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Research Article

Investigation of the Fluorescence Imaging Properties of *Thymus Zygiodes* (Thyme) Extract and Its Interaction with Plasmid DNA

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Abstract

Organic chemical structures are known to be synthesized in laboratories or isolated from natural sources. Organic structures isolated from plants can be used as therapeutic and colorant in pharmaceutical, cosmetic, food and textile industries. In this study, powder thyme (*Thymus Zygiodes*) plant obtained from the markets of the Kayseri province. The extract of the plant with distilled water was prepared in 1:2 ratio. Agarose gel electrophoresis method was used to examine the interaction status of the extract substances and plasmid DNA (pBR322). The extract of thyme opened the supercoiled structure of plasmid DNA form I. Accordingly, the concentration of the form II structure increased. The nicked form II structure transformed into the form III linear DNA structure. In addition, the onion root cells that were incubated with the extract were imaged in three separate filters under fluorescent microscope. Since the purpose of the study was to investigate the fluorescent properties of the thyme extract and its interaction with DNA, it was determined that thyme extract has fluorescent substances and one of them interacts with DNA. However, it can be determined which substance it is with isolations, chromatographic and spectroscopic analysis. Thus, Thyme extract can be used as a DNA marker.

Keywords

Agarose Gel; Fluorescent; *Thymus Zygoides*; pBR322 DNA

Introduction

Natural resources that include both organic and inorganic materials are known to consist of plants, animals, and minerals [1-3]. While plants are mostly used for dyeing textile materials consisting of cellulose and wool (protein), they are used less in foodstuffs, medicine, cosmetics, and texture painting [4,5]. Alongside this, plant extracts are used as a source of electrons in metallic nanoparticle provision [6]. While the oil extracts obtained from plants are used for aromatherapy, water based extracts have treatment related characteristics that kill cancer cells alone or as mixture teas [7-9]. *Thymus* (Labiatae) thyme structure contained by the thyme plant has the chemical formula $C_{50}H_{82}O_4$ and a weight of 747,202 g/mol and shows antifungal and antibacterial properties [10,11]. Through chromatographic analyses, the thyme plant was found have a phenol content that changes in the summer and winter. Through studies, the thyme plant was found to contain 40-60% essential oils, have a phenol content that decreases during the winter, and have

two types of oil, namely rectified and non-rectified oils [12,13]. In its structure, thyme contains a water soluble, coloring material named luteolin beside oils [14]. Luteolin is an organic coloring material in a yellow crystal form that has the chemical formula $C_{15}H_{10}O_6$ and a weight of 286.24 molecules (Figure 1). It is soluble in water, however it is only present in small amounts in the structure of thyme, which gives meals odor. For this reason, vivid, dark coloring may not be achieved with coloring performed with thyme [15]. Poliens, aromatics, and their derivatives are fluorescent compounds [16]. Since luteolin structurally contains these compounds, it was thought that it could show fluorescent characteristics, and it was decided to use the compound in this study.

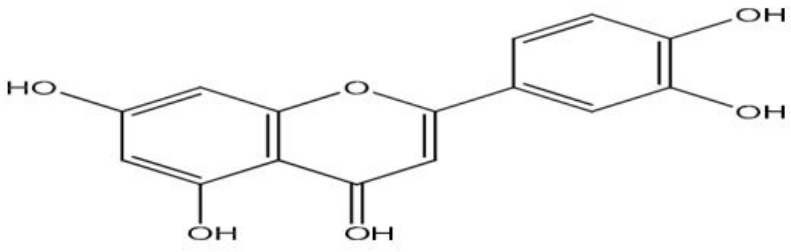


Figure 1: The chemical structure of the Luteolin molecule in the thyme extract [17].

DNA is a polymeric biomolecule formed by monomer structures called nucleotides by adding together and growing. In monomer structures, only the names and numbers of base couples change. In the structure of a double chain DNA helix, there are big and small holes called major and minor grooves, and these holes enable the DNA to interact with proteins and similar molecules [17]. Since the size of the molecule in the structure of the thyme plant would remain in micro dimension compared to the macro molecule size of the DNA, it is thought that an interaction with the holes in the structure of the DNA could occur.

There is a double helix chromosomal DNA molecule in the cytoplasm of the bacterial cell. These are not present in certain bacteria and in the form of small, double helix loops called plasmid in others. These do not perform the same tasks as chromosomal DNA and the bacteria can keep alive and genetically active without these loops [18]. Since bacteria are prokaryotic creatures, their plasmids and bacterial DNA are in a loop form. The DNA structure of eukaryotic creatures, on the other hand, is in the form of a straight chain [19].

