



(RESEARCH ARTICLE)



## Some bioactive component analysis of herbal capsules

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### Abstract

Since the beginning of the evaluation of plants in terms of human health, the bioactivity properties of plants have been studied in the laboratory and thus standards have been brought to the treatment methods with plants. For this purpose, some bioactive component analyzes of 18 herbal capsules and 2 liquid herbal syrups produced by Naturin Company were performed in our current study. In this context, the total oxidant and antioxidant status of these plant mixtures were evaluated. The free radical capacities of the samples were determined by 1, 1-diphenyl-2-picrylhydrazil (DPPH) method and their DNA protective activities were determined using pBR322 plasmid DNA. Total antioxidant level (TAL) and Total oxidant level (TOL) activities were performed using Rel Assay diagnostic kit. All 3 of the stinging nettle samples showed DNA protective activity. The clean sample containing milk thistle extract also showed DNA Protective activity. In both the syrup samples (My guard and DTX-19) results were detected in the direction of positive protection on DNA. When the DPPH radical scavenging capacity was examined, it was determined that the best results were in juniper, nettle and thistle thistle samples, and the samples with syrup had a very good radical scavenging effect, and all of the samples showed antioxidant activity. This study is a preliminary and supportive study in order to elucidate the properties that can be used as drug active ingredients in traditional medicine, especially in the field of pharmacology.

**Keywords:** DPPH, DNA, Pbr 322, TAL, TOL

### 1. Introduction

Plants are the most important source of natural medicines used in traditional treatment methods. In recent years, worldwide studies have reported that approximately 72,000 of the 422,000 flowering plants (corresponding to approximately 17%) of the 422,000 flowering plants spread around the world have a therapeutic value (1). The term "phytotherapy" which means "treatment with medicinal plants" was used for the first time by the French doctor Henri Leclerc (1870-1955). (2). In these traditional methods, active substances of plants, which we will describe as secondary metabolites, are used. These secondary metabolites, which are not responsible for the growth and development of the plant, are the active substances of plants (3). In this way, it has been accepted as a strong possibility that the bioactive substances carried by a plant species with a well-known medicinal value are also found in other plant species related to this species, and in this way, the variety of plants that can be used as herbal medicine has increased rapidly. According to the World Health Organization (WHO) data. The number of medicinal plants used for therapeutic purposes is around 20,000. Throughout the history of humanity, many diseases (diabetes, jaundice, shortness of breath, etc.) have been tried to be treated by using plants. Scientists have conducted different kinds of studies for centuries to determine these therapeutic aspects (4). The World Health Organization (WHO) reports that approximately 4 billion people in the world (80% of the world population) try to solve their health problems with herbal medicines in the first place (5). In addition, approximately 25% of prescription drugs in developed countries consist of active ingredients of herbal origin

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(vimbilastine, reserpine, quinine, aspirin, etc.) treatment approach. This approach presents a uniquely different perspective on diseases and treatments. The perspective of the RTM system on diseases is also different, and it is considered as the adaptation process to the new formation that occurs in the body. These adaptations are actually positive new conditionings that the body creates in order to sustain life in the face of internal and external factors. In the RTM model, diseases are seen as the reflection of epigenetic changes resulting from gene-environment mismatch on phenotype. The treatment strategy is mainly based on restoring health by rehabilitating deteriorated structures.

Considering that most disease-causing epigenetic changes are potentially reversible, it has been clinically observed that epigenetic changes are resolved when appropriate treatment protocols are applied. The effectiveness of RTM, which was introduced to the medical literature as a new diagnosis and treatment model, has been demonstrated for a long time (approximately 25 years) in different diseases (approximately 130,000 patients) with clinical experience. For this purpose, it is important to analyze the drugs obtained from plants under standard laboratory conditions and to publish and share the results (7). The efficiency of the analysis of herbal capsules used for therapeutic purposes in the RTM system was investigated by various studies, and some bioactive components were analyzed in this study. In these studies, the toxic effect of My Guard® herbal mixture syrup used in the regeneration therapy system was evaluated, and no toxic effects of My Guard® product were observed at the indicated dose in acute and subacute periods (8). In our study, it is ensured that the herbal preparations are standardized scientifically and ethically by making bioactive component analyzes of the same product. In recent years, detailed laboratory studies on plants have become widespread. Among the reasons for this are the high side effects of synthetic drugs, the inability to plan personalized treatments, and the fact that each drug does not show the same effect-benefit relationship in every patient. Same time; Many reasons can be listed, such as frequent and unnecessary use of antibiotics, and acquired resistance against existing bacteria (9). This situation supports the use of herbal medicines as a traditional treatment tool as well as being complementary in their use with synthetic drugs that are toxic and expensive on the organism. Looking at the literature, thousands of phytochemicals derived from plants have been found to be safe and have very few side effects. The causes of these side effects and the results of the analysis should be evaluated and standards should be determined. For this purpose, the analyzes of herbal and supplementary capsules based on the RTM system produced by the nataurin company were examined in this study.

