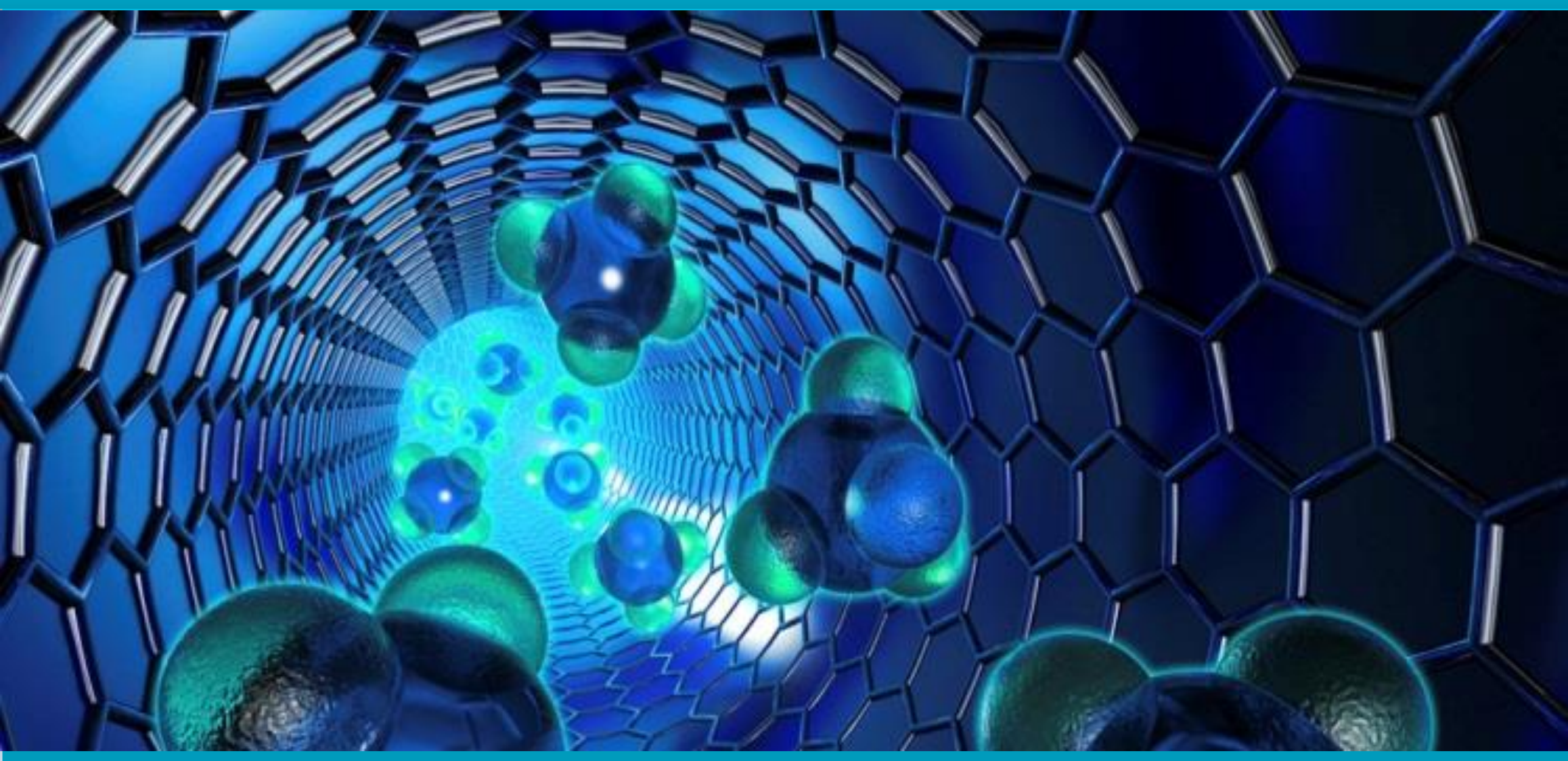
e-ISSN: 2602-277X

International Journal of Chemistry and Technology



Volume: 6, Issue: 1 30 June 2022

E - Journal http://dergipark.org.tr/pub/ijct

e-ISSN: 2602-277X

International Journal of Chemistry and Technology

JOURNAL INFO

Journal Name	International Journal of Chemistry and Technology
Journal Initial	IJCT
Journal Abbreviation	Int. J. Chem. Technol.
ISSN (Online)	2602-277X
Year of Launching	2017, August
Editor-in-Chief and Managing Editor	Prof. Dr. İbrahim Demirtaş
Manager of Publication	Dr. Oğuzhan Koçer
Scope and Focus	Chemistry, Material Science, Technology
Review Type	Peer Review Double-Blinded
Ethical Rules	Plagiarism check, copyright agreement form, conflict of interest, ethics committee report
Access Type	Open Access
Publication Fee	Free
Article Language	English
Frequency of Publication	Biannualy
Publication Issue	June, December
Publisher	Prof. Dr. İbrahim DEMİRTAŞ
Web Page	http://dergipark.org.tr/pub/ijct
Contact E-mail address	ijctsubmission@gmail.com, ijctsubmission@yahoo.com
Contact Address and Executive address	Kilis 7 Aralik University, Faculty of Science and Arts, Department of Chemistry, 79000, Kilis
Contact Telephone	90 5535983054 (Editor-in-Chief), 90 530 3645222 (Manager of Publication), 90 532 233 17 38 (Secretary)
Publication Date	28/12/2018
Technical Editor	Assist. Prof. Dr. Evrim BARAN AYDIN
Spelling Editor	Dr. Oğuzhan Koçer, MSc. Rabia Acemioğlu
Language (Grammar) Editor	Assist. Prof. Dr. Muhammet KARAMAN, Assist. Prof. Dr. Muhittin KULAK, Dr. Lawali YABO DAMBAGI
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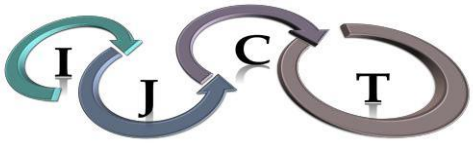
All detailed information including instructions for authors, aim and scopes, ethical rules, manuscript evaluation, indexing info, and manuscript template etc. can be found on the main web page of IJCT (http://dergipark.gov.tr/ijct).

e-ISSN: 2602-277X



International Journal of Chemistry and Technology

Volume: 4, Issue: 1, 30 Tuesday 2020

e-ISSN: 2602-277X

International Journal of Chemistry and Technology

Advisory Editorial Board

e-ISSN: 2602-277X

International Journal of Chemistry and Technology

Advisory Editorial Board

e-ISSN: 2602-277X

International Journal of Chemistry and Technology

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DOI: http://dx.doi.org/10.32571/ijct.986667 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Optimization of extraction parameters of cationic dye using emulsified liquid membrane process. Application of Box-Behnken design

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Received: 24 August April 2021; Revised: 1 February 2022; Accepted: 4 February 2022

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Citation: Bendebane, S.; Bendebane, H.; Bendebane, F.; Ismail, F. *Int. J. Chem. Technol.* **2022**, 6 (1), 1-6.

ABSTRACT

Cationic dye (Methylene blue) was extracted from wastewater by using emulsified liquid membrane process. The membrane is composed of two phases: organic and aqueous. D2EHPA, SPAN 80, and acid are the composition of the aqueous phase, however, the fuel oil represents the organic phase. The effect of different parameters such as the initial concentration of cationic dye (MB), the addition of salt (KCl/ NaCl/ Na_2SO_4), acid (HCl/ H_2SO_4/H_3PO_4) and the extractant concentration (10, 30 and 50 ppm) was examined using Box-Behnken design. The optimization of the extraction has been shown that the extraction efficiency reach100% for the following optimum operating conditions: 30 ppm for the initial concentration of $[BM]_0$, 10%w for the extracting (D2EHPA) with the presence of Na_2SO_4 and H_2SO_4. It can be said that the ELM method is efficient for the removal of methylene bleu.

Keywords: Emulsified liquid membrane, extraction, methylene bleu, modelling, optimization.

Emülsifiye sıvı membran prosesi kullanılarak katyonik boyanın ekstraksiyon parametrelerinin optimizasyonu. Box-Behnken tasarımının uygulanması

ÖZ

Emülsifiye sıvı membran prosesi kullanılarak atık sudan Katyonik boya (Metilen mavisi) ekstrakte edilmiştir. Membran organik ve sulu olmak üzere iki fazdan oluşmaktadır. Sulu fazın bileşiminde D2EHPA, SPAN 80 ve asit bulunur, ancak fuel oil organik fazı temsil eder. Katyonik boyanın başlangıç konsantrasyonu (MB), tuz ilavesi (KCl/ NaCl/ Na2SO4), asit (HCl/ H2SO4/H3PO4) ve ekstraktan konsantrasyonu (10, 30 ve 50 ppm) gibi farklı parametrelerin etkisi Box-Behnken tasarımı kullanılarak incelenmiştir. Ekstraksiyonun optimizasyonu aşağıdaki optimum çalışma koşulları için ekstraksiyon verimliliğinin %100'e ulaştığını göstermiştir: 30 ppm [BM]0 başlangıç konsantrasyonu, Na_2SO_4 ve H_2SO_4 varlığında yapılan ekstraksiyonun %10'u (D2EHPA). Metilen mavisinin giderilmesinde ELM yönteminin etkili olduğu söylenebilir.

Anahtar Kelimeler: Emülsifiye sıvı membran, ekstraksiyon, metilen bleu, modelleme, optimizasyon.

1. INTRODUCTION

Basic dye family represent the most important class of commercial dyes and are mainly used in textile, leather, paper, plastics, etc.[1-3]Among these dyes, methylene blue (MB) is a harmful pollutant and has been long been known for its detrimental effects on human health and the presence of low concentration improves water pollution. The treatment and elimination of dyes from contaminated waters have been the subject of great attention in the last few years.[4] There are several methods for dyes removal such as adsorption,[5,6] extraction,[7] biological treatment, coagulation/flocculation, chemical oxidation and photocatalytic process etc.[8] Emulsion liquid membrane (ELM) has achieved a lot of attention seen in their advantages.[9]

DOI: http://dx.doi.org/10.32571/ijct.986667 E-ISSN: 2602-277X

The main objective of this work was to model and optimize the influence of various factors affecting the emulsion liquid membrane formation and its stability for removing MB. To achieve total elimination of dye, four factors such as the initial concentration of cationic dye (MB), the addition of salt (KCl/ NaCl/ Na_2SO_4), acid (HCl/ H_2SO_4/H_3PO_4) and the extractant concentration (10, 30 and 50 ppm) were followed applying a Box-Behnken design

The four parameters take each three levels according to the Box-Behnken design. The experiment matrix consists of 27 experiments. The Box-Behnken design makes it possible to minimize the number of experiences,[10-13] save time, money.

2. MATERIALS AND METHODS

2.1. Materials and compounds

Methylene bleu (MB) ($C_{16}H_{18}N_3ClS$) is a cationic dye supplied by Sigma Aldrich. The emulsified liquid membrane used for the extraction of MB consisted of monooleate of sorbitan (SPAN 80), an anionic commercial surfactant, di-2-ethylhexylphosphoric acid (D2EHPA) as the extractant, the fuel oil as the thinner. Sulfuric acid (95-97%, Fluka), phosphoric acid H_3PO_4 (85%, Merck), and hydrochloric acid HCl (37.25%, Merck) are the internal phase tested. The fuel oil used in this study is a fraction of Algerian petroleum. Demineralized water (pH=5.0–6.5) was used to prepare the external aqueous phase.

The addition of salt in the external aqueous phase was carried out. The salts used are sodium sulfate (Na_2SO_4) (99%, Merck), KCl (99%, Sigma-Aldrich), NaCl (99%, Sigma-Aldrich).

The homogenizer Moulinex active flow technology, with a capacity of 700 Whats was used to generate a stable emulsion for the extraction. Then, to ensure the dispersion of the emulsified membrane into a beaker containing the external aqueous phase, a mechanical stirrer type RW20 Kjank & Kunkel was used.

2.2. Methods

The ELM method consists to contact the aqueous phase containing the pollutant with an emulsion (W/O) which was formed of an organic phase (membrane) and an internal aqueous solution. Before extraction, a preliminary study of the ELM was essential to deduce the favorable conditions for the emulsion stability. The different parameters affecting the stability of membrane are the concentration of extractant, the concentration of internal phase, the time of emulsification, stirring speed, the ratio $V_{internal}$ / $V_{external}$ and $V_{externe}/V_{emulsion}$. The stability of the membrane has been verified in an interior study.[14] They found that the membrane is very stable. And the most favorable conditions for the stability of emulsified liquid membranes are: stirring speed = 200 rpm, SPAN 80 = 6%w, D2EHPA= 8 %w ; time of emulsification = 3 mn, ratio V_{Org}/V_{int} = 1, ratio $V_{internal}$ / $V_{external}$ = 5 and $[H_2SO_4]_{internal}$ = 1M.

The emulsion (W/O) was prepared by mixing two phases organic and aqueous with the homogenizer for three minutes (a time that is already optimized). The organic phase (membrane) consisted of SPAN 80, D2EHPA, and fuel oil. However, the aqueous phase was the acid. The emulsions are prepared according to the matrix given by the MINITAB 18.

The samples were analyzed using a visible spectrophotometer (PRIM-SECOMAM) (at 664nm) to determine the concentration of the residual MB. The extraction efficiency was calculated by equation (1).

$$R\% = [1 - \frac{[BM]_f}{[BM]_0}] \times 100 \qquad (1)$$

Where:
R : extraction yield (%).
$[MB]_f$: final concentration of MB in the external phase (ppm).
$[MB]_o$: initial concentration of MB in the external phase (ppm).

3. RESULTS AND DISCUSSION

3.1. Box-Behnken design

The industrial effluents are released into the aquatic environment loaded with various types of salts. In order to study the effect of the presence of salts in the solution, we have chosen three salts KCl, NaCl and Na_2SO_4 for levels (-1), (0) and (+1) respectively. The other factors studied are the weight percentage of D2EHPA (from 5 to 10% w), the initial concentration of MB dye (10 to 50ppm) and the type of acid in the internal phase (HCl (-1), H_2SO_4 (0) and H_3PO_4 (+1)). For this, an experimental design of second degree was envisaged (Table 1).

3.2. Anova

The ANOVA statistical analysis of experimental results are grouped in Table 2.

From the results of Table 2 it can be seen that the (internal phase) is a highly significant term on MB extraction with a p-value equal to zero. The linear effect and the square effect of the salt are significant terms where the value of the p-value is less than or equal to 0.05 (5%) with the values of p-value respectively equal to 0.05 and 0.03. We also observe that no interaction is significant.

DOI: http://dx.doi.org/10.32571/ijct.986667 **E-ISSN: 2602-277X**

Table.1: Experimental yields of MB extraction according to Box-Behnken design.

Essay	$[BM]_0$	Effect of salts	Internal phase	[D2EHPA]	R exp. %	R theo. %
1	30	KCl	H_2SO_4	5	98.98	99.79
2	50	NaCl	HCl	7.5	76.24	79.08
3	10	KCl	H_2SO_4	7.5	99.56	92.43
4	30	KCl	HCl	7.5	76.25	79.61
5	50	NaCl	H_3PO_4	7.5	82.32	77.59
6	30	NaCl	HCl	5	82.66	78.27
7	10	NaCl	HCl	7.5	61.34	70.16
8	30	NaCl	HCl	10	82.50	77.29
9	50	NaCl	H_2SO_4	5	99.07	98.34
10	30	Na_2SO_4	HCl	7.5	92.48	87.05
11	50	KCl	H_2SO_4	7.5	96.75	94.86
12	50	Na_2SO_4	H_2SO_4	7.5	99.81	102.00
13	30	NaCl	H_2SO_4	7.5	96.26	93.54
14	10	Na_2SO_4	H_2SO_4	7.5	100.00	98.56
15	10	NaCl	H_2SO_4	10	91.25	92.11
16	30	KCl	H_2SO_4	10	94.55	93.81
17	30	Na_2SO_4	H_2SO_4	5	98.90	103.00
18	50	NaCl	H_2SO_4	10	86.43	88.07
19	30	Na_2SO_4	H_3PO_4	7.5	93.99	90.75
20	30	KCl	H_3PO_4	7.5	78.67	84.24
21	30	NaCl	H_3PO_4	5	83.43	84.40
22	30	NaCl	H_2SO_4	7.5	94.08	93.54
23	10	NaCl	H_2SO_4	5	89.29	87.77
24	30	NaCl	H_3PO_4	10	79.30	79.46
25	30	Na_2SO_4	H_2SO_4	10	100.00	103.00
26	10	NaCl	H_3PO_4	7.5	78.71	79.97
27	30	NaCl	H_2SO_4	7.5	90.28	93.54

Table.2 : Estimated coefficients for the yield of MB extraction for coded units.