Fluorescent microscopy is a very sensitive method widely used for the examination of the distribution of molecules in the cell. Fluorescent dye is used to mark a molecule in a living or fixed cell. The light of fluorescent dye molecule absorbs light in one wavelength and emits in a second wavelength. However, the emissions of certain fluorescent dyes can be affected by changes in the environment. For example, changes in calcium, potassium, and pH affect the emissions of specific fluorophore. Through this, by choosing an appropriate fluorescent dye, changes in intracellular ion concentrations can be detected in real time. The tool that shows the emissions of the molecules is the Fluorescent microscope. The fluorescence microscope contains a strong light source, special filters, and an additional tool for fluorescent sample labeling, and is connected to a basic light microscope. Additionally, the use of fluorescent labeled antibodies and proteins, and nucleic acid fluorescent dyes give many different ways and samples to label a sample that naturally contains an addition of fluorescent proteins [20,21].

Materials and Methods

Preparation of the Thyme Extract (TE)

Dry, powdered thyme and was obtained from the markets of Kayseri province. After 50 g of powdered thyme was boiled for 30 minutes in 100 mL of distilled water. It was filtered twice with a blue band filter paper (Whatman). The extract was used in interaction with the plasmid DNA.

The provision of the onion roots

Each of the clean, medium size, dry (top) onions after onion add these sentence -obtained from the markets of Kayseri province were placed in beakers with water from their tops. The next day, the 0.5-1.0 cm roots emerging from the tops of the onions in water were cut with a scalpel and kept in acetic acid for 30 minutes. Then the roots were left in 70% ethyl alcohol for five minutes twice, washed with pure water, and put in a 70% ethyl alcohol solution in a topped container and kept at +4°C.

Dyeing on the onion root cells with thyme

80 mL of the prepared thyme extract was taken into a beaker. 2-3 onion roots were put into the beaker the solution was

kept on a 50°C stove (Heto/Cell House 200) for an hour. The onion roots were taken out of the solution and washed. Preparates were formed by pressing between a lam and lamella. The preparates were examined under a fluorescent microscope (Olympus BX50) with three different filters (yellow, blue, green) and pictures were taken.

Thyme extract and DNA interaction

Interactions between thyme extract and pBR322 plasmid DNA were studied by agarose gel electrophoresis. The extract in DMF and water were prepared and used freshly. Then 40 µL aliquots of decreasing concentrations of extract ranging from 50% to 3.125% were added to 1 µL of plasmid DNA (conc: 0.5 µg/mL) in a buffer solution containing TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4). The mixtures were left in an incubator at 37°C for 24 h in the dark. Then 10 µL aliquots of extract/DNA mixtures were loaded onto the 1% agarose gel with loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol). Electrophoresis was carried out in TAE buffer (0.05 M Tris base, 0.05 M glacial acetic acid, 1 mM EDTA, and pH 8.0) for 3 h at 60 V. After electrophoresis, the gel was subsequently stained by ethidium bromide (0.5 µg/mL) and visualized under UV light using a Trans illuminator and photographed with a video camera and saved as a jpeg file. The experiments were performed 3 times [22].

Results and Discussion

The water extract of thyme was combined with onion root cells, which are easy to obtain and work on. The images of the cells were taken with three different filters under a fluorescent microscope. The images can be seen in figure 2.

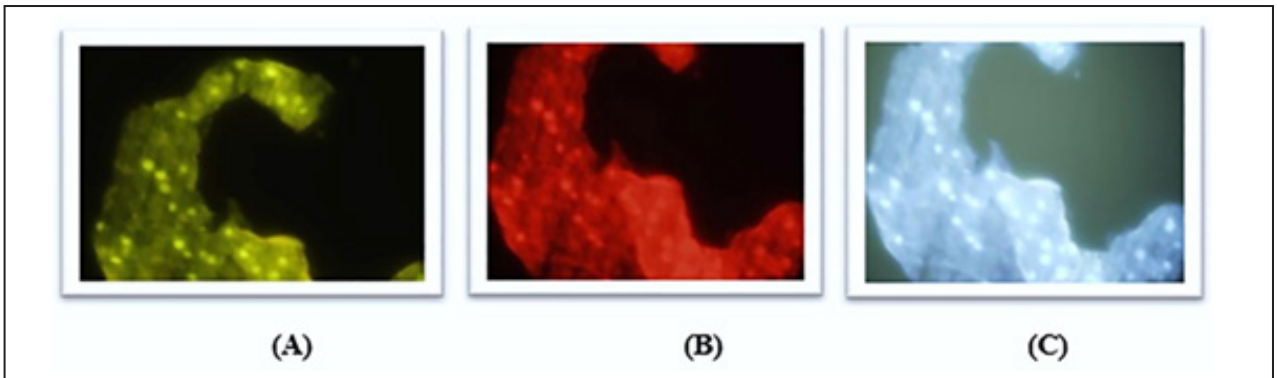


Figure 2: The images of the onion root cells stained with thyme extract at 50°C. (A): Wb (440-485 nm); (B): Wg (500-565 nm); (C): Wu (565-590 nm).