## 2. Material and methods

### 2.1. Materiel

In this study, 18 samples as herbal capsules and 2 samples as syrups belonging to Naturin Company (Natural Products and Pharmaceutical Raw Materials Industry Trade Limited Company) were included in the study.

### 2.2. Antioxidant-Oxidant Activity Study

Determination of Antioxidant Activity by DPPH Radical Scavenging Capacity. The free radical scavenging capacities of the samples were determined using 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical. The DPPH solution added to the samples causes a decrease in the absorbance value at the optical density at 517 nm, and the discoloration of the compound is an indication that the sample has a good radical removal capacity (9). Values were evaluated by calculating the % inhibition value as shown in Table 1.

**Table 1** % inhibition values of DPPH

Samples	% inhibition (%)	Samples	% inhibition (%)
Timoksin	45,71	My System	52,38
IST-ARD	49,52	Curcimax	40
ISY-REG	53,33	IST-Glio	50,48
IST-REM	40,00	Slimax	43,81
ARD-REM	46,67	DVD-REG	52,38
MY CODE	56,19	My Clean	40,95
Regulin	47,62	My Guard	54,29
DVD-ARD	50,48	DTX-19	53,33
ARDZ REM	49,52	ROMX	55,24
KT-REM	52,38	KL21	43,81

### 2.3. Determination of Total Antioxidant Level (TAS) by ABTS Method

Commercially available kits were used to determine the antioxidant capacity of the samples. Antioxidant substances in the sample convert the radical into the dark blue-green colored ABTS (2,2-AzinoBis (3-Ethyl Benzo Thiazoline-6-Sulfonic Acid) form of reduced ABTS, and the change in absorbance at 660 nm indicates the total antioxidant level of the sample. Trolox, a synthetic antioxidant and Vitamin E analogue, was used as a positive control (10). Rel Assay Diagnostics-TAS Assay Kit was used in this study.

### 2.4. Determination of Oxidant Activity by Total Oxidant Level (TOS)

This test is calibrated with hydrogen peroxide. Ferrous ion chelator in the presence of oxidant. The complex oxidizes to the ferric ion. Presence of oxidants in the reaction medium. As a result, the oxidation reaction intensity increases. Ferric ion acidic forms a colored complex in the medium. In the samples evaluated colorimetrically, the oxidant value is determined according to the intensity of the color. The TOS value is determined by comparing the results obtained with mmol H<sub>2</sub>O<sub>2</sub> Equiv. /L (11)

## 3. Results

Considering the DPPH radical scavenging capacities, it was determined that the best results were in juniper, nettle and thistle samples, and the syrupy samples had a very good radical scavenging effect. The DPPH solution added to the samples causes a decrease in the absorbance value at the optical density at 517 nm, and the discoloration of the compound is an indication that the sample is a radical scavenger. The % inhibition values are shown in table 2.