Terms	**Coef**	**SE Coef**	**T**	**P**
Constant	93.54	3.19	29.29	0.000
$[BM]_0$	1.64	1.60	1.02	0.33
Salts	3.49	1.60	2.18	**0.05**
internal Phase (Acids)	2.08	1.60	1.30	0.22
D2EHPA	-1.48	1.60	-0.93	0.37
$[BM]_0{}^2$	-2.56	2.40	-1.07	0.31
$(Salts)^2$	6.15	2.40	2.57	**0.03**
$(internal\ phase)^2$	-14.28	2.40	-5.96	**0.000**
$(D2EHPA)^2$	0.59	2.40	0.25	0.81
$[BM]_0\times$ salts	0.42	2.77	0.15	0.88
$[BM]_0 \times$ internal phase	-2.82	2.77	-1.02	0.33
$[BM]_0\times$ (D2EHPA)	-3.65	2.77	-1.32	0.21
Salts× internal phase	-0.23	2.77	-0.08	0.94
Salts×(D2EHPA)	1.51	2.77	0.55	0.60
internal Phase ×(D2EHPA)	-0.99	2.77	-0.36	0.73

DOI: http://dx.doi.org/10.32571/ijct.986667 E-ISSN: 2602-277X

3.3. Mathematical model

The mathematical model is a second degree and relates the extraction yield to the different factors, their squares and their interactions. For coded units the model is represented by equation 2:

$$\mathbf{R(\%)} = 93.5420 - 1.6353 \times \mathbf{[BM]_0} - 3.4873 \times \mathbf{(salt)} - 2.0793 \times \mathbf{(internal\ phase)} + 1.4818 \times \mathbf{(Extractant)} + 2.5611 \times \mathbf{[BM]_0^2} - 6.1477 \times \mathbf{(salt)^2} + 14.2781 \times \mathbf{(internal\ phase)^2} - 0.5935 \times \mathbf{(Extractant)^2} - 0.4216 \times \mathbf{[BM]_0 \times (salt)} + 2.8231 \times \mathbf{[BM]_0 \times (internal\ phase)} + 3.6508 \times \mathbf{[BM]_0} \times \mathbf{(Extractant)} + 0.2315 \times \mathbf{(salt)} \times \mathbf{(internal\ phase)} - 1.5076 \times \mathbf{(salt) \times (Extractant)} - 0.9902 \times \mathbf{(internal\ Phase) \times (Extractant)} \quad (2)$$

3.4. Effects of factors

A factor may be a qualitative or quantitative variable, continuous or discontinuous, controllable or uncontrollable.[15] Figure 1 shows the effect of the factors studied in the chosen domain on the yield of MB extraction.

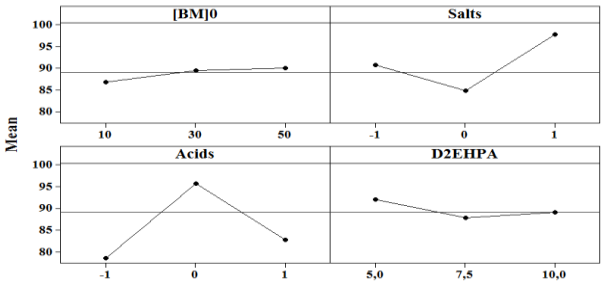

Figure1. Effects of factors.

Two effects are observed; the first is a negative effect of 5 to 7.5% by mass and a second positive effect of 7.5 to 10% by mass (with a weak slope). It can be said that the yield is good at a minimum level of the mass percentage of extractant. This is explained with a high mass percentage extractant (D2EHPA) the membrane becomes a little viscous, which means that the transfer is difficult.[16]

It is observed that there are two slopes, a negative one from KCl (-1) to NaCl and a positive slope from NaCl to Na_2SO_4. Therefore, we can say that the presence of Na_2SO_4 promotes the extraction of methylene blue which it has been obtained a yield of 97.76%. It was observed that there is a positive effect from HCl to H_2SO_4 and a negative effect from H_2SO_4 to H_3PO_4. It was also found that the H_2SO_4 acid gives the best removal efficiency (95.77%) of methylene blue dye. So, we can explain this result by the presence of hydrogen ions ($2H^+$) which promotes the discoloration of aqueous solutions of MB.[17] It was observed that there is a positive effect of the initial dye concentration with a moderately high slope (low inclination). It was found that increasing the dye concentration increases the yield of the dye extraction (from 86.83 to 90.10%). Therefore, the concentration of 50 ppm in methylene blue gives the best result.[18,19]

3.5. Henry's line of residual values

Generally, Henry's line is useful for checking the normality of the model. We see that the points tend to form a line.

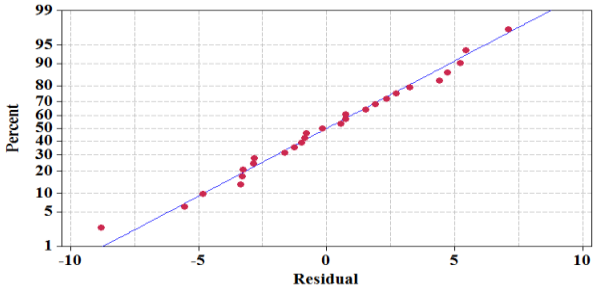

Figure 2. Henry's right of residual values for yield.

3.6. Graphics contour and surface responses

At an initial concentration of $[BM]_0$ equal to 50 ppm and in the presence of Na_2SO_4, it can be seen that the good yields are of the order of 100% in an average level for the acid (internal phase is sulfuric acid H_2SO_4) and at a concentration of minimum value of D2EHPA (5% m). The response surface is convex, giving the same findings of the contour.

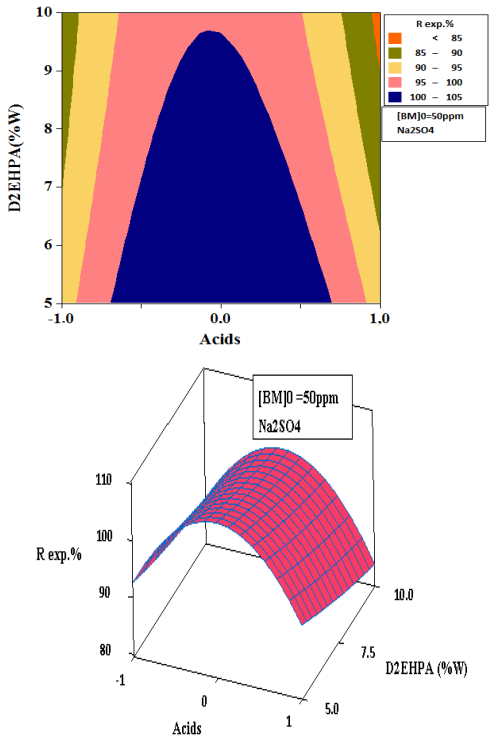

Figure 3. Graphic contour and surface responses as function of acid-D2EHPA with $[BM]_0$ = 50 ppm and Na_2SO_4 salt.

DOI: http://dx.doi.org/10.32571/ijct.986667 E-ISSN: 2602-277X

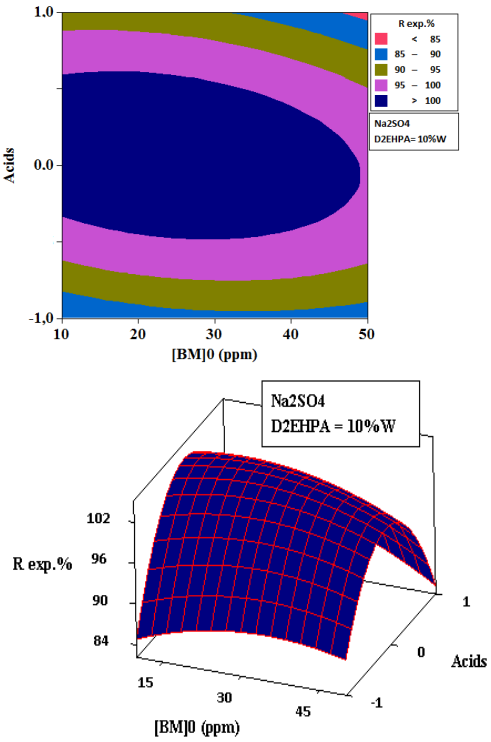

Figure 4. Graphic response contour as a function of $[BM]_0$ - acids with salt Na_2SO_4 and D2EHPA = 10% m.

By fixing the salt Na_2SO_4 and the mass percentage of D2EHPA at 10% m, it is noted that the good yields take a large place in the contour surface under the conditions $[BM]_0$ of (10ppm-50ppm) and in the presence of the phase internal H_2SO_4. The response surface indicates the same observations

3.7. Experimental yield depending on the theoretical yield

From Figure.5 it can be seen that the experimental and estimated yields are distributed around the regression line (R^2 = 0.9553). The fit is very good, especially above 80%.

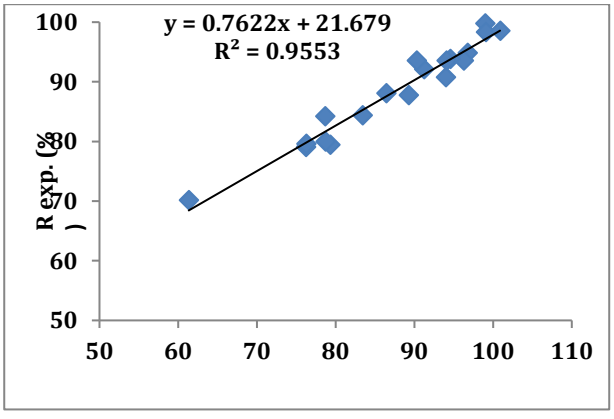

Figure 5. Graphical representation of measured responses as function of theoretical responses for BM extraction.

4. OPTIMISATION

The goal is to maximize the extraction yield. A constraint was imposed on studied factors. The best elimination of MB dye is obtained under optimal conditions: 30 ppm for the initial concentration of $[BM]_0$, 10%w for the extracting (D2EHPA) with the presence of Na_2SO_4 and H_2SO_4.

Finally, we can say that in total we could extract the entire MB using the emulsified liquid membrane technique.

5. CONCLUSIONS

The elimination of the cationic pollutant "methylene blue" is influenced by several factors that have demonstrated the possibility of using the emulsified liquid membrane extraction process.

A membrane consists of a surfactant (span80), a diluent (fuel oil) and an extractant (D2EHPA) in the organic phase, varying the type of acids as an internal phase. In addition, an external aqueous phase of the MB dye mixture with salt at different concentrations of pollutant (MB) has been studied in order to find the optimal conditions that promote the extraction yield of the cationic dye MB.

From the Box-Behnken design, we obtained several information which increase the experimental yield of MB extraction. Thus, after optimizing the extraction of MB, we found the best optimal conditions, which are: a weight percentage of extractant equal to 10%w, the presence of sulfuric acid as the internal phase, the initial concentration of dye is 30 ppm, the salt that promotes extraction is Na_2SO_4 for total extraction of MB.

ACKNOWLEDGEMENTS

We thank Prof. Abbes Boukhari Director of the LOMOP Research Laboratory and Dr Fethi Saoudi Head of the Process Engineering Department, BadjMokhtar University of Annaba, for providing us with the necessary material resources.

Conflict of interests

I declares that there is no a conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.986667 **E-ISSN: 2602-277X**

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DOI: http://dx.doi.org/10.32571/ijct.1003871 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Synthesis and biological activities of new hybrid chalcones with benzoic acid ring

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Received: 17 November 2021; Revised: 11 March 2022; Accepted: 14 March 2022

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Citation: Kurşun, Aktar, B. S.; Sıcak, Y.; Oruç-Emre, E.E. *Int. J. Chem. Technol.* **2022**, 6 (1), 7-14.

ABSTRACT

A series of *E*-4-(3-oxo-3-(substituted)prop-1-en-1-yl)benzoic acid derivatives (**1**-**5**) were synthesized by the Claisen-Schmidt condensation of various ketones with 4-formylbenzoic acid. The anticholinesterase (AChE and BChE), tyrosinase, and urease inhibition activities of the synthesized compounds (**1**-**5**) were examined. It was found that the most active compound against AChE enzyme in anticholinesterase inhibition activity was compound **1**. Compound **4** was the most active compound in tyrosinase inhibition activity, while compound **3** was the most active compound in urease psychological activity.

Keywords: Chalcone, anticholinesterase activity, tyrosinase inhibition activity, urease inhibition activity.

Benzoik asit halkalı yeni hibrit kalkonların sentezi ve biyolojik aktiviteleri

ÖZ

4-formilbenzoik asit ile çeşitli ketonların Claisen–Schmidt kondensasyonuyla bir dizi E-4-(3-okso-3-(sübstitüe)prop-1-en-1-il)benzoik asit türevleri (1-5) sentezlenmiştir. Sentezlenen bileşiklerin (**1**-**5**), antikolinesteraz (AChE ve BChE), tirosinaz ve üreaz inhibisyon aktiviteleri incelendi. Antikolinesteraz inhibisyon aktivitesinde AChE enzimine karşı en aktif bileşiğin, bileşik **1** olduğu tespit edilmiştir. Bileşik **4**, tirosinaz inhibisyon aktivitesinde en aktif bileşik iken bileşik **3**, üreaz inhibisyon aktivitesinde en aktif bileşiktir.

Anahtar Kelimeler: Kalkon, antikolinesteraz aktivite, tirosinaz inhibisyon aktivite, üreaz inhibisyon aktivite.

1. INTRODUCTION

There are two types of cholinesterase enzymes in our body, AChE (Acetylcholinesterase) and BChE (Butyrylcholinesterase). AChE is abundant in the healthy adult brain and is called true cholinesterase. BChE, on the other hand, is present in limited amounts and is referred to as "serum cholinesterase, pseudocholinesterase or nonspecific cholinesterase" .[1] AChE is widely distributed in all excitable tissues such as the lungs, spleen, brain, muscles, erythrocyte membranes, and nerve endings. BChE, on the other hand, is synthesized in the liver and released into plasma at high levels and is found only in the central and peripheral nervous systems.[2,3]

Tyrosinase is an enzyme that specifically affects the concentration of melanin pigment which is one of the factors that influence human skin and hair color [4,5]. Melanin plays a protective role in the development of skin cancer, absorbs ultraviolet (UV) rays, protects the skin from UV damage and reactive oxygen species (ROS), and purifies the organism from toxic substances and drug residues.[6] The increase in melanin pigmentation causes undesirable symptoms in people. These undesirable symptoms, which are visible on the skin, often affect the psychological state of the person.

Helicobacter pylori bacteria colonize and secrete urease. This enzyme acts on urea as a substrate, and as a result of its hydrolysis, CO_2 and NH_3 are formed. This protects the bacteria from the low pH of gastric juice. However, NH_3 is toxic to the epithelial cells of the stomach and also increases the effect of cytotoxins secreted by the agent by reducing intercellular adhesion.[7] Although this formation

DOI: http://dx.doi.org/10.32571/ijct.1003871 E-ISSN: 2602-277X

causes diseases such as gastritis, peptic ulcers, and gastric cancer, it has the risk of leading to some new diseases triggered by these disorders. Proton generating inhibitors are used in the treatment of *H. pylori* infection.[8] To prevent this bacterial infection, many researchers have focused their studies on new proton-generating agents.

The term "chalcone" was first used by Kostanecki, who made synthesis studies of some natural chromophoric products. It forms a benzylidene acetophenone scaffold in which two aromatic structures are linked by a three-carbon α,β unsaturated carbonyl bridge.[9] It and its derivatives can be classically synthesized by the Claisen-Schmidt reaction[10], as well as by the solid phase Claisen-Schmidt reaction[11], can also be synthesized by various methods such as the solvent-free Claisen-Schmidt reaction[12], the Suzuki Miyaura reaction[13], the coupling reaction[14], the carbonylative Heck coupling reaction[15], the one-pot reaction of chalcones[16], microwave method[17], solid acid catalyst[18], the Sonogashira isomerization connection[19], Friedel Crafts reaction[20], Juliae Kocienski Olefination.[21] Chalcones have various pharmacological activities such as anti-platelet[22], antidiabetic[23], antineoplastic[24], antiangiogenic[25], antiretroviral[26], antiinflammatory[27], antigout[28], antihistaminic[29], antioxidant[30], antiobesity[31], hypolipidemic[32], antitubercular[33], antifilarial[34], antiinvasive[35], antimalarial[36], antiprotozoal[37], antibacterial[38], antifungal[39], antiulcer[40], antisteroidal[41], immunosuppressant[42], hypnotic[43], anxiolytic[44], antispasmodic[45], antinociceptive[46], and osteogenic.[47]

Our aim in this study was to synthesize new chalcones hybridized with 4-formylbenzoic acid and to investigate the anticholinesterase (AChE and BChE), tyrosinase, and urease inhibition activities. Because the number of Alzheimer's patients is increasing day by day. And when the drugs on the market are used for a long time, the body shows resistance and the drug does not show its effect. Therefore, new drugs are needed. In addition to these activities, ADME study was also conducted, and other parameters were used to investigate whether the synthesized molecule could be a drug candidate.