When we looked at the fluorescent images of the onion root cells in figures with yellow, blue, and green filters, it was seen that the thyme extract gave weak light emissions in the 500-565 nm wavelength interval and that there were strong emissions in the cell nucleus in the 440-485 nm and 565-590 nm wavelength intervals. This shows the presence of fluorescent staining based on the interaction between the coloring material in the extract and the molecules in the cell nucleus such as DNA and proteins. Thinking that the thyme extract, which was found to have fluorescent characteristics, would be in interaction with the DNA in the nucleus, the same extract was combined with plasmid DNA and the agarose gel electrophorese technique was applied. The gel image obtained after the agarose gel electrophorese was given in (Figure 3). The line 1 only has pBR322 DNA and the line 2 only has thyme extract. In line 2, 3, 4, 5 and 6 the solutions of thyme extract with an addition of pBR322 in various solutions diluted with DMF were present. The middle of the three lines in each column in (Figure 3) belongs to pBR322 DNA. As a result of the analysis, the *Thymus zygoides* extract was seen to show interaction with the plasmid DNA form I super helix structure, opening the helix structure. As a result, the density of the form II structure has increased, transforming the unstable form II structure into the form III linear DNA structure. Since the phenolic compounds of the *Thymus zygoides*, especially the components that are soluble in water of DMF, seek intercalation or groove attachment, this has caused the plasmid DNA to transform into different forms. The expansion in the DNA band when thyme was added to the tail showed that the thyme extract interacted with the pBR322 DNA, increasing the intensity of fluorescent emission in the environment.

Thyme extract, which interacts with plasmid DNA and causes transformations in the DNA structure, contains polar and a polar molecules that can affect the structure of bacteria. While most of the materials in the structure of the thyme are a polar molecules that are soluble in oil. The polar molecule in the structure of thyme that is soluble in water or DMF and is abundant is luteolin [23]. Since the water extract of thyme was used in the study, the molecule that interacted with the DNA can be thought to be luteolin as well. Since bacterial DNA

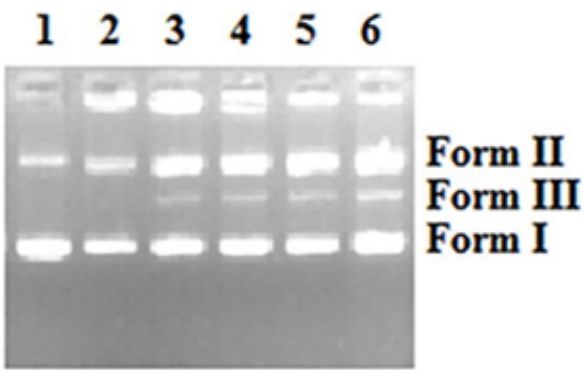


Figure 3: The UV images of the pBR322 DNA and thyme extract after gel electrophoresis. Line 1: pBR322, line 2: 3.125 % TE, line 3: 6.25 % TE, line 4: 12.5 % TE, line 5: 25 % TE, line 6: 50% TE. The top and the bottom bands correspond to form II (single nicked open circular), form III (linear) and form I (covalently closed circular or supercoiled) plasmids, respectively.

was used in this study, the results are interpreted accordingly.

The study could have been performed for DNA and RNA molecules isolated from human blood. However, for the clear explanation of the interaction, the use of pBR322 DNA, which contains purer, non-isolated DNA molecules, was found to be more appropriate. For the determination of whether the extract of *Thymus zygoides* damages DNA when interacting with it, methods such as comet will also be used. According to the results that will be obtained from these studies, the points that should be given attention in the consumption of the extracts of plants such as *Thymus zygoides* will be taken into consideration, limiting the amount of consumption through step by step concentration changes. Additionally, since the light emission in the environment increased with the interaction of DNA and *Thymus zygoides* extract during electrophorese, it can be suggested that *Thymus zygoides* can be used as a natural fluorescent material in the qualitative determination of DNA. After it was determined through literature research that flavons are structurally fluorescent, luteolin, which is a coloring material soluble in water and a structural fluorescent, was preferred in the study. After the onion root cells that were prepared to prove that the fluorescent characteristic of luteolin continues inside the cell were made to interact with the extract, they were examined under a fluorescent microscope with filters in three different wavelengths. Since the fluorescent emissions occurred in the nuclei of onion root cells, the research was continued with the thought that the extract would have interacted with the DNA molecule in the nucleus. PBR322 DNA and the water extract of thyme was combined in agarose gel electrophorese. Thus, it was found that the changes in the structure of pBR322 DNA molecules were caused by thyme extract, or even more specifically, the luteolin molecule that is abundant in the extract and soluble in water. Making explanations for the interaction of the extract of *Thymus Zygoides*, which is known to have antifungal and antibacterial characteristics [12,13], with biomolecules and suggesting the use of the extract in cytogenetic and molecular biology studies forms the Eigen value of the study. As a result, the identification of natural biomarkers constitutes the originality and future of the study. Combining with biomaterials after obtaining and characterizing organic structures with differential distillation in thyme extract will lead this study to more promising results.

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