/ml, (b) 1 µg/ml, (c) 0.1 µg/ml, (n) NIP combined with the highest *Datura innoxia Miller* concentration.

Selectivity of the atropine-MIPs

To evaluate the selectivity of atropine-MIPs, tropic acid and tropine, which were atropine-like substances at concentrations of 10 and 100 µg/ml, as well as concentrations 0.01, 0.1, 1, 10 and 100 µg/ml of atropine, were prepared and were observed in combination with atropine-MIPs under UV light. In addition, acetylsalicylic acid, which was selected as a non-alkaloid substance, also tested in two concentrations of 10 and 100 µg/ml and were observed in combination with atropine-MIPs below UV light.

The results observed under UV light showed that the lowest concentration of atropine produced in reaction with MIP was 0.1 µg/ml and increased with increasing observed fluorescent light concentration (Figure 4). Also atropine-MIPs combined with tropic acid and tropine at both concentrations, produced fluorescent radiation (Figures 5,6), but of atropine-MIPs combined with acetylsalicylic acid did not show fluorescent radiation (Figure 7). The results of this study confirmed the selectivity of atropine-MIPs for atropine and atropine-like compounds.

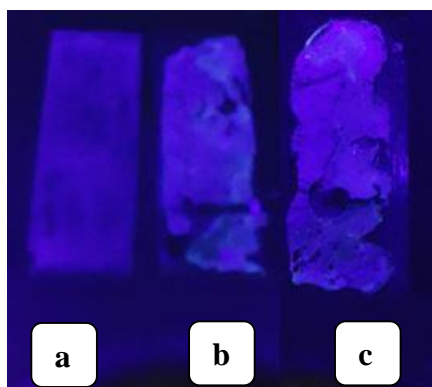**Figure 4.** MIP slides combined with atropine concentrations under UV light
   (a) NIP slide combined with a concentration of 100 µg/ml atropine under UV light;
   (b) MIP slide combined with a concentration of 0.01 µg/ml atropine under UV light;
   (c) MIP slide combined with a concentration of 0.1 µg/ml atropine under UV light;
   (d) MIP slide combined with a concentration of 1 µg/ml atropine under UV light;
   (e) MIP slide combined with a concentration of 10 µg/ml atropine under UV light;
   (f) MIP slide combined with a concentration of 100 µg/ml atropine under UV light.
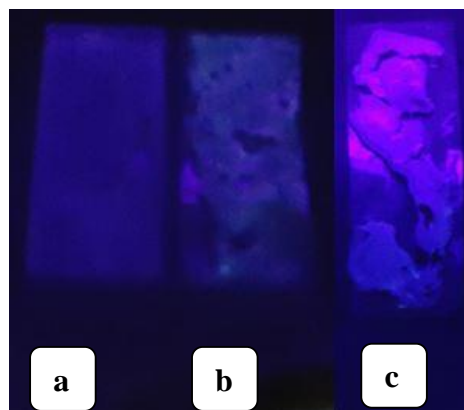
Figure 5. MIP slides combined with 10 and 100 µg/ml tropine concentrations under UV light
 (a) NIP slide combined with a concentration of 100 µg/ml tropine under UV light;
 (b) MIP slide combined with a concentration of 10 µg/ml tropine under UV light;
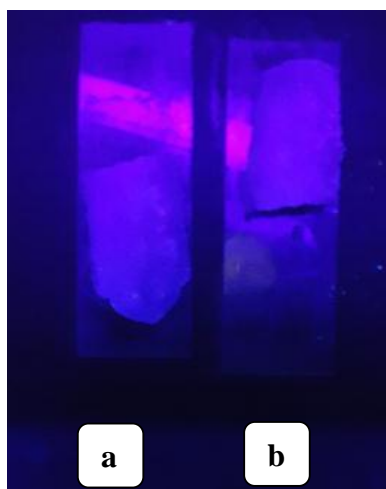 (c) MIP slide combined with a concentration of 100 µg/ml tropine under UV light.

Figure 6. MIP slides combined with 10 and 100 µ*g*/*ml* tropic acid concentrations under UV light
 (a) NIP slide combined with a concentration of 100 µ*g*/*ml* tropic acid under UV light;
 (b) MIP slide combined with a concentration of 10 µ*g*/*ml* tropic acid under UV light;
 (c) MIP slide combined with a concentration of 100 µ*g*/*ml* tropic acid under UV light.

Figure 7. MIP slides combined with 10 and 100 µ*g*/*ml* acetylsalicylic acid concentrations under UV light
 (a) MIP slide combined with a concentration of 10 µ*g*/*ml* acetylsalicylic acid under UV light;
 (b) MIP slide combined with a concentration of 100 µ*g*/*ml* acetylsalicylic acid under UV light.

DISCUSSION

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The MIPs were designed in this study caused fluorescent radiation in UV light at 365 nm by reacting with atropine and atropine-like compounds. The presented atropine MIPs were successfully used to detect atropine and atropine-like compounds.

In case of *Datura innoxia Miller* there was also the atropine-like alkaloid that contained tropine, atropine and hyoscyamine [22]. According to the combination of *Datura innoxia Miller* extract with atropine-MIP, it could be concluded that tropine, atropine and hyoscyamine present in this plant extract bind to MIP. *Lactuca serriola* L. root contains hyoscyamine [20]. Hyoscyamine is very similar to atropine and according to the combination of *Lactuca serriola* L. extract with atropine-MIP, it could be concluded that hyoscyamine present in this plant extract bind to MIP. The atropine-like compound found in *Withania somnifera* (L.) *Dunal* and *Physalis alkekengi* is tropine [21]. Therefore, the fluorescent irradiation observed was due to the interaction of atropine with atropine-MIP in the extract of these plants with atropine-MIP.

According to research [19], Dragendorff reagent precipitates alkaloids, hence the alkaloid extracts in the present study were

precipitated by combination with Dragendorff. In general, it could be concluded that atropine-MIP reacts well with atropine and atropine-like compounds and under the UV light at 365 nm, fluorescent radiation was observed, so atropine-MIP could be used to detect atropine and atropine-like compounds. In a study using ephedrine as a template for the design of Molecularly Imprinted Solid Phase Extraction (MISPE), ephedrine in Chinese Ephedra pre-treatment was efficient [23]. MIP has been used to determine the concentration and extraction of Sinomenium in *Sinomenium acutum*, that showed this method could be used for the analysis of *Sinomenium acutum* in biological samples [24]. Extraction of caffeine and theophylline from *Camellia sinensis* was performed using MIP. The solid phase extraction of MIP-based sorbent was comparable to C18 material [25].

CONCLUSION

Consequently, based on the results of this experiment on atropine and atropine-like compounds, we found that the application of such a method for early evaluation of the presence of the active ingredients in question could be valuable. And the use of

MIP in the identification of metabolites can reduce the cost and time of research and thus be effective and practical.

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