2. MATERIALS AND METHODS

2.1. General

Chemicals and solvents were in analytical grade and were purchased from Merck and Sigma-Aldrich. All chemical reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F254 plates. Melting points were determined using the 18 Stuart SMP20 automatic melting point apparatus and were uncorrected. FTIR spectra were determined with a Perkin Elmer 1620 model FTIR spectrophotometer. Elemental analyses (CHNS) were performed using a VarioMICRO elemental analyzer. ^{1}H and ^{13}C NMR spectra were recorded by Agilent 600 MHz spectrometer. The mass spectra of all compounds were recorded using an Agilent 1260 infinity LC. 6210 HPLCTOF/MS spectrometer in electrospray mode.

2.2. General procedure for chalcones (1-5)

Substituted acetophenone (0.01 mol) and 4-formylbenzoic acid (0.02 mol) were dissolved in methanol (25 mL) and solid NaOH was added and then mixed at room temperature in a magnetic stirrer for 24 hours. The mixing process continued for 24 hours. After the completion of the reaction was determined by TLC, and extracted with dichloromethane. After extraction, the organic part was dried with $MgSO_4$. The mixture was filtered and removed from $MgSO_4$. The liquid organic phase was evaporated in the evaporator, and the solid was crystallized with the appropriate solvent to obtain a pure substance.[48]

2.3. *E*-4-(3-oxo-3-(4-pyrrolidin-1-yl)phenyl)prop-1-en-1-yl)benzoic acid (1)

Yellow solid, yield: 39.71 %, m.p: 191.4. °C. MS m/z: (321.37) 321.90 [M]+.

2.4. *E*-4-(3-oxo-3-(4-piperazin-1-yl)phenyl)prop-1-en-1-yl)benzoic acid (2)

Yellow solid, yield: 46.80 %, m.p: 178.5 °C. MS m/z: (336.38) 336.90 [M]+.

2.5.*E*-4-(3-(4-(*N*-(cyclohexylcarbamoyl)sulfamoyl) phenyl)-3-oxoprop-1-en-1-yl) benzoic acid (3)

Cream solid, yield: 68.00 %, m.p: 162.9 °C. MS m/z: (456.51) 456.80 [M]+.

2.6. *E*-4-(3-oxo-3-(4-trifluoromethyl)phenyl)prop-1-en-1-yl)benzoic acid (4)

Cream solid, yield: 50 %, m.p: 190.6 °C. MS m/z: (320.26) 320.80 [M]+.[49]

2.7. *E*-4-(3-(4-bromophenyl)-3-oxoprop-1-en-1- yl) benzoic acid (5)

Yellow solid**,** yield: 60 %, m.p: 166.6 °C. MS m/z: (331.16) 332.80 [M+H]+.[49]

2.8. Preparation of the Solution of the Compounds

The compounds and assay standards were dissolved in dimethyl sulfoxide (DMSO). The solutions of the synthesized chalcones **(1-5)** were prepared at four different concentrations i.e. 400, 200, 100, and 50 µM for the anticholinesterase and urease inhibition tests, and 400, 200, 100, and 50 mM for the tyrosinase inhibition test.

Int. J. Chem. Technol. **2022**, 6 (1), 7-14
DOI: http://dx.doi.org/10.32571/ijct.1003871
E-ISSN: 2602-277X

Table 1. FTIR, ^{1}HNMR and ^{13}C NMR spectral data of chalcone **1-5**

Compound	FTIR ν_{max} (cm^{-1})	Elemental analysis		^{1}H NMR(δ) †	^{13}C NMR(δ) ††
		Anal. Calcd (%)	Found (%)		
1	2960, 2846 (aromatic CH), 2674, 2557 (aliphatic CH), 1679 (acid C=O), 1651 (ketone C=O), 1588, 1587, 1543, 1505 (C=C), 986 (trans C=C).	$C_{20}H_{19}NO_3$: C: 74.75 H: 5.96 N: 4.21	C: 74.50 H: 5.93 N: 4.21	1.95 (s, 4H, H_{15}), 3.36 (s, 4H, H_{14}), 6.47 (d, 2H, *J*=7.8 Hz, H_{12}), 7.65 (d, 1H, *J*=15.6 Hz, H_8), 7.95-8.05 (m, 5H, $H_{5,7,11}$), 8.11 (d, 2H, *J*=7.2 Hz, H_4), 13.27 (s, 1H, OH).	25.38, 47.76, 111.46, 125.09, 129.01, 130.00, 130.38, 131.48, 136.06, 139.31, 140.47, 151.35, 167.02, 193.48.
2	2923, 2851 (aromatic CH), 2742, 2508 (aliphatic CH), 1688 (acid C=O), 1660 (ketone C=O), 1593, 1505, 1455, 1407 (C=C), 973 (trans C=C).	$C_{20}H_{20}N_2O_3$: C: 71.41 H: 5.99 N: 8.33	C: 71.00 H: 5.83 N: 8.40	3.42 (s, 4H, H_{15}), 3.63 (s, 4H, H_{14}), 7.08 (d, 2H, *J*=8.40 Hz, H_{12}), 7.69 (d, 1H, *J*=15.60 Hz, H_8), 7.80-7.96 (m, 4H, $H_{11,5}$), 8.04 (d, 1H, *J*=15.60 Hz, H_7), 8.10 (d, 2H, *J*=7.80 Hz, H_4), 13.25 (s, 1H, OH).	42.65, 44.17, 114.31, 129.21, 130.01, 130.14, 130.38, 131.22, 132.21, 141.53, 153.99, 167.32, 186.94, 193.48.
3	3278 (urea NH), 3072 (aromatic CH), 2928, 2855 (aliphatic CH), 1668 (acid C=O), 1604 (ketone C=O), 1567, 1538, 1427 (C=C), 989 (trans C=C).	$C_{23}H_{24}N_2O_6S$: C: 60.51 H: 5.30 N: 6.14 S: 7.02	C: 61.00 H: 5.41 N: 6.10 S: 7.08	6.50 (s, 1H, H_{14}), 7.81 (d, 1H, *J*=15.6 Hz, H_8), 7.99-8.00 (m, 4H, $H_{4,5}$), 8.02 (d, 1H, *J*=15.6 Hz, H_7), 8.06 (d, 2H, *J*=7.80 Hz, H_{12}), 8.32 (d, 2H, *J*=7.80 Hz, H_{11}), 10.57 (s, 1H, H_{16}), 13.09 (s, 1H, OH)	24.65, 25.39, 32.68, 48.66, 124.44, 128.14, 129.53, 129.61, 129.67, 130.19, 132.99, 138.97, 141.11, 144.05, 150.83, 167.28, 189.00.
4	2951, 2828 (aromatic CH), 2642, 2547 (aliphatic CH), 1682 (acid C=O), 1661 (ketone C=O), 1607, 1567, 1505, 1426 (C=C), 1177 (C-F), 985 (trans C=C).	$C_{17}H_{11}FO_3$: C: 63.76 H: 3.46	C: 63.64 H: 3.49	7.81 (d, 1H, *J*=15.6 Hz, H_8), 7.93 (d, 2H, *J*=6.60 Hz, H_5), 7.99-8.01 (m, 4H, $H_{11,12}$), 8.05 (d, 1H, *J*=15.6 Hz, H_7), 8.33 (d, 2H, *J*=7.80 Hz, H_4), 13.17 (s, 1H, OH).	
5	2954, 2829 (aromatic CH), 2661, 2545 (aliphatic CH), 1682 (acid C=O), 1657 (ketone C=O), 1608, 1583, 1567, 1505 (C=C), 986 (trans C=C).	$C_{16}H_{11}BrO_3$: C: 58.03 H: 3.35	C: 58.70 H: 3.49	7.78-8.00 (m, 3H, $H_{8,5}$), 7.97-8.05 (m, 3H, $H_{7,12}$), 8.10-8.12 (m, 4H, $H_{4,11}$), 13.27 (s, 1H, OH).	124.26, 129.48, 130.00, 130.16, 130.38, 131.12, 132.36, 136.72, 143.48, 167.28, 188.39, 193.46.

DOI: http://dx.doi.org/10.32571/ijct.1003871 E-ISSN: 2602-277X

2.9. Enzyme Inhibition Assays

The all enzyme inhibition activity assays of chalcones **1-5** were tested at four µM concentrations (i.e. 400-200-100-50 µM for anticholinesterase and urease inhibition activity, 400-200-100-50 mM for tyrosinase inhibition activity) in triplicate measurements. The results of all enzyme inhibition activities were given as 50 % concentration (IC_{50}).

Anticholinesterase inhibition activity

The *in vitro* anticholinesterase inhibition activity against AChE and BChE of chalcones **1-5** was performed according to Ellman's method using 96 well microplate readers. For these assays were used AChE and BChE obtained from electric eel and horse serum, respectively. The acetylthiocholine iodide and butyrylthiocholine chloride were utilized as substrates in assays. DTNB (5,50-dithiobis(2-nitrobenzoic) acid was used as a coloring agent to measure the anticholinesterase inhibition activity.[50]

Tyrosinase inhibition activity

The spectrophotometric analysis of tyrosinase inhibition activity was performed according to the slightly modified literature procedures of Hearing.[51] DMSO with kojic acid and L-mimosine, respectively, was used for the control and tyrosinase standards for the determination of tyrosinase inhibition activity.

Urease inhibition activity

The spectrophotometric analysis of urease inhibition activity was performed by measuring ammonia production using the indophenol method according to procedures described in the literature as.[52] DMSO and thiourea were used as control and urease positive standards, respectively.

2.10. Absorption, Distribution, Metabolism and Excretion (ADME) properties

The basic parameters affecting drug metabolism such as the absorption, distribution, metabolism and excretion (ADME) properties of chalcones **1-5** in the body, were carried out with the web-based program SwissSimilarty (SwissADME) according to the rules of Lipinski and Veber.[53] The drug likeness model score properties of chalcone compounds **1-5** were performed using the Molsoft software program.[54]

3. RESULTS AND DISCUSSION

3.1. Chemistry

Benzoic acid hybridized chalcones were synthesized by Claisen-Schmidt condensation between a different ketone and 4-formylbenzoic acid in the presence of NaOH in methanol (Figure 1). Compounds **1-5** were crystallized in a hexane/DCM mixture. The yields were in the range of 39.71-60.00 %. The IR spectra of the chalcones (**1-5**) showed the aromatic CH stretching band at 2923-3278 cm^{-1}, the acidic C=O stretching band at 1668-1688 cm^{-1}, and the ketone C=O stretching band at 1604-1661 cm^{-1}.

The ^1H NMR spectra of compounds **1-5** showed that the Hα and H_β protons resonated as doublets at 7.65-7.98 ppm (j=14.4-15.6) and 7.95-8.08 ppm (j=15.6-17.4), respectively. Therefore, all compounds were in the trans structure.

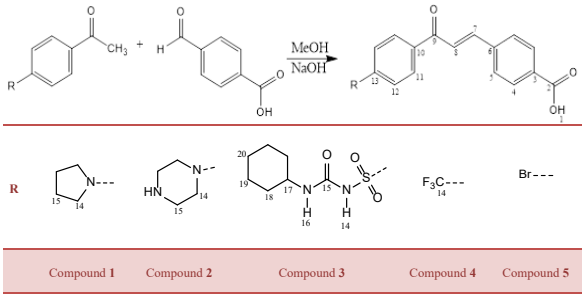


Figure 1. Synthetic route of target compounds (**1-5**)

3.2. Biological Activity

The anticholinesterase, tyrosinase, and urease inhibition activities of compounds **1-5** were determined at 100, 50, 25, and 5 µMi respectively. The IC_{50} values of the enzyme inhibition activities of compounds **1-5** were given in Table 2. The anticholinesterase inhibition activity of compounds **1-5**, compound **1** was determined to be the most active compound against each AChE enzyme.

Compounds **1** and **2** were found to be more active against BChE enzyme than galantamine, the standard of the assay. The IC_{50} values of the synthesized compounds against AChE were: Compound **2**> Compound **1**> Compound **3**> Compound **4** > Compound **5** > Galantamine, while against BChE were: Compound **2**> Compound **1**> Galantamine> Compound **3**> Compound **4** > Compound **5**.

In the tyrosinase inhibition activity of compounds **1-5**, compound **4** exhibited the best activity in the series. The IC_{50} values of tyrosinase inhibition activity of the synthesized compounds were: Kojic acid ~ L-mimosine> Compound **4**> Compound **5**> Compound **3**> Compound **1** > Compound **2**.

In the urease inhibition activity of compounds **1-5**, compound **3** displayed the best activity in this series. The IC_{50} value of urease inhibition activity of the synthesized compounds were: Compound **3**> Thiourea> Compound **2**> Compound **1**> Compound **4** > Compound **5**.

DOI: http://dx.doi.org/10.32571/ijct.1003871 E-ISSN: 2602-277X

Table 2. The IC_{50} values of enzyme inhibition activities of compounds **1-5**.

Compound	Anticholinesterase Inhibitory Activity		Tyrosinase Inhibitory Activity	Urease Inhibitory Activity
	AChE assay IC_{50} (µM)	BChE assay IC_{50} (µM)	Tyrosinase assay IC_{50} (mM)	Urease assay IC_{50} (µM)
1	47.09±0.73	47.10±0.51	47.01±0.46	31.77±0.58
2	40.21±0.67	40.32±0.28	56.82±0.54	28.30±0.91
3	53.05±0.49	62.17±0.36	38.22±0.77	20.18±0.51
4	69.20±0.12	78.44±0.69	21.73±0.24	43.07±0.22
5	75.65±0.85	83.50±0.19	30.42±0.16	59.64±0.86
Galantamine [b]	4.9±0.36	47.23±0.77	**NT**	**NT**
Kojic acid[b]	**NT**	**NT**	0.64±0.38	**NT**
L-mimosine[b]	**NT**	**NT**	0.75±0.14	**NT**
Thiourea[b]	**NT**	**NT**	**NT**	25.33±0.36

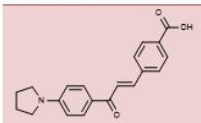
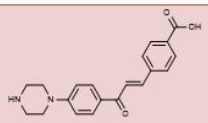
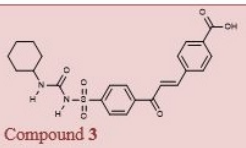
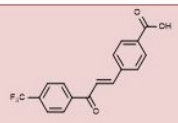
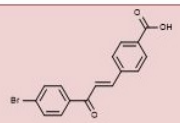
[a] Values expressed are means ± S.E.M. of three parallel measurements. $p< 0.05$, significantly different with the student's *t*-test. [b] Reference compounds. NT: Not tested.

3.3. Molecular properties, Lipinski rule, and ADME

The molecular weight of all compounds was between 321.37-456.51g (150 g/mol <MW<500 g/mol). The iLOGP values were in the range that should be between 2.20-2.70. All compounds, except **3**, passed the brain barrier. All compounds except compound **3** had a high gastrointestinal absorption value. The TPSA values of compounds **1**, **2**, **4** and **5** were in the range of 54.37-69.64 Å², while compound **3** was not in the required value range (20 Å²<TPSA<130 Å²) with a value of 138.02 Å².