According to the results in Table 2, all samples have antioxidant activity. The oxidant activity was found to be high in all samples and was interpreted by calculating the OSI values. IST-REM, Regulin, with an OSI value above 1 were detected as thyme. The oxidative stress index (OSI) is an indicator that shows the level of oxidative stress and is calculated as follows:

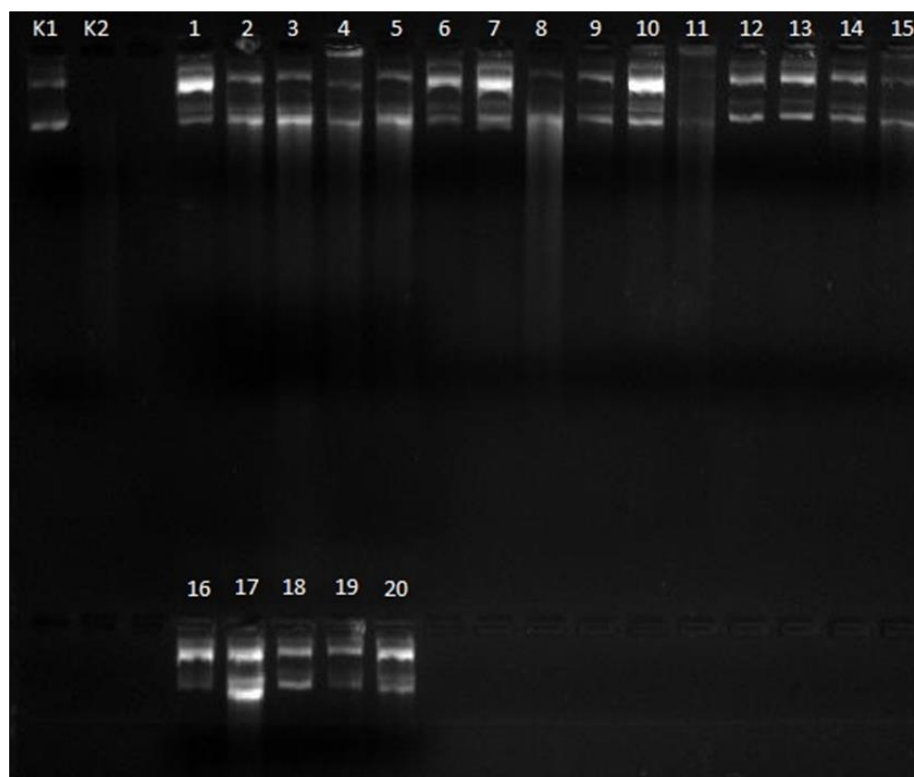
$$\text{OSI} = (\text{TOS}, \mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / (\text{TAS}, \mu\text{mol Trolox equivalent/L}) \times 100 \text{ (Ulaşvd., 2013)}$$

**Table 2** TAL, TOL and OSI values of products

Samples	TAL (μmol/L)	TOL (μmol/L)	OSI values
Timoksin	3,813	37,382	0,98
IST-ARD	4,112	15,766	0,38
ISY-REG	4,255	19,931	0,46
IST-REM	3,464	61,577	1,77
ARD-REM	4,206	15,171	0,36
MY CODE	3,850	29,945	0,77
Regulin	4,273	56,321	1,31
DVD-ARD	3,31	9,122	0,274
ARDZ REM	4,273	16,857	0,39
ROMX	4,258	36,490	0,85
KT-REM	2,918	15,868	0,54
KL21	4,273	60,9	1,42
My System	4,266	36,589	0,85
Curcimax	2,794	3,867	0,138
IST-Glio	3,191	29,152	0,91
Slimax	4,247	18,344	0,43
DVD-REG	3,858	10,312	0,26
My Clean	4,258	34.110	0,801
My Guard	4,307	18,047	0,41
DTX-19	3,169	12,395	0,39

### 3.1. DNA Protective Activity Potential

The pBR322 plasmid DNA (vivantis) was used to determine the protection levels of the samples from UV-C and oxidative damage. Plasmid DNA was added with 5 µl of sample in each sample and was damaged by applying H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxide) and UV. Imaging on 1.25% agarose gel was performed using the DNA protective activity determination method (12,13). As seen in the DNA bands in Figure 1, K1 and K2 coded samples are our control and the comparison of 20 samples was made accordingly. When the results were evaluated, all 3 of the nettle samples showed DNA protective activity. The clean sample containing milk thistle extract also showed DNA Protective activity. In both of the syrup samples, the results were determined in the direction of positive protection on DNA.