The Brain or Intestine Predictive Permeability (BOILED-Egg) method was used to obtain a visual cue for the drug candidate of the synthesis molecules of the new compounds based on the oral absorption potential with respect to the polarity and lipophilicity of the small molecules. The visual estimates of gastrointestinal absorption and blood-brain barrier (BBB) penetration of chalcones **1-5** were shown in Table 3. According to the BOILED-Egg plot, chalcones (**1-5**) were located in the yellow circle (compounds **1**, **2**, **4**, and **5**) expressing good intestinal absorption and the gray area (compound **3**) representing poor intestinal absorption of BBB. Compound **2** was also found to be in the blue spot, indicating its good bioavailability. It appeared that this compound could be used as an alternative substrate for P-glycoprotein, and would decrease the absorption and penetration of this compound in the brain.[55,56]

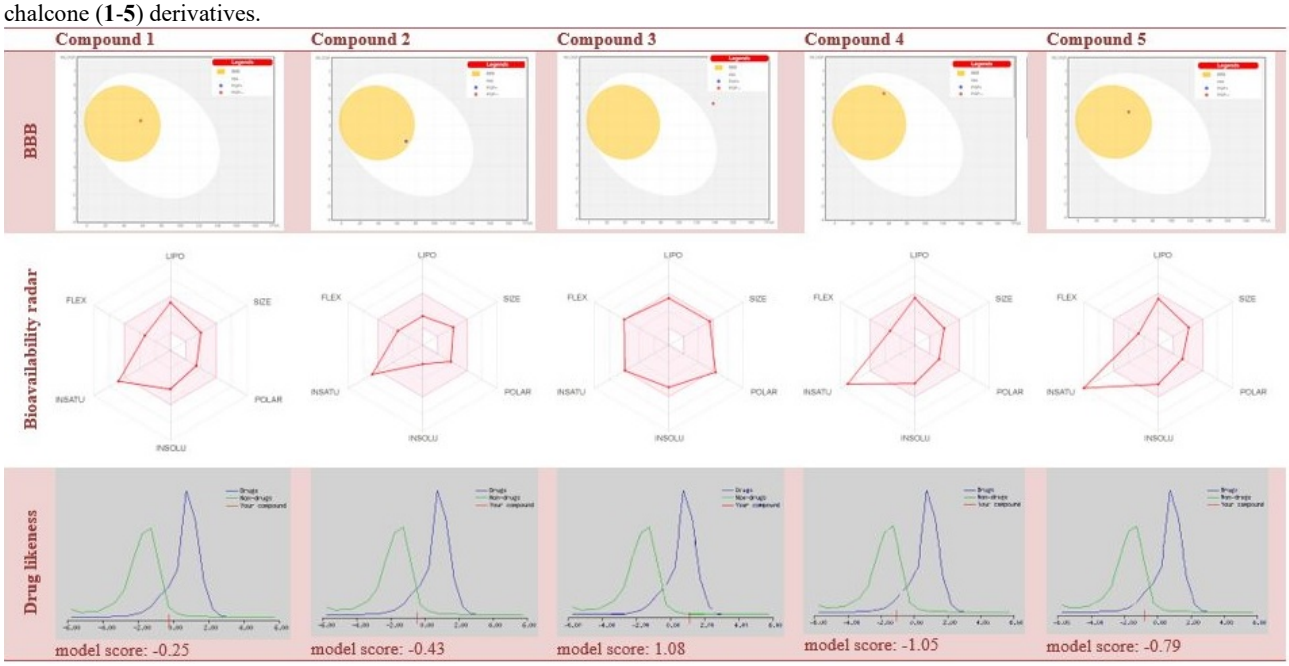
Table 3. Drug-likeness properties of the synthesized benzoic acid hybridized chalcone derivatives (**1-5**).

	Compound				
	 Compound 1	 Compound 2	 Compound 3	 Compound 4	 Compound 5
Molecular Formula	$C_{20}H_{19}NO_3$	$C_{20}H_{20}N_2O_3$	$C_{23}H_{24}N_2O_6S$	$C_{17}H_{11}F_3O_3$	$C_{16}H_{11}BrO_3$
MW (g/mol)	321.37	336.38	456.51	320.26	331.16
iLOG P	2.70	2.69	2.20	2.21	2.48
BBB	Yes	Yes	No	Yes	Yes
GI	High	High	Low	High	High
TPSA Å²	57.61	69.64	138.02	54.37	54.37
Synthetic accessibility	2.56	2.76	3.49	2.60	2.51
Lipinski	Yes	Yes	Yes	Yes	Yes

*These parameters were determined with SwissSimilarity and Molsoft software

DOI: http://dx.doi.org/10.32571/ijct.1003871 E-ISSN: 2602-277X

Table 4. Boiled-Egg, Bioavailability radar and Drug likeness model score properties of the synthesized benzoic acid hybridized chalcone (**1-5**) derivatives.



Bioavailability radar of the **1-5**. The pink area represents the optimal range for each properties (LIPO: Lipophilicity, SIZE: Molecular weight, POLAR: Total Polar Surface Area, INSOLU: Insolubility, INSATU: Instauration, FLEX: Flexibility). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. CONCLUSIONS

In this study, especially compound **3**, which showed the best activity in urease inhibition activity, can be developed as an agent that can also eliminate the symptoms that cause urease inhibition, as a result of the satisfactory results to be obtained from the further cytotoxic activity study. According to the other enzyme inhibition results, for anticholinesterase enzyme inhibition activity, AChE and BChE enzymes showed good inhibition activity against both enzymes, while compound **2** was the most active component in the tyrosinase enzyme inhibition activity. It can be concluded that the particularly compound **4** can be the potential candidate for the treatment of skin diseases and hyper-pigmentation caused by melanin.

ACKNOWLEDGEMENTS

The authors are grateful to YYÜ Scientific Research Projects Presidency for their financial support for project numbered 2013-FBE-D058.

Conflict of interests

I declare that there is no conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.936862 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Calculation of usability as anti-corrosion of pyrimidine-type bases by density functional theory

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Received: 29 January 2022; Accepted: 18 Fabruary 2022

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Citation: Akbaş, E.; Kahraman, H.; Akbaş, B. Ç. *Int. J. Chem. Technol.* **2022**, 6 (1), 15-20.

ABSTRACT

Corrosion of metals is an important problem in many industries. This problem can be reduced by coating the metal surface. With this application, the metal can be isolated from the corrosive environment. Surface coating materials may be inorganic compounds as well as compounds of organic origin. In the case of organic compounds, these compounds are selected from groups rich in functional groups, containing free electron pairs and/or π electrons. In this study, pyrimidine derivatives were selected which is synthesized Robins et al. These compounds are very rich in functional group, free electron pairs and π electrons. To calculate the anticorrosive effects of the compounds theoretically, quantum chemical calculations were performed with the Gaussian09W software package using the density functional theory method (DFT) of all compounds, the 6-31G (d, p) base set and B3LYP functions. The quantum chemical computations showed that the studied compound was adsorbed differently on the metal surface all depend to the nature of the molecular structure.

Keywords: Pyrimidine-type bases, density functional theory, anti-corrosion, chemical calculation.

Yoğunluk fonksiyonel teorisi ile pirimidin tipi bazların korozyon önleyici olarak kullanılabilirliğinin hesaplanması

ÖZ

Metallerin korozyonu birçok endüstride ciddi bir sorundur. Metal yüzey kaplanarak bu sorun azaltılabilir. Bu uygulama ile metal, korozif ortamdan izole edilebilir. Korozyon önleyici, yüzey kaplama malzemeleri inorganik bileşikler olabileceği gibi organik kökenli bileşikler de olabilir. Organik bileşikler söz konusu olduğunda, bu bileşikler, serbest elektron çiftleri ve/veya π elektronları içeren fonksiyonel gruplar açısından zengin gruplardan seçilir. Bu çalışmada, antikorozif özelliklerini incelemek üzere Robins ve ark.'nın sentezlediği pirimidin türevleri seçildi. Bu bileşikler fonksiyonel grup, serbest elektron çiftleri ve π elektronları bakımından çok zengindir. Bileşiklerin antikorozif etkilerini teorik olarak hesaplamak için tüm bileşikler için yoğunluk fonksiyonel teorisi (DFT) yöntemi, 6-31G (d, p) baz seti ve B3LYP fonksiyonları kullanılarak Gaussian09W yazılım paketi ile kuantum kimyasal hesaplamaları yapıldı. Hesaplamalar, incelenen bileşiğin, moleküler yapının doğasına bağlı olarak metal yüzeyinde farklı şekilde adsorbe edildiğini gösterdi.

Anahtar Kelimeler: Pirimidin tipi bazlar, yoğunluk fonksiyonel teorisi, korozyon önleyici, kimyasal hesaplama.

1. INTRODUCTION

Acid solutions, which are widely used in industrial production, especially in cleaning processes, cause significant mass losses on the surface. These mass losses are due to corrosion on the metal surface. Many techniques have been developed to prevent corrosion occurring on the surface. One of these techniques is the use of organic compounds as corrosion inhibitors.

Organic compounds containing aromatic ring, heteroatom and π electrons show high inhibition property. Due to the high nucleophilic character of sulfur, it adheres to the methal surface more easily than oxygen and nitrogen atoms. Accordingly, the inhibition effect of heteroatoms will be O < N < S. [1] If the compounds containing heteroatoms also including π-electrons, further increasing the adsorption.

DOI: http://dx.doi.org/10.32571/ijct.936862 E-ISSN: 2602-277X

It is desirable that the compounds used for corrosion preventive purposes are not harmful to nature. Therefore, pyrimidine derivatives are of great interest. Pyrimidine derivatives exhibit wide biochemical effects due to the activity of aromatic ring system, N atoms and π electrons. Due to these properties, pyrimidine compounds are promising for corrosion inhibition.

Many different experimental methods are used for corrosion inhibition. These are often very costly methods. They also fail to explain the inhibition mechanism of corrosion. For this reason, computational methods have recently been used frequently as an alternative to experimental methods. The quantum chemical calculation method is one of these computational methods. With this method, the working cost and time are reduced and the mechanism can be explained.

The inhibition effect is mostly connected to the electronic structure of the compounds. In the case of using organic compounds as inhibitors, the selected molecules must be able to donate electrons to the empty d-orbital of the metal and also be suitable to form anti-feedback bonds. Various methods have been developed to elucidate the electronic structures of compounds. One of these methods is the quantum chemical calculation method. In this study, the Gaussian09.6[2] package program was used which is one of the programs that can best use the quantum chemical calculation method. According to the frontier orbital theory, the reaction is due to an interaction between the Highest Molecular Orbital (HOMO) and Lowest Occupied Molecular Orbital (LUMO) boundary orbits of the compounds.

Therefore, correct interpretation of these energy levels is important to understand the inhibition effect. To understand the mechanism, it must be calculated in the energy gap (ΔE). The energy gap equals the difference between ELUMO and EHOMO energies. Low values of ΔE will provide perfect inhibition effect.

In order to determine the ability of the molecule to prevent corrosion that may occur on the metal surface, the absolute I, A, S, ω, ΔN and ΔEback-donation properties must be calculated. In this study, all these calculations were carried out according to Shojaie et al.[3] using Gaussian09.

2. MATERIALS AND METHODS

In this work, Gaussian package program was used for the quantum chemical calculations by density functional theory (DFT) at the B3LYP / 6-31G (d, p) level. With this program, many calculations that can be made with the quantum mechanical method can be done easily. These calculations allow us to predict the molecular properties, molecular structures, vibrational frequencies and reactions of the compounds. It is possible to calculate the optimum geometry, minimum energy, bond lengths, bond angles and dihedral angles for a compound.

As the geometric optimization calculations of large molecules are made with high level methods, they are very time consuming processes. Large molecules can have a large number of conformers whose global minimum and energies make it difficult to find local minimums as low as fairly large molecules can have. There are many special methods for conformational scanning whose aim is to find the low energy conformer. Due to the large number of conformers in question, energy calculations in conformational scanning with large molecules are usually made by molecular mechanics. The aim of this work is to determine the effectiveness of pyrimidine compounds with various bonded functional groups as corrosion inhibitors. For this purpose, quantum chemical parameters such as HOMO, LUMO, MEP, ΔE, the ionization potential, the electron affinity, chemical hardness and softness, general electrophilic index, transmitted electron fraction index and recovery (ΔEback-donation) were calculated.

3. RESULTS AND DISCUSSION

The geometric optimizations and quantum chemical parameters used to determine their corrosion inhibition potential of previously synthesized pyrimidine compounds[4] (Figure 1) has been studied by DFT calculations.

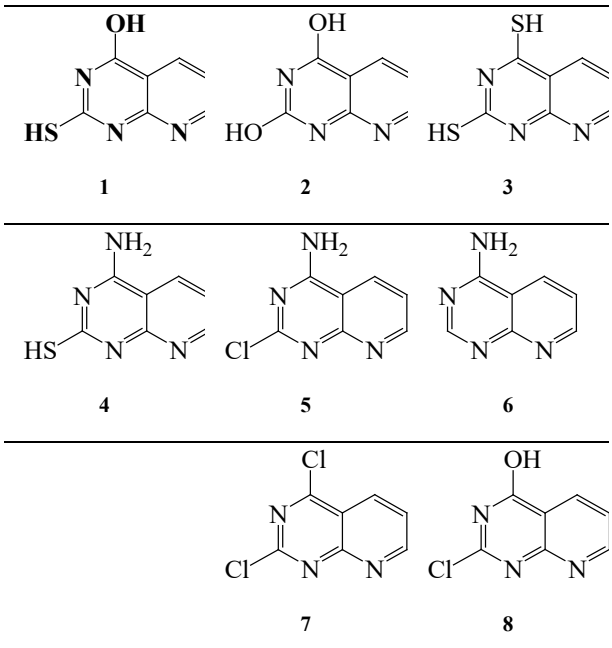


Figure 1. Molecular structures and schematic representation of pyrimidine derivatives

Theoretical calculations led to the development of experimental work. With theoretical calculations, corrosion activity parameters of the analyzed molecules against metal atoms can be calculated. Theoretical

DOI: http://dx.doi.org/10.32571/ijct.936862 E-ISSN: 2602-277X

calculations have demonstrated that the molecule's filled highest energy orbital and empty lowest energy orbital values are the most important parameters in estimating the corrosion inhibition activity of the molecules against metal atoms. It can be found that molecules for which quantum chemical calculations are made are active molecules with parameters like EHOMO, ELUMO, ΔE, χ, μ, η, ω, σ. [5,6]

$$\mu = -\chi = \left(\frac{\partial E}{\partial N}\right)_{\vartheta(r)} \quad (1)$$

$$\eta = \frac{1}{2}\left(\frac{\partial^2 E}{\partial N^2}\right)_{\vartheta(r)} = \frac{1}{2}\left(\frac{\partial \mu}{\partial N}\right) \quad (2)$$

The I, A, χ, σ, η values of the studied molecules are obtained by EHOMO and ELUMO with the following equations are obtained:

$$\chi = -\mu = \left(\frac{-E_{HOMO}-E_{LUMO}}{2}\right) = \left(\frac{I+A}{2}\right) \quad (3)$$

$$\chi = -\mu = \left(\frac{-E_{HOMO}-E_{LUMO}}{2}\right) = \left(\frac{I+A}{2}\right) \quad (4)$$

σ is a chemical illustrator that surveys molecular stability and reactivity. σ is defined as the reverse of η.

$$\sigma = \frac{1}{\eta} \quad (5)$$

The ω is a survey of the energy drop because of the maximum electron run between donor and acceptor. It can be represented as a function of χ and η as shown in Eq. (6).

$$\omega = \frac{\mu^2}{2\eta} = \frac{\chi 2}{2\eta} \quad (6)$$

The ω surveys the ability of molecules to receive electrons. As the ω value of a molecule increases, its electrophilic character increases, and as it decreases, its nucleophilic character increases.

The electronegativity value of molecules is a parameter that helps to compare the reactivity of molecules. The value of this parameter is given to estimate the electron transfer between metal and inhibitor. The molecule with a high electronegativity value hardly gives any valence electrons to this molecule. Because these electrons are attracted to the nucleus more than other molecules. According to Sanderson's electronegativity equation[7], we calculate the value of the electrons transferred from the anti-corrosion molecule with the following equation.

$$\Delta N = \frac{\chi_M - \chi_{inh}}{2(\eta_M + \eta_{inh})} \quad (7)$$

Here χM and χinh are the electronegativity value of the metal and the inhibitor molecule, respectively. ηM and ηinh are the chemical hardness of the metal and the inhibitor molecule, respectively.

According to the simple charge transfer model, the electron donation and recovery process can be expressed as an electronic donation back process between the inhibitor molecule and the metal surface.

$$\Delta E_{back\ donation} = -\frac{\eta}{4} \quad (8)$$

The ΔEback donation implies that When η > 0 and ΔEback donation <0 the charge transfer to a molecule, followed by a back donation from the molecule, is energetically favored.

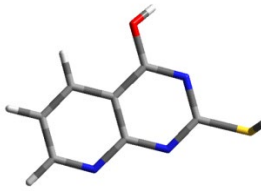
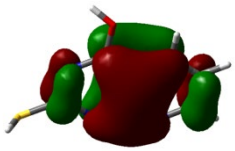
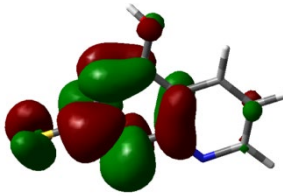
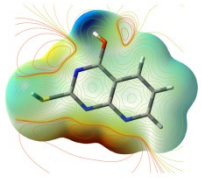
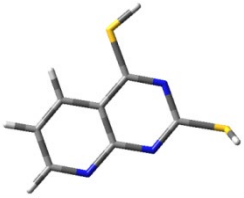
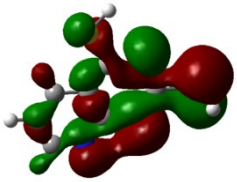
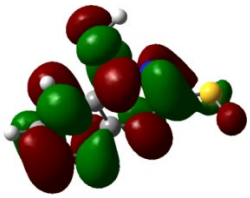
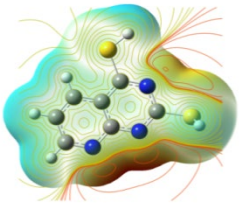
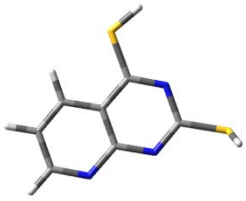
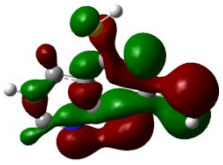
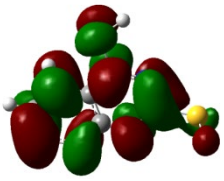
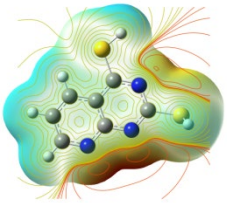
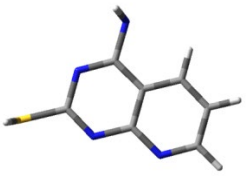
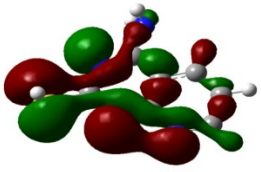
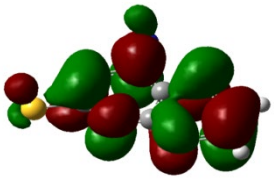
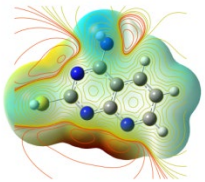
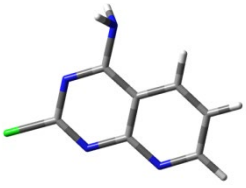
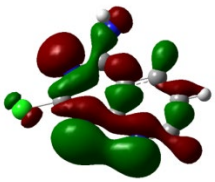
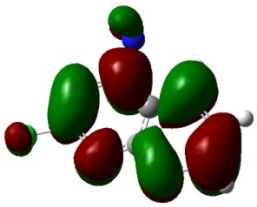
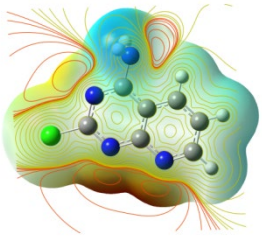
Fully geometric optimizations of all molecules, HOMO-LUMO diagrams, molecular electrostatic potential maps (MEPs) and corrosion inhibition parameters were calculated with DFT and B3LYP (d, p) base set in Gaussian09 program (Figure 2 and Table 1). It has been determined in some studies that this calculation method, which is carried out theoretically, is used to examine the relationship between corrosion inhibition efficiency and electronic properties of molecules.

MEPs that provide information about the molecular distribution of electrons are represented by different colors. In Figure 1, the negative (red) areas of the MEPs are associated with electrophilic reactivity and positive (blue) areas with nucleophilic reactivity. Electrostatic potential increases during red> orange> yellow> green> blue. The highest potential is on oxygen atoms.

The chemical reactivity properties of the inhibitor molecule depend on the interaction between HOMO and LUMO orbitals. The energy level of HOMO is defined as the skill of a molecule to donate electrons. Therefore, the molecule with a higher EHOMO value shows a better tendency to electron donation and increases the adsorption on the metal. Therefore, it provides better inhibition efficiency. The LUMO energy level is considered the ability of the molecule to accept electrons. A low LUMO energy level indicates that the molecule can gain electrons more easily. The high HOMO-low LUMO values of the inhibitor increase its adhesion to the metal surface.

One of the important parameters determining the reactivity of the inhibitor is the energy gap value (ΔEgap). The ΔEgap value of the inhibitor indicates its ability to bind to the metal surface. The value of ΔEgap is calculated from the difference between the LUMO energy and the HOMO energy. The low energy gap value (ΔEgap) means that the inhibitor will stick to the metal surface more easily.

DOI: http://dx.doi.org/10.32571/ijct.936862 E-ISSN: 2602-277X

		HOMO	LUMO	MEPs
1				
2				
3				
4				
5				

DOI: http://dx.doi.org/10.32571/ijct.936862 E-ISSN: 2602-277X

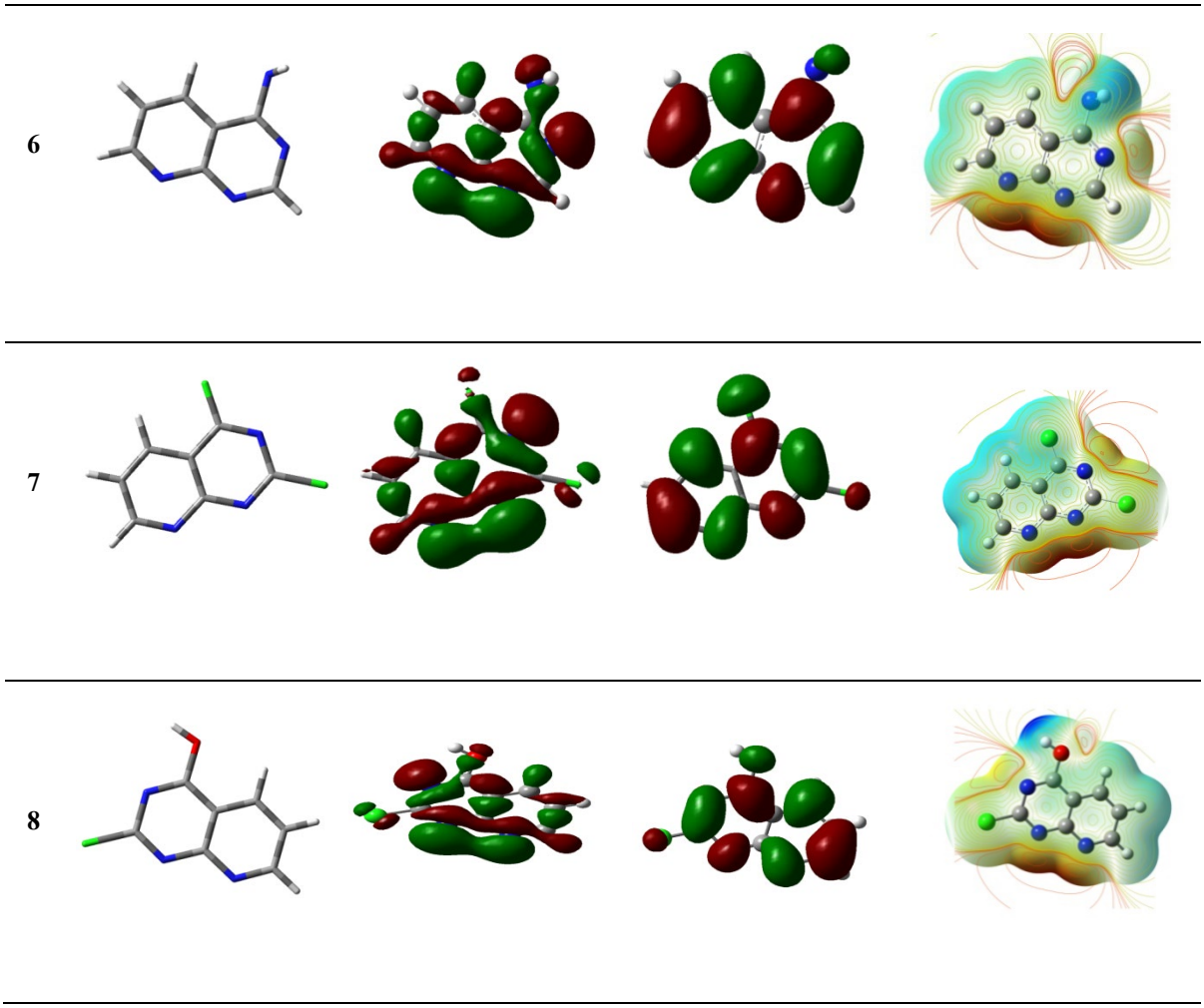


Figure 2. Structures, HOMO-LUMO diagrams and MEPs of optimized pyrimidine molecules

Table 1. Calculated quantum chemical parameters of the studied molecules in gas phase (e*V)*

Compound	E_{HOMO}	E_{LUMO}	ΔE	I	A	η
1	-7.4868	-0.9317	6.5551	7.4868	0.9317	6.5551
2	-6.6661	-2.6284	4.0377	6.6661	2.6284	4.0377
3	-6.6661	-2.6284	4.0377	6.6661	2.6284	4.0377
4	-2.5054	-6.5883	-4.0829	2.5054	6.5883	-4.0829
5	-2.5252	-6.8149	-4.2897	2.5252	6.8149	-4.2897
6	-2.2849	-6.5208	-4.2359	2.2849	6.5208	-4.2359
7	-6.9309	-2.7076	4.2233	6.9309	2.7076	4.2283
8	-6.9689	-2.4523	4.5166	6.9689	2.4523	4.5166

Compound	S	χ	μ	ω	ΔN	$\Delta E_{back\text{-}donation}$
1	0.1526	4.2093	4.4935	1.5169	0.2129	-1.6388
2	0.2477	4.6472	4.2138	2.1988	0.2914	-1,0094

DOI: http://dx.doi.org/10.32571/ijct.936862 E-ISSN: 2602-277X

Table1 continued

3	0.2477	4.6477	4.2240	2.2095	0.2913	-1,0094
4	-0.2449	4.5469	4.8316	-2.8588	-0.2655	1,0207
5	-0.2331	5.9326	5.0226	2.9404	-0.1244	1.0725
6	-0.2360	4.4028	3.8636	-1.7621	-0.3065	1.0589
7	0.2365	4.8193	5.3886	3.4336	0.2579	-1.0571
8	0.2214	4.7106	4.6964	2.4417	0.2528	-1.1292

The inhibition properties of organic molecules also depend on the chemical hardness (η) and chemical softness (σ) of the molecule. The term chemical hardness (η) is used against electron cloud polarization and chemical degradation. The chemical hardness, global softness values of the Koopman theory[8] replaced the HOMO and LUMO energy values. If hard molecules have a high ΔEgap value, this molecule is not a good corrosion inhibitor.

The global electrophilic index (ω) surveys the ability of molecules to receive electrons. As the ω value of a molecule increases, its electrophilic character increases, and as it decreases, its nucleophilic character increases. If the transferred electron (ΔN) is < 3.6, it helps to increase the inhibitory efficiency by increasing the skill of these inhibitors to donate electrons to the metal surface. [9] The highest electron fraction is connected with the best inhibitor. In the light of this information it can be said that the compounds 4, 5 and 6 may have high inhibition potential.

4. CONCLUSIONS

In this study, quantum chemical calculations of previously synthesized pyrimidine compounds[4] were studied. The electronic properties of the molecules, corrosion prevention parameters and electrostatic potential maps (MEP) properties were theoretically calculated on the DFT-B3LYP / 6-31 G (d, p) base set. As a result, it was determined that compound 4-6 is theoretically the most active structure in terms of corrosion prevention potential.

ACKNOWLEDGEMENTS

We would like to thank Van YYU BAP Coordination of Turkey FYL-2020-9273.

Conflict of interests

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.1024979 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Phenolic profile, antioxidant, DNA protection, acetylcholinesterase, butyrylcholinesterase and urease inhibition activities of *Coriandrum sativum* L. leaf, seed and flower extracts

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Received: 18 November 2021; Revised: 15 January 2022; Accepted: 19 January 2022

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Citation: Ozen, T.; Yenigun, S.; Toka, M. *Int. J. Chem. Technol.* **2022**, 6 (1), 21-32.

ABSTRACT

In this study, chemical content, antioxidant, enzyme inhibition, and DNA protection activities of extracts obtained from different solvents of the *Coriandrum sativum* leaf, flower and seed were determined. Total phenol and flavonoid contents of *C. sativum* leaf, seed and flower hexane extracts were higher than water, methanol, ethanol and ethyl acetate extracts. The highest anthocyanin content was found in the flower part of *C. sativum*. From the antioxidant activity tests, total antioxidant activity of flower aqueous extract, reducing power of seed aqueous extract, H_2O_2 scavenging activity of leaf ethyl acetate extract, $OH^{\cdot}$ radical scavenging activity of leaf hexane extract, free radical scavenging activity of flower methanol extract, metal chelate activity of leaf ethyl acetate extract, superoxide anion scavenging activity of leaf aqueous extract and lipid peroxidation inhibition activity of the leaf ethyl acetate extract had the highest. It was found that the urease inhibition activity of the seed methanol extract and the acetylcholinesterase and butyrylcholinesterase inhibition activities of the seed ethanol extract presented effective inhibition activity as 80.30±0.20%, 112.83±10.75 µg/mL, and 334.28±23.09 µg/mL, respectively. The leaf hexane, flower ethyl acetate, and leaf methanol extracts showed the highest DNA protection activities with values of 71.86%, 70.89%, and 69.38%, respectively. According to the phytochemical content and biochemical activity results, this study is a valuable report proving that the *C. sativum* plant is a natural effective product.

Keywords: *Coriandrum sativum* L. (kinzi), antioxidant activity, enzyme inhibition, DNA protection activity

Coriandrum sativum L. yaprak, tohum ve çiçek ekstraktlarının fenolik profili, antioksidan, DNA koruma, asetilkolinesteraz, butirilkolinesteraz ve üreaz inhibisyon aktiviteleri

ÖZ

Bu çalışmada *Coriandrum sativum* yaprağı, çiçeği ve tohumunun farklı çözücülerinden elde edilen ekstraktların kimyasal içeriği, antioksidan, enzim inhibisyon ve DNA koruma aktiviteleri belirlendi. *C. sativum* yaprak, tohum ve çiçek hekzan ekstraktlarının toplam fenol ve flavonoid içerikleri su, metanol, etanol ve etil asetat ekstraktlarından daha yüksek bulundu. En yüksek antosiyanin içeriği *C. sativum* çiçek kısmında bulunmuştur. Antioksidan aktivite testlerinden, çiçek sulu ekstraktının toplam antioksidan aktivitesi, tohum sulu ekstraktının indirgeme gücü, yaprak etil asetat ekstraktının H_2O_2 süpürme aktivitesi, yaprak hekzan ekstraktının $OH^{\cdot}$ radikal süpürme aktivitesi, çiçek metanol ekstraktının serbest radikal süpürme aktivitesi, metal yaprak etil asetat ekstraktının şelat aktivitesi, yaprak sulu ekstraktının süperoksit anyon temizleme aktivitesi ve yaprak etil asetat ekstraktının lipid peroksidasyon inhibisyon aktivitesi en yüksek değerleri gösterdi. Tohum metanol ekstraktının üreaz inhibisyon aktivitesinin ve tohum etanol ekstraktının asetilkolinesteraz ve butirilkolinesteraz inhibisyon aktivitelerinin sırayla %80.30±0.20, 112.83±10.75 µg/mL ve 334.28±23.09 µg/mL olarak etkin inhibisyon aktivitesi gösterdiği bulunmuştur. Yaprak hekzan, çiçek etil asetat ve yaprak metanol ekstraktları, sırasıyla %71.86, %70.89 ve %69.38 değerleriyle en yüksek DNA koruma aktivitelerini göstermiştir. Fitokimyasal içerik ve biyokimyasal aktivite sonuçlarına göre bu çalışma, *C. sativum* bitkisinin doğal etkili bir ürün olduğunu kanıtlayan değerli bir rapordur.

Anahtar Kelimeler: *Coriandrum sativum* L. (kinzi), antioksidan aktivite, enzim inhibisyonu, DNA koruma aktivitesi.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

1. INTRODUCTION

One of the main changes that occur during preparing and consuming food is oxidation. Lipid oxidation, which initiates other changes in the nutritional system, affects the quality, nutrition, color, smell, structure, and safety of nutrients. The reactive oxygen species (ROS) and antioxidant protective systems can interact with the chemical changes in biological relevant macromolecules. This imbalance provides appropriate pathobiochemical mechanisms which start and develop many diseases. One process that can apply to eliminate the adverse effects of these ROS is using antioxidant substances.[1]

Antioxidants are low concentrations of organic molecules preventing free radical oxidation of different compounds. In the last century, synthetic antioxidants were used for preservation purposes in the food industry since they were especially effective for a more extended period. However, recent reports of the findings of the carcinogenic effects of synthetic antioxidants, legal restrictions on the use of synthetic antioxidants have begun to introduce in many countries.[2] Recently, the demand for natural antioxidants of herbal has been raised for the food industry and pharmaceutical medicine. As a natural result, attention to natural antioxidants continues to grow, especially those of plant origin.[3] Some plant phenolics have recently been recognized as antioxidants and are produced commercially. In this respect, it is crucial to know the biological availability and required levels of these antioxidants that provide a protective effect on the diet. Natural antioxidants have therapeutic potential as medicinal plants, singlet oxygen suppressors, reducing agents, and free radical scavengers. These plant antioxidant activities are due to bioactive compounds such as isocatechins, flavones, lignans, flavonoids, coumarins, isoflavones, catechins, and anthocyanins.

Nowadays, pharmacological research on natural antioxidants with low or no side effects increases to use in preventive medicine. These spices are known to have a health effect (diuretic, expectorant, laxative, antibacterial, antipyretic) and have been used effectively in local treatments in many countries. Moreover, those natural products' (plants) in vivo physiological effects have been determined by applying many models of experimental animals such as beneficial effects on lipid metabolism, antidiabetic activities, ability to stimulate anti-inflammatory, antipathogenic, antioxidant, and digestion studies.[1]

Coriandrum sativum, which has nutritional and medicinal properties, is widely used and distributed spices due to its monoterpenoid-linalool and essential fatty acids, especially in its seeds. *C. sativum* is used to prepare many home remedies used for flu, seasonal fever, nausea, vomiting, and stomach ailment treatments; it is also used for indigestion, intestinal worms, rheumatism, and joint pain. Many of *C. sativum* curative properties are attributed to its special phytonutrient status, and for this reason, it is referred to as a source of bioactive compounds.[4] *C. sativum* is among the essential plants that produce essential oil globally, with 750 tons. In addition to the production of essential oil, *C. sativum* is used as a spice with fresh and dried herbs, called Chinese parsley or "Cilantro".[5] The homeland of *C. sativum* is Anatolia and the Caucasus. Additionally, it is found naturally in Asia and Europe. *Coriander* cultivation, which belongs to the *Umbelliferae* (umbrella flower) family, is cultivated in Hungary, Russia, Poland, England, Bulgaria, Netherlands, Egypt, and Morocco. In Turkey, it is grown in the Lakes Region, Ankara, Eskişehir, and Konya.[6,7] Although the green parts of *C. sativum* are used as "Chinese parsley" in some countries, the principal used parts of the plant are the seeds (fruits). *C. sativum* seeds use as whole or powdered by mixing them into candies, sauces, milk, and meat products to impart flavor and smell. Linalool is a significant raw material in perfume and cosmetic production. It is also used as a protective material in pharmaceutical and food products for bactericidal and fungicidal.[8] Further, the green part of coriander is used as a spice, either fresh, dried, or in brine.[9] Also, it is known that *C. sativum* is used in various drug preparations to remove foul odors.[10] Because it shows drug properties, *C. sativum* also has delicious and gas-digesting properties.[4] Thus, determining the antioxidant activity of the *C. sativum* plant, which has many properties, will contribute to the literature.

In the literature, *C. sativum* has an anxiolytic effect in mice[11] and antibacterial activity to *S. choleraesuis*, *B. megaterium* and *E. coli*.[12,13] It has been reported to reduce triglyceride and cholesterol levels in rats,[14] be effective in treating inflammatory bowel diseases, and have *in vivo* antidiabetic properties.[15] Five components (Q-carotene, Q-cryptoxanthin epoxide, violaxanthin, neoxanthin, and lutein-5,6-epoxide) were obtained from *C. sativum* ether extract and compared their antioxidant activities with synthetic antioxidant, BHT.[16] Although these components did not show antioxidant activity as much as BHT. Q-carotene showed the most remarkable effect among these fractions. Besides, *C. sativum* ether crude extract showed more antioxidant activity, which attributed to a synergistic effect between carotenoid fractions.

C. sativum seeds and leaves extracted with different polarities were evaluated for their antioxidant activities and the inhibition of Fe^{+2}-induced phospholipid peroxidation and observed a correlation between their antioxidant activity and total phenol content.[17] In addition, it was determined that the ethyl acetate extract, which has a medium polarity, showed more effect than the other extracts. In the study by de Almeida Melo and co-worker,[18] the aqueous extract of *C. sativum* was obtained by successive extraction. Four different fractions were also acquired using silica gel column chromatography. Caffeic acid was determined as 4.34

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

and 2.64 µg/mL in the first and third fractions, respectively, and protocatechuic acid and glycate in the second and fourth fractions, 6.43 and 3.27 µg/mL, respectively. Additionally, using the Q-carotene/linoleic acid model, they suggested that the antioxidant activities of these fractions were the same, and thus the antioxidant activity of the *C. sativum* aqueous extract was due to its content of phenolic acids. *C. sativum* whole seeds or powdered ones are mixed into candies, sauces, milk and meat products, and alcoholic and non-alcoholic beverages to impart flavor and smell. Linalool, the main component of essential oil, is a significant raw material in perfume and cosmetic products. It is also used as a preservative in pharmaceutical products and food due to its bactericidal and fungicidal. In addition, the green parts of *C. sativum* using as a spice, either fresh, dried, or in brine. It stated that *C. sativum* is used in various drug preparations to remove foul odors. *C. sativum* also has delicious and gas-digesting properties because it shows drug properties.[19]

Although there are studies on antioxidant activity for *C. sativum* leaf and seed extracts in the literature, no study has been found on flower extracts. In addition, there are no studies on the inhibition activities of urease and esterases (acetylcholinesterase, butyrylcholinesterase); also, no one involves DNA protection activities of *C. sativum* seed, leaf, and flower extracts. In this study, considering the integrity of the aerial part of the *C. sativum* plant of the extracts of three different parts obtained with five solvents were investigated phytochemically and biochemically by tests for the chemical contents, antioxidant, enzyme inhibition, and DNA protection activities.

In this work, bioactive compounds (total phenolic, flavonoid, and anthocyanin), antioxidant activity tests (total antioxidant, metal chelating, lipid peroxidation, superoxide radical, free radical, hydroxyl radical, H_2O_2 scavenging activity, and reducing power capacity,) and enzyme inhibition activity tests (acetylcholinesterase, butyrylcholinesterase, and urease) were applied in the extracts of three different parts of the plant, obtained from different polarity solvents (ethanol, methanol, aqueous, hexane, and ethyl acetate). Thus, a significant potential of the *C. sativum* plant in Turkey in terms of the production of medicinal and spice plants was investigated, and its advantages and valuable results were obtained.

2. MATERIALS AND METHODS

2.1. Chemicals

Methanol, ethanol, ethyl acetate, hexane, acetone, gallic acid, catechin, acetylcholinesterase (AChE), urease, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), butyrylcholinesterase (BChE), gel loading dye (blue), ethidium bromide, galantamine, trolox, tert-butyl hydroquinone (TBHQ), α-tocopherol, ammonium molybdate, sodium phosphate, $K_3Fe(CN)_6$, $FeCl_3$, KCl, HCl, Na_2CO_3, $NaNO_2$, $AlCl_3$, NaOH, nitro blue tetrazolium (NBT), trichloroacetic acid (TCA), $FeSO_4$, phenazine meta sulfate (PMS), thiobarbituric acid (TBA), nicotinamide adenine dinucleotide (NADH), $FeCl_2$, ferrozine, salicylic acid, $FeSO_4$, $FeCl_2$, potassium iodide (KI), $Na_2S_2O_3$, DTNB, phenol, sodium nitroprusside, NaOCl from Sigma-Aldrich; Folin-Ciocalteu, ascorbic acid, thiourea from Merck; 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) from Fluka; pBR322 plasmid DNA from ThermoFisher and ethylenediaminetetraacetic acid (EDTA) from Carlo Erba were purchased.

2.2. Sample

The *C. sativum* growing in the natural environment was purchased from the local market in Samsun, Atakum, İncesu Village in 2019. The plant was identified by Prof. Dr. Erkan Yalçın, Ondokuz Mayıs University, Faculty of Arts and Sciences, Department of Biology, and its voucher numbers were OMUB 8679 and OMUB 1861. The leaves, seeds, and flower parts of the plant were dried in a cool and air-flowing condition and ground into powder in the grinding mill.

2.3. Extraction

The extraction processes were applied to the seed (10 g), flower (10 g), and leaf (10 g) parts of the *C. sativum* plant with different polarities (methanol, ethanol, hexane, and ethyl acetate) using a soxhlet device and filtered on Whatman no:1 paper. The solvents were evaporated rotary at 40 °C and received dry crude extracts. Aqueous extracts were prepared with hot aqueous using a magnetic stirrer and filtered on Whatman no:1 paper. The clear extract was lyophilized at -50 °C, and under low pressure, the crude extract was obtained in powder form. The crude extracts were stored at -20 °C for chemical content analysis and activity determination.

2.4. Chemical Component Analysis

2.4.1. Total phenol determination

The total phenol content of the extracts was determined by expressing gallic acid equivalent (GAE)/g.[20] 1 mL of extract, and 1 mL of Folin & Ciocalteu's reagent solutions were mixed in the beaker. Then, 3 mL of 2% Na_2CO_3 solution was added to the reaction mixture. After the mixture was kept in the dark for 2 hours at room temperature, its absorption was read at 760 nm. A calibration curve was drawn using gallic acid as a standard (0.025-6.25 mg/mL; y= 0.0639x - 0.0054, R^2= 0.99) and determined the total phenolic contents of the extracts.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

2.4.2. Total flavonoid determination

The total flavonoid quantity of the extracts was calculated by stated as catechin equivalent (CE)/g.[21] 250 µL of extract solution and 75 µL of 5% $NaNO_2$ were mixed homogeneously. After 5 minutes, 150 µL of 10% $AlCl_3$ and 500 µL of 1 M NaOH solutions were inserted into the mixture. Finally, after adding 275 µL of aqueous to the reaction mixture, the absorption of the mixture was read at 510 nm. A calibration curve was drawn using catechin as a standard (0.06-1000 µg/mL; y= 0.2268x + 0.0216, R^2= 0.99) and calculated the total flavonoid contents of the extracts.

2.4.3. Total anthocyanin determination

The total anthocyanin content of the extracts was applied by modifying the previously applied methods.[22] 1 g of dry ground plants was mixed homogeneously with 1% HCl solution and centrifuged. The supernatant of the 0.2 mL extract with 1.8 mL of the buffer was mixed. In the analysis, KCl buffer solution for the first pH=1 buffer solution and sodium acetate buffers for the second pH=4.5 buffer solution was used. The extracts were diluted using pH 1.0 and pH 4.5 buffers. The absorbance of the mixtures was then measured at 520 nm. After 15 minutes, absorbance values of the mixture at 700 and 520 nm were recorded. The total anthocyanin concentration of the plant was expressed as mg cyanidin 3-glucoside equivalent/mL.

2.5. Antioxidant activity

The antioxidant activity of *C. sativum* extracts was determined by the following spectroscopic methods. BHA, TBHQ, BHT, α-tocopherol, ascorbic acid, and trolox were used as standard antioxidants. All activity tests were applied at a dose of 500 µg/mL of the extracts and performed in triplicate. While the reducing power capacities and total antioxidant activities of the extracts were expressed as absorbance values, the results of other activity tests were given as %.

2.5.1. Determination of total antioxidant activity

The activity was determined by the ammonium molybdenum method that is based on the reduction of molybdenum in an acidic medium and the formation of the green color phosphate/Mo(V) compound formed at 695 nm.[23] It was mixed with 1 mL of reagent solution (28 mM sodium phosphate:0.6 M sulfuric acid:4 mM ammonium molybdate) and 0.1 mL of extract.

The reaction medium was incubated in a lidded tube in an aqueous shaker bath for 90 minutes at 95 °C. Then the mixture was cooled to 25 °C, and it was recorded the absorbance values at 695 nm.

2.5.2. Determination of reducing power

The reducing capacities of *C. sativum* extracts were observed with Fe^{3+} to Fe^{2+} reduction assay spectroscopically.[24] Briefly, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ was mixed with 1 mL of extract. The mixture waited in an aqueous bath at 50 °C for 20 minutes. After, 2.5 mL of 10% TCA was added to the mixture medium, and the mixture was centrifuged at 2500 x g. The 2.5 mL of the obtained supernatant was mixed with 0.5 mL of 0.1% $FeCl_3$ and 2.5 mL of ddH_2O. The reducing power capacities of the standards and extracts were measured at 700 nm, and changes in absorbance were followed.

2.5.3. Determination lipid peroxidation inhibition

The lipid peroxidation inhibition capacities of the extracts were obtained by monitoring the level of linoleic acid peroxidation.[25] Extract solution 550 µL of linoleic acid (40 µM), 150 µL of ascorbic acid (10 µM), and 500 µL of phosphate buffer (100 µM, pH 7.4) were mixed homogeneously. Linoleic acid peroxidation is initiated by adding 0.1 mL of $FeSO_4$ (10 µM) to the mixture. The reaction mixture is incubated at 37 °C for 60 minutes in the dark. After incubation, 1.5 mL of 10% TCA solution prepared in 0.5% HCl, 3 mL 1% TBA solution prepared in of 50 mM NaOH was added to the reaction mixture. The TBA/extract (or standard) mixture was incubated at 95 °C. Later the mixture was cooled to 25 °C, 3 mL of *n*-butanol was added. The inhomogeneous mixture was centrifuged, and the pink supernatant was removed. The absorption of the mixture was read at 532 nm. The percent inhibition of linoleic acid peroxidation level was estimated according to the following formula;

Activity (%): $((A_0-A_1)/A_0) \times 100$

A_0: absorbance of the control, A_1: absorbance of the sample

2.5.4. Determination of free radical scavenging activity

This assay was evaluated by a 1,1-diphenyl-2-picryl-hydrazyl (DPPH˙) radical scavenging assay.[26] 3 mL of the extract and solutions of standard antioxidant substances were mixed with 1 mL of 0.1 mM DPPH˙ solution. Changes in absorbance at 517 nm were recorded. The activity of the samples was calculated according to the formula written below.

Activity (%): $((A_0-A_1)/A_0) \times 100$

A_0: absorbance of the control, A_1: absorbance of the sample

2.5.5. Determination of superoxide anion scavenging activity

This method of the extracts and antioxidant substances was determined according to the Nishikimi Method.[27] In the method, the superoxide radical was produced by the

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

NADH-PMS system by reduction of NBT and oxidation of NAD. In the experiment, NBT (156 µM, 1.0 mL), 1 mL of different concentrations of extract and solutions of antioxidant substances, and NADH (468 µM, 1.0 mL) was stirred thoroughly. The reaction was initiated by adding PMS (100 µM, 0.4 mL) to the reaction mixture, incubated, and left at ambient temperature for 5 minutes. The absorbance of the mixture was read at 560 nm, and the superoxide anion scavenging activity of the samples was calculated according to the formula written below;

Activity (%): $((A_0-A_1)/A_0) \times 100$

A_0: absorbance of the control, A_1: absorbance of the sample

2.5.6. Determination of metal chelating activity

The complexation activity of the standard and extract antioxidant substances with Fe^{2+} was evaluated by measuring the absorbance of the Fe^{2+}-ferrozine complex at 562 nm.[28] 0.4 mL of extract and 0.05 mL of 2 mM $FeCl_2$ were mixed by vortex thoroughly. The reaction is initiated by adding 0.2 mL of 5 mM ferrozine. The final volume is complete to 4 mL with ethanol. The mixture was thoroughly mixed by vortex and left at 25 °C for 10 minutes. The absorbance of the mixture is read at 562 nm, and the % inhibition of Fe^{2+}-ferrozine complex formation was estimated according to the equation given below;
Activity (%): $((A_0-A_1)/A_0) \times 100$
A_0: absorbance of the control, A_1: absorbance of the sample

2.5.7. Determination hydroxyl radical ($OH^{\cdot}$) scavenging activity

The activity of the extracts and standard antioxidant substances was determined by modifying the method developed by Smirnoff, Cumbes.[29] $OH^{\cdot}$ radical was formed with a mixture of $FeSO_4$ and H_2O_2 and determined by measuring the spectrophotometric absorption of the compound formed by the radical with salicylic acid. The absorbance of the 3.0 mL reaction mixture consisting of 1.0 mL $FeSO_4$ (1.5 mM), 0.3 mL salicylic acid (20 mM), 0.7 mL H_2O_2 (6 mM), and 1.0 mL plant extract was measured at 562 nm, and percent $OH^{\cdot}$ scavenging activity was calculated according to the formula below.
Activity (%): $((A_0-A_1)/A_0) \times 100$
A_0: absorbance of the control, A_1: absorbance of the sample

2.5.8. Determination H_2O_2 scavenging activity

The activity was evaluated by a titration assay modified by Zhao and co-workers[30]. 1.0 mL, 0.1 mM H_2O_2, and 1.0 mL extract (or standard antioxidant substance) were added and mixed well, then 0.1 mL of 3% ammonium molybdate, 10 mL of sulfuric acid (2M), and 7.0 mL of potassium iodide (1.8 M) were added, sequentially. The mixture was titrated with $Na_2S_2O_3$ (5 mM). According to the obtained volume values, the percent activity was calculated according to the formula below.
Activity (%): $((V_0-V_1)/V_0) \times 100$
V_0 represents the $Na_2S_2O_3$ volume (mL) spent in the presence of H_2O_2 (without extract or standard substance; ddH_2O is used),
V_1 denotes the $Na_2S_2O_3$ volume (mL) in the mixture containing the extract or standard substance.

2.6. Enzyme inhibition activities

Enzyme inhibition activities of *C. sativum* extracts were observed by the following spectroscopic methods. Galantamine was used as a standard in acetylcholinesterase and butyrylcholinesterase inhibition activities, and thiourea was used as a standard in urease inhibition activities. Enzyme inhibition activity tests were applied to the extracts and standard substances under the same conditions and performed in triplicate.

2.6.1. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activity

AChE and BChE inhibition levels of the extracts were measured spectrophotometrically.[31] 750 µL of 100 mM Na-K buffer (pH 8.0), 50 µL of different concentrations (0.025-5.0 µg/mL) of extract solutions (or galantamine), 100 µL of enzyme solution (0.03 U/mL, 100 mM pH 8.0, Na-K buffer) into the tubes was mixed, respectively and incubated for 15 minutes at 25 °C. 50 µL of 3.3 mM DTNB and 50 µL of 1 mM acetylcholine iodide (or butyrylcholine chloride) were added to the mixture. The absorption values of each mixture were recorded at 412 nm. The IC_{50} values of the extract samples and galantamine were determined and expressed as µg/mL. IC_{50} values (µg/mL) were also presented with effective concentration inhibition of AChE (or BChE) for inhibition activities.

2.6.2. Urease inhibition activity

The urease inhibition activities of the extracts were determined by spectrophotometrically.[32] 125 µL of 0.47 U urease (100 mM pH 6.8 PBS), 25 µL of extract (or thiourea, 50-500 µg/mL), 275 µL of 0.2 mM urea were placed in test tubes, respectively. The mixture was kept waiting for 15 minutes at 30 °C in a shaking aqueous bath. 225 µL of phenol reagent (0.005%, w/v sodium nitroprusside + 1%, w/v phenol) and 350 µL of alkaline reagent (1%, w/v NaOH + 0.075%, v/v NaOCl) were added, respectively. The mixture was incubated for 50 minutes at 30 °C in a shaking aqueous bath. The absorptions of each mixture were measured at 630 nm, and percent urease inhibition activity was calculated according to the formula below. The activities of the extracts and thiourea at 100 µg/mL were expressed as %.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

Inhibition activity (%): $((A_0-A_1)/A_0) \times 100$
A_0 is the absorbance of the control, A_1 is the absorbance of the extract or standard substance

2.7.DNA Protection Activity

The DNA protection activity of *C. sativum* extracts was estimated using the agarose gel electrophoresis method. The capacities of the extracts to protect plasmid DNA (pBR322, ThermoFisher) from the oxidizing effects of H_2O_2 and UV treatment were evaluated by their DNA breaking forms.[33,34] In summary, assay 5 µL of extract (1000 µg/mL), 3 µL of plasmid DNA (1:3, v/v), and 1 µL of H_2O_2 (30%) were mixed. The mixture of 6 µL H_2O and 3 µL plasmid DNA (1:3, v/v) as negative control (C1) and 6 µL H_2O, 3 µL plasmid DNA (1:3, v/v) as other positive control (C2), and 1 µL of H_2O_2 (30%) were formed. In addition, quercetin (1000 µg/mL) was used as a standard. The reaction was initiated by the application of UV irradiation for 5 minutes. After irradiation, 2 µL of loading dye was mixed into the mixture and loaded on a 1% agarose gel to which 2 µL of ethidium bromide was added. Electrophoresis was performed at 90 volts for 60 minutes and then photographed with a UV transilluminator (320 nm, 8000 µW/cm). In addition, the percent protection level of the super-coiled DNA form (Form I) and broken DNA form (Form II) of the extracts and quercetin were calculated using the ImageJ Program.

2.8. Statistical analysis

Each parameter of *in vitro* biological activity studies was expressed as triplicate analysis results ± standard deviation values. All data were analyzed in the IBM Statistical Package for Social Studies (SPSS) 20.0 program. ANOVA was used because the mean of more than two independent groups between the analysis means and the data was with normal distribution variance and homogeneous. Tukey HSD[a,b] was used for multiple comparisons based on the data obtained. The statistical significance of the values was compared with the activity analysis result group, and the level of significance was expressed with $p<0.05$ values and considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Extracion yield, total phenol, flavonoid, and anthocyanin contents

Phenolic compounds exhibit high antioxidant activity. They have an important role in the protection of plants against UV radiation, beast of prey, and pathogens.[35] Phenolics are all-important plant components because of their capacity to scavenge radicals such as active oxygen types like singlet oxygen and hydroxyl and free radicals.[3,36] Flavonoids are phenolic compounds that impressive against microbial infections and are synthesized by plants. And also, they are abundantly present in nearly whole plants and have many effects like antioxidant, antiviral and antimutagenic.[37] In recent, bioactive compounds from natural sources obtaining interest increase. Therefore, vast areas of bioactivity methods and assays have been developed.[38,39]

The yields of the extracts were obtained from seed, leaf, and flower extracts of *C. sativum*. Total flavonoid and phenolic contents were applied to methanol, aqueous, ethanol, hexane, ethyl acetate, and extracts of *C. sativum* flower, seed, and leaf parts. The results of the total phenolic, anthocyanin, and flavonoid constituents of *C. sativum* leaf, seed, and flower extracts and dried samples were summarized in Table 1. The amounts of total phenol contents were determined as the GAE using a calibration equation and calculated from the GAE graph ($y= 0.0639x - 0.0054$, $R^2= 0.99$). The highest total phenolic contents identified in leaf hexane extract, seed hexane extract, and flower hexane extract as 250.40±17.84, 423.65±21.15, and 238.82±10.46 mg GAE/g dry plant, respectively, while leaf methanol extract, aqueous seed extract, and flower ethyl acetate extract exhibited the lowest phenolic compounds as 5.46±0.07, 30.07±0.16 and 25.80±10.46 mg GAE/g dry plant, respectively. The previous study done by Wangensteen and co-workers[17] found that the total phenolic contents of *C. sativum* seed ethanol, dichloromethane, ethyl acetate, and *n*-butanol extracts as 0.15±0.01, 0.09±0.01, 1.89±0.08, and 1.16±0.01 g GAE/100 g plant, respectively. In our study, the total phenolic contents of *C. sativum* seed ethanol and ethyl acetate extracts were determined to be higher. Demir and Korukluoglu[40] determined the total phenol contents of *C. sativum* seed methanol and ethanol extracts as 4.20±0.30 and 2.10±0.40 mg GAE/g. However, in our study, the total phenol contents of *C. sativum* seed methanol and ethanol extracts were determined to be higher. In a different study, Muñiz-Márquez and co-workers[41] calculated the total phenol content of all parts of *C. sativum* ethanol extract as 1.38±0.06 mg GAE/g plant. In contrast, in our study, the total phenol contents of *C. sativum* seed, leaf, and flower ethanol extracts were calculated to be higher. On the other hand, Yildiz[42] observed that the phenolic content of all parts of *C. sativum* ethanol extract was 14.97±0.05 mg GAE/g. In our study, the total phenol contents of *C. sativum* seed, leaf, and flower ethanol extracts were determined to be higher. Harsha and Anilakumar[43] found that the total phenol contents of *C. sativum* leaf ethanol extract was 133.74 µg GAE/mg extract. However, in our study, the total phenol content of *C. sativum* leaf ethanol extract was determined to be higher. The work of Msaada and co-workers[44] reported that the total phenol contents of *C. sativum* fruits methanol extracts obtained from Tunisia, Syria, and Egypt were as 1.00±0.06, 1.09±0.02, and 0.94±0.05 mg GAE/g, respectively. In our study, the total phenol contents of *C. sativum* fruit methanol extract was determined to be higher. In a different study, Gallo

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

and Co-workers[45] observed that the total phenol contents of aqueous and ethanol extract obtained using *C. sativum* seed ultrasound and microwave were determined as 41.81±2.77 and 82.09±8.43 mg GAE/100 g plant, respectively. In our study, the total phenol contents of *C. sativum* seed ethanol and aqueous extracts were determined to be higher. Sreelatha and Inbavalli[46] reported the phenol contents of *C. sativum* seed and leaf ethanol extracts as 15.14±1.62 and 25.23±2.17 g GAE/100 g plant, respectively, while, in our study, the total phenol contents of *C. sativum* seed and leaf ethanol extracts were determined to be higher. The total flavonoid contents were performed as the catechin equivalent (CE) using a graph equation drawn from a standard quercetin graph (y= 0.2268x + 0.0216, R^2= 0.99). The highest total flavonoid contents identified in leaf aqueous extract, seed, and flower hexane extract as 32.46±1.10, 132.35±14.08, and 68.66±9.09 mg CE/g dry plant, respectively, while leaf methanol extract, aqueous seed extract, and flower ethyl acetate extract exhibited the lowest phenolic compounds as 3.12±0.02, 17.61±0.20 and 11.43±0.13 mg CE/g dry plant, respectively. Harsha and Anilakumar[43] found the total flavonoid contents of *C. sativum* leaf ethanol extract as 44.5 □g CE/mg extract. However, in our study, the total flavonoid contents of *C. sativum* leaf ethanol extract were determined to be higher.

Table 1. Extraction yield, total phenolic, flavonoid, and anthocyanin contents of *C. sativum* leaf, seed, and flower extracts

Samples	Code	Yield, %	Total phenolic, mg GAE/g	Total flavonoid, mg CE/g	Total anthocyanin, µg cy-3-glu/g
***C. sativum* leaf (1 g)**	**CSL**	-	-	-	136.56±0.16
***C. sativum* leaf aqueous extract**	**CSLA**	23.90	87.77±0.43	32.46±1.10	-
***C. sativum* leaf methanol extract**	**CSLME**	1.80	5.46±0.07	3.12±0.02	-
***C. sativum* leaf ethanol extract**	**CSLET**	12.00	57.09±0.75	26.60±0.90	-
***C. sativum* leaf ethyl acetate extract**	**CSLEA**	3.40	26.35±0.84	15.25±0.38	-
***C. sativum* leaf hexane extract**	**CLHE**	10.70	250.40±17.84	22.78±0.81	-
***C. sativum* seed (1 g)**	**CSS**	-	-	-	14.07±0.21
***C. sativum* seed aqueous extract**	**CSSA**	8.76	30.07±0.16	17.61±0.20	-
***C. sativum* seed methanol extract**	**CSSME**	18.50	62.47±1.03	35.34±0.79	-
***C. sativum* seed ethanol extract**	**CSSET**	38.00	141.53±2.38	68.25±3.04	-
***C. sativum* seed ethyl acetate extract**	**CSSEA**	30.60	99.26±1.47	49.89±0.16	-
***C. sativum* seed hexane extract**	**CSSHE**	26.30	423.65±21.15	132.35±14.08	-
***C. sativum* flower (1 g)**	**CSF**	-	-	-	184.26±0.74
***C. sativum* flower aqueous extract**	**CSFA**	9.30	39.20±0.17	20.28±0.21	-
***C. sativum* flower methanol extract**	**CSFME**	19.50	93.18±0.93	34.79±0.87	-
***C. sativum* flower ethanol extract**	**CSFET**	11.80	42.72±0.93	21.64±1.78	-
***C. sativum* flower ethyl acetate extract**	**CSFEA**	5.30	25.80±0.10	11.43±0.13	-
***C. sativum* flower hexane extract**	**CSFHE**	5.40	238.82±10.46	68.66±9.09	-

In the work of Msaada and co-workers[44] reported that the total flavonoid contents of *C. sativum* fruits obtained from Tunisia, Syria, and Egypt were as 2.03±0.04, 2.51±0.08, and 2.07±0.05 mg CE/g, respectively. In this study, the total flavonoid content of *C. sativum* fruit methanol extract was determined to be higher. Sreelatha and Inbavalli[46] reported the total flavonoid contents were as 18.41±2.85 and 19.15±2.33 g QE/100 g plant, respectively, while, in our study, the total flavonoid contents of *C. sativum* seed and leaf ethanol extracts were determined to be higher. The total anthocyanin concentration of the plant was expressed as mg cyanidin 3-glucoside equivalent/mL. The anthocyanin compound amounts of *C. sativum* leaf, seed, and flower (1 g plant) were 136.56±0.16, 14.07±0.21, and 184.26±0.74 µg cyanidin-3-glucoside equivalent/g, respectively.

3.2. Antioxidant activity

Free radicals to include their orbitals unpaired electrons. These molecules or atoms generated consistently in the body contain ROS. Reactive radicals that carry unpaired electrons can quickly bond with biomolecules.[47] Free radicals are the reason for most of the diseases.[48] Many serious diseases such as cancer and cardiovascular have been deliberated to be the conclusion that this radical does harm lipids, nucleic acid, and proteins. The reduced ability of a compound can concern with antioxidant capacity. Those are the ability to transfer electrons into reactive radicals, thereby its reducing unreactive species and into more stable.[47] Some plants have the capacity to both balance and avoid series radical reactions owing to definite specific reducing substances in their structure.[49] Eight different antioxidant activity tests were applied to test the methanol, aqueous, ethyl acetate, hexane, and ethanol extracts of *C. sativum* leaf, seed, and flower parts at 500 µg/mL, and their results were exhibited in Table 2. The extracts of *C. sativum* seed, flower, and leaf exhibited relatively effective antioxidant activities. The results of activities were significant differences between control and extracts, statistically ($p < 0.05$). The total antioxidant activities of *C. sativum* of leaf hexane, seed aqueous, and flower aqueous extracts were observed the highest as 1.043, 0.557, and 0.272, respectively.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

Sreelatha and Inbavalli[46] determined the total antioxidant activities as 55.36±0.28% and 64.56±0.51% for the *C. sativum* seed and leaf ethanol extracts. However, in our study, total antioxidant activities for the *C. sativum* seed and leaf ethanol extracts were calculated to be lower. This is thought to be due to the phenolic and flavonoid content it contains. The reducing power capacity of *C. sativum* of leaf methanol, seed aqueous, and flower aqueous extracts was determined to be the highest as 0.564, 0.235, and 0.370. Harsha and Anilakumar,[43] IC_{50} values of reducing power capacities of *C. sativum* leaf ethanol extract was found as 251.80 µg/mL. In another study, Msaada and co-workers,[44] observed the EC_{50} values of reducing power activities as 122.01±13.25, 54.20±6.22, and 56.11±7.45 µg/mL of methanol extracts of *C. sativum* fruits obtained from Tunisia, Syria, and Egypt. Martins and co-workers[50] found the EC_{50} values of reducing power capacities of *C. sativum* seed hydro-methanolic extract as 2069.00±55.00 µg/mL. In our study, the reducing power activity results were expressed as absorbance. Therefore, it could not be compared with the data in the literature due to unit differences. The H_2O_2 scavenging activities of *C. sativum* of leaf ethyl acetate, seed hexane, and flower ethyl acetate extracts were observed to be the highest activities as 25.21, 23.57, and 21.05%. In the literature researches, no data on the content of H_2O_2 scavenging activities could occur. The $OH^{\cdot}$ radical scavenging activities of *C. sativum* of seed hexane, leaf hexane, and flower hexane were calculated as 52.60, 60.25, and 55.79%. %. The free radical scavenging activities of *C. sativum* of flower methanol, seed methanol, and leaf methanol extracts were the most effective as 98.86, 97.25, and 98.11%. Wangensteen and co-workers[17] determined that $DPPH^{\cdot}$ scavenging activities of *C. sativum* leaf and seed ethanol extracts were as 510.00±12.00 and 389.00±5.00 µg/mL. Demir and Korukluoglu[40] determined that the free radical scavenging activities of *C. sativum* seed methanol and ethanol extracts were as 2.20±0.20 and 5.60±0.20 mg/mL. Harsha and Anilakumar,[43] free radical scavenging activities of *C. sativum* leaf ethanol extract was found as 217.20 µg/mL. In another study, Msaada and co-workers[44] observed that free radical scavenging activities of methanol extracts of C. sativum fruits obtained from Tunisia, Syria, and Egypt were the IC50 values as 27.00±6.57, 36.00±3.22, and 32.00±2.87 µg/mL. In our study, the free radical scavenging activity results were expressed as %. Therefore, it could not be compared with the data in the literature due to unit differences. In a different study, Gallo and co-workers [45] observed the $DPPH^{\cdot}$ activities of ethanol-aqueous extracts obtained by using *C. sativum* seed, via ultrasound and microwave extraction, as 74.38 and 25.56%, however, in our study, free radical scavenging activities for the *C. sativum* seed ethanol extracts were calculated to be higher. Sreelatha and Inbavalli[46] determined the IC_{50} values of free radical scavenging activities as 20.36±0.63 µg/mL and 25.32±0.54 µg/mL for the *C. sativum* seed and leaf ethanol extracts. Mathew and Subramanian[51] found the free radical scavenging activity as 0.21±0.10% of 0.1 mg/mL concentration of *C. sativum* leaf methanol extract. Ahmed and co-workers[52] observed the free radical scavenging activities for the *C. sativum* seed methanol (maceration), methanol (soxhlet), chloroform, and petroleum ether extracts at 1 mg/mL concentration as 40.60, 28.36, 48.30, and 32.80% while in our study, free radical scavenging activities for the *C. sativum* seed methanol extracts were calculated to be higher. Conversely, free radical scavenging activities of *C. sativum* seed, flower, and leaf methanol (soxhlet) extracts were observed to be higher in our study. Martins and co-workers[50] found the free radical scavenging of *C. sativum* seed hydro methanolic extract as 1930.00±24.00 µg/mL.

In this work, the metal chelating activity of C. sativum leaf ethyl acetate, seed ethyl acetate, and flower ethyl acetate was observed as 39.84±0.30, 35.37±0.61, and 30.90±1.41%, respectively. Harsha and Anilakumar,[43] metal chelating activities of *C. sativum* leaf ethanol extract was found as 368.12 µg/mL. The superoxide anion scavenging activities of *C. sativum* of leaf aqueous, seed aqueous, and flower aqueous extracts were exhibited the highest as 75.40, 66.01, and 60.95%, respectively. The lipid peroxidation inhibition capacities of C. sativum of leaf ethyl acetate, seed ethyl acetate, and flower ethyl acetate were found effective with high values of 93.88, 85.58, and 87.89%, respectively. Harsha and Anilakumar,[43] IC_{50} values of lipid peroxidation inhibition activities of *C. sativum* leaf ethanol extract was found as 518.60 µg/mL.

3.3. Urease, AChE and BChE inhibition activities

Three different enzyme inhibition activity tests were applied to the methanol, aqueous, ethanol, hexane, and ethyl acetate extracts of *C. sativum* leaf, seed, and flower parts. Their results were exhibited in Table 3.

Today's, inhibition of significant enzymes of diseases relevant to community health such as Alzheimer's enter into vital significance.[53] AChE is concerned with the growth of cells and aids the maturing of neurons and regeneration of nerves.[54] This enzyme inhibition induces the continuous and excessive acetylcholine (ACh) collection in nerve synapses. AChE, first of all, pervades in the nervous tissue, quickly intervenes in the hydrolysis of the neurotransmitter ACh, and reasons the cancellation of the conduction of nerve impulse, thereby providing a normal physical function of the body. AChE is a vital member of the nervous system. Thus, adverse effects on AChE activity can induce neurotoxicity.[55] BChE is connected in many physical factors, the most distinct the hydrolysis of not only noncholine but also choline esters. Consequently, it has a significant role in neurotransmission and anesthesia.[56] A vital rise in the acetylcholinesterase activity is spied in Alzheimer's disease early phase.

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

The activity of BChE progressively advances in Alzheimer's late phases. Therefore, both AChE and BChE are considerable medicinal goals to the improvement of the cholinergic explicit and idea the AD.[57] In the AChE inhibition activity test, it was determined the IC_{50} values of *C. sativum* of leaf ethanol, seed ethanol, and flower ethanol as 145.07±8.09, 112.83±10.75, and 187.38±5.77 µg/mL extracts had the highest activity. These extracts have higher AChE inhibitory activity than galantamine (IC_{50}, 418.20±9.55 µg/mL). It shows that ethanol extracts of leaves, seeds, and flowers can be used as drugs for this enzyme.

Mathew and Subramanian[51] determined the AChE inhibition activity of *C. sativum* leaf methanol extract was 36.25±5.30% at 0.1 mg/mL. In our study, the AChE inhibition activity results were expressed as IC_{50} value. In the BChE inhibition activity test, it was determined the IC_{50} values of *C. sativum* of leaf ethanol, seed ethanol, and flower aqueous as 553.45±11.55, 334.28±23.09, and 430.67±14.06 µg/mL extracts had more effective than that of alanthamine (IC50, 409.52±15.84 µg/mL).

Table 2. Antioxidant activities of *C. sativum* leaf, seed, and flower extracts at 500 µg/mL.

Samples/ Standards	Total antioxidant	Reducing power	H_2O_2 scavenging	$OH^{\cdot}$ radical scavenging	Free radical scavenging	Metal chelating	Superoxide anion scavenging	Lipid peroxidation
	Abs., 695 nm	Abs., 700 nm	Activity, %					
CSLA	0.443±0.000^{o}	0.301±0.000^{f}	13.04±1.57^{a}	44.85±0.41^{ij}	95.32±0.28^{g}	35.40±0.33^{g}	75.40±0.58^{l}	63.79±0.24^{c}
CSLME	0.372±0.001^{m}	0.564±0.006^{h}	18.64±1.21^{cd}	36.31±0.93^{d}	98.11±0.22^{hi}	28.94±0.46^{d}	54.88±0.06^{h}	58.27±2.09^{b}
CSLET	0.303±0.001^{l}	0.201±0.000^{d}	18.96±2.35^{cd}	51.46±0.39^{kl}	95.34±0.14^{g}	18.63±0.37^{a}	62.88±0.07^{jk}	64.24±0.42^{c}
CSLEA	0.103±0.001^{d}	0.144±0.000^{ab}	25.21±1.49^{f}	22.31±0.58^{ab}	80.11±0.35^{b}	39.84±0.30^{h}	38.32±0.67^{def}	93.88±0.14^{h}
CLHE	1.043±0.000^{t}	0.169±0.000^{c}	20.72±1.84^{de}	60.25±0.03^{n}	92.57±0.56^{f}	25.81±0.24^{b}	40.27±2.05^{f}	83.87±0.22^{f}
CSSA	0.557±0.000^{r}	0.235±0.002^{e}	16.55±1.05^{abc}	43.53±0.27^{hi}	88.97±0.34^{d}	27.46±0.72^{c}	66.01±0.03^{k}	61.56±1.67^{c}
CSSME	0.245±0.001^{i}	0.226±0.000^{e}	17.10±0.47^{bcd}	41.60±0.10^{fghi}	97.25±0.25^{h}	31.04±0.33^{ef}	58.02±0.20^{hi}	54.50±1.34^{d}
CSSET	0.096±0.001^{c}	0.200±0.000^{d}	14.14±1.88^{ab}	50.76±0.36^{kl}	84.71±0.29^{c}	31.52±0.07^{f}	35.25±0.05^{d}	76.20±0.13^{de}
CSSEA	0.065±0.001^{a}	0.136±0.000^{a}	19.18±2.01^{cd}	37.87±0.29^{def}	89.51±0.10^{e}	35.37±0.61^{g}	37.10±1.05^{def}	85.58±0.37^{fg}
CSSHE	0.080±0.000^{b}	0.127±0.000^{a}	23.57±1.33^{ef}	52.60±0.68^{lm}	86.59±0.52^{d}	34.74±0.33^{g}	35.44±0.06^{d}	75.69±1.51^{de}
CSFA	0.272±0.000^{k}	0.370±0.000^{g}	17.76±1.53^{bcd}	43.04±0.07^{ghi}	85.24±0.93^{cd}	25.93±0.52^{b}	60.95±0.07^{ij}	54.82±0.29^{a}
CSFME	0.260±0.000^{j}	0.313±0.000^{f}	19.40±1.71^{cd}	40.50±0.19^{efgh}	98.86±0.07^{i}	30.03±0.59^{de}	38.71±0.14^{ef}	74.24±0.43^{d}
CSFET	0.233±0.001^{h}	0.200±0.000^{d}	17.87±1.04^{bcd}	48.18±0.33^{jk}	94.07±0.97^{g}	29.75±0.19^{de}	43.56±1.05^{g}	75.02±1.12^{de}
CSFEA	0.221±0.001^{g}	0.200±0.001^{d}	21.04±2.16^{de}	21.02±0.49^{a}	95.39±0.27^{g}	30.90±1.41^{ef}	31.33±3.88^{c}	87.89±0.24^{g}
CSFHE	0.167±0.000^{f}	0.145±0.029^{c}	21.05±1.51^{de}	55.79±0.51^{m}	91.26±0.28^{f}	25.72±0.85^{b}	35.88±0.30^{de}	83.61±2.79^{f}
BHA	0.449±0.001^{p}	0.131±0.000^{a}	17.65±0.22^{bcd}	37.49±0.15^{de}	77.37±0.40^{a}	NT	25.19±0.18^{a}	63.51±0.03^{c}
BHT	0.633±0.001^{s}	0.131±0.000^{a}	19.52±0.10^{cd}	39.17±5.21^{defg}	77.46±0.29^{a}	NT	22.35±0.45^{b}	76.95±0.33^{de}
Trolox	0.392±0.007^{n}	0.147±0.000^{ab}	17.44±0.77^{bcd}	42.55±2.73^{ghi}	78.24±0.44^{a}	NT	25.55±1.28^{a}	74.32±0.37^{d}
TBHQ	0.372±0.001^{m}	0.161±0.000^{bc}	18.86±0.10^{cd}	24.41±0.27^{ab}	78.19±0.47^{a}	NT	21.14±0.19^{a}	77.62±0.43^{e}
α-tocopherol	0.393±0.003^{n}	0.156±0.000^{bc}	20.39±0.19^{cde}	25.43±0.34^{b}	77.68±0.53^{a}	NT	19.49±0.62^{i}	77.31±0.25^{de}
Ascorbic acid	0.144±0.001^{e}	0.194±0.000^{d}	18.53±0.10^{cd}	31.41±0.04^{c}	91.40±0.82^{f}	NT	59.04±0.07^{i}	83.44±1.05^{f}
EDTA	NT	NT	NT	NT	NT	85.61±0.49^{i}	NT	NT

NT: not tested, Variance analysis ($p<0.05$)

Urease is an enzyme to provide the hydrolysis of urea to form carbon dioxide and ammonia. The most significant part is to preserve the bacteria in the acidic ambiance of the stomach.[58] These enzyme inhibitors can enter into a vital to oppose impact the negative effect of urease in living organisms. These inhibitors are efficient against a few acute infections induced by the secretion of urease by *Helicobacter pylori* that contain gastric tract

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

syndromes and urinary tract infections.[59] It is now commonly accepted that duodenal ulcers and gastric are usually caused by *H. pylori* that grow and survive in an acidic medium.[60,61] This organism releases urease that changes urea into ammonia, and the excretion of ammonia defends it from the acidic medium of the stomach.[62] World Health Organization have classified *H. pylori* as a first-class carcinogen.[63] Several enzyme inhibitors were artificially developed for pharmacology. However, they could command some side effects like liver damage and gastrointestinal disturbances. Therefore, it increased significant interest in exploring safe and new inhibitors from natural sources.[64-66]

The urease inhibition activities of the leaf methanol, seed methanol, and flower methanol extracts were exhibited the highest activity as 68.75±0.97, 80.30±0.20, and 69.64±0.68%, respectively. These extracts have higher urease inhibition activity than thiourea (38.76±6.13%).

Table 3. Enzyme inhibition activities of leaf, seed, and flower extracts of C. sativum.

Samples/ Standards	AChE	BChE	Urease
	IC_{50}, µg/mL		
CSLA	247.36±11.55^{d}	NA	35.66±0.02^{b}
CSLME	NA	743.07±20.82^{e}	68.75±0.97^{e}
CSLET	145.07±8.09^{b}	553.45±11.55^{c}	33.65±0.02^{b}
CSLEA	NA	NA	41.84±0.02^{c}
CLHE	NA	NA	35.39±0.05^{b}
CSSA	440.91±0.00^{f}	367.63±23.09^{a}	32.62±0.11^{b}
CSSME	NA	NA	80.30±0.20^{f}
CSSET	112.83±10.75^{a}	334.28±23.09^{a}	33.81±0.19^{b}
CSSEA	NA	NA	32.31±0.09^{b}
CSSHE	648.21±28.17^{h}	NA	16.14±0.05^{a}
CSFA	533.22±102.63^{g}	430.67±14.06^{b}	34.31±0.05^{b}
CSFME	767.44±80.83^{i}	573.14±46.19^{e}	69.64±0.68^{e}
CSFET	187.38±5.77^{c}	940.47±28.28^{f}	33.79±0.17^{b}
CSFEA	NA	NA	56.75±0.05^{d}
CSFHE	634.45±40.62^{h}	640.83±24.38^{d}	15.19±0.26^{a}
Galantamine	418.20±9.55^{e}	409.52±15.84^{b}	NT
Thiourea	NT	NT	38.76±6.13^{c}

NA: not activity, NT: not tested, Variance analysis ($p<0.05$)

3.4.DNA protection activity

The leaf, seed, and flower methanol, aqueous, ethanol, hexane, and ethyl acetate extracts of *C. sativum* were analyzed in an anaerobic medium to observe the transformations in forms I, II, and III of plasmid DNA, to determine DNA protection activity (Figures 1 and 2). Two forms were observed in the gel image and these forms; form I, which is the super-coiled circular DNA, where plasmid DNA walks fast, and form II, which is the truncated DNA form, where it migrates slowly. Also, controls, extracts, and quercetin were observed to have higher DNA protection activity in form I than in form II. It was noted that when UV and H_2O_2 were applied together (Lane 2), it would cause DNA damage in form II. This damage can be reduced in the presence of the quercetin (1000 µg/mL) (Lane 18).[67] Further, the addition of *C. sativum* extracts to the H_2O_2 reaction mixture provided significant protection from damage to super-coiled circular DNA (Lane 3-17). While the form I DNA protection activity of the standard and the extracts was observed over 70% of the leaf hexane and flower ethyl acetate extracts, also, it was determined that the form II DNA protection activity was above 60% of the flower acetone and flower hexane extracts. In their study, Harsha and Anilakumar[43] determined that *C. sativum* leaf ethanol extract has DNA protection activity. In another study, Divya and co-worker[68] observed that carotenoids isolated from *C. sativum* fractions had DNA protection activity. In our study, the DNA protection activity results of *C. sativum* extract obtained from different polar and nonpolar solvents were comparable to that of quercetin and expressed as %.

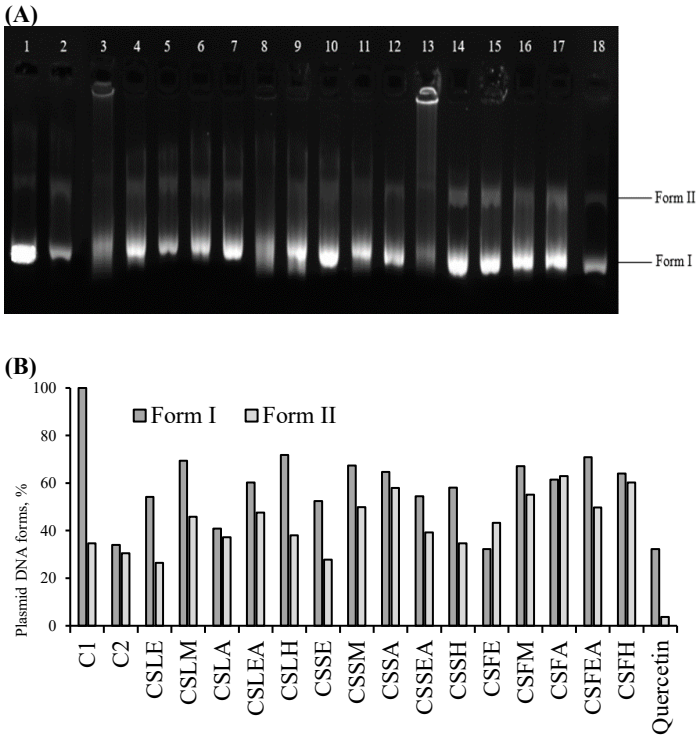

Figure 1. The effect of DNA protection activity of crude *C. sativum* leaf, seed and flower extracts.

(A) Agarose gel electrophoresis; Lane 1: Negative control (C1); Lane