**Figure 1** DNA band profiles of samples

K1: Control 1 K2: Control 2

1: Timoksin 2: IST- ARD 3: IST-REG 4: IST REM 5: ARD-REM 6: My CODE 7: Regulin 8: DUD-ARD 9: ARDZ-REM 10: ROMX 11: KT-REM 12: KL2 113: My System 14: Curcimax 15: IST-GLIO 16: Slimax 17: DVD-REG 18: My Clean 19: My Guard 20: DTX-19

## 4. Discussion

Plants provide the oxygen necessary to sustain human life. They are essential for human life in terms of food and health. Since ancient times, people have preferred to discover the therapeutic and protective properties of plants and to benefit from them for a healthy life. For this reason, studies on herbal preparations and extracts are becoming more and more common. Among the reasons why herbal drugs are preferred, countries that do not have a suitable developed pharmaceutical industry are turning to herbal preparations, which are an easy and cheap treatment method. However, it is extremely important that these herbal preparations have passed the same tests as synthetic chemical drugs and that their standards have been determined. (7.14).

This situation supports the use of herbal medicines as a traditional treatment tool as well as being complementary in their use with synthetic drugs that are toxic and expensive on the organism. For this purpose, it is becoming increasingly important to analyze and report the bioactive component of herbal capsules. In this study, some analyzes of naturine natural products, which are recommended for treatment and preventive purposes in traditional medicine, were made. The contents of 18 plant capsules and 2 syrup samples used in the RTM system and produced by Naturin company were formulated as a mixture of herbs in different combinations. The effectiveness of these plant mixtures on oxidative stress index and DNA protection was examined in this study. In our study; the free radical scavenging effects of these compounds were also tested on DPPH, a stable radical. UV rays are divided into 3 classes as A, B and C, and the most

harmful part of them is UV-C. It is known that these rays, which reach the world with the damage of the atmosphere layer in recent years, have negative effects on all kinds of living things. Both enzymatic and non-enzymatic antioxidants provide protection against the harmful effects of these UV rays. Therefore, in our study, oxidant states and protective activity against UV rays were evaluated together. UV rays can methylate the hereditary material DNA and cause permanent damage and cause different types of cancers (15). Many herbs have been reported to have anti-inflammatory, antimicrobial, antioxidant, antidiarrheal, analgesic, and wound healing benefits (16)

While it was determined that plant mixtures containing stinging nettle and milk thistle extract showed protective activity on DNA, it was also determined that these samples had high antioxidant capacity. At the same time, it was determined by studying the DPPH radical that nettle, thistle and hawthorn-based plant mixture capsules have very good radical scavenging properties. It was determined that syrup samples containing plant extracts also had a protective potential on DNA and showed good antioxidant properties. While free radicals cause damage to cells, the strong radical OH. It can cause nucleophilic attack on DNA and cause damage. For this reason, DNA protective activity studies should be studied as a whole, not only by evaluating the DNA protection potential, but also by looking at the antioxidant activity.

OSI values below  $< 1$  in all 20 samples, and high antioxidant activity detected in all of them and OSI values  $< 1$ , all of the samples have antioxidant potential. Studies on the oxidant and DNA protective activity of these plant capsules have been conducted for the first time and are planned to be supported by further studies.

In addition to the use of plant capsules alone, it is also extremely important to use it as an alternative treatment method in order to benefit from its complementary effect. In recent years, bioactivity studies of medicinal aromatic plant extracts, which reduce cancer risks by preventing DNA damage and are frequently used in preventive medicine, have been widely performed. As a result, it is extremely important to illuminate the structures of plants that have been used for centuries for both treatment and prevention of diseases. At the same time, the detection of side effects with a high risk of danger seen in some of the synthetic chemical-based substances has increased the necessity of using less harmful natural products. We see this effect in phytochemicals using the RTM system. We believe that biochemical and molecular analyzes of the secondary metabolites of plants containing bioactive components will be very enlightening in terms of the usability of extracts to be obtained from these components in personalized traditional treatment methods.

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## 5. Conclusion

In this study, in which the bioactive component analyzes of these herbal capsules used in the RTM treatment system were studied, it was concluded that the products in question could play a protective role in diseases caused by DNA damage, but they should be supported by clinical studies to test their clinical efficacy.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

There is no conflict interest between authors.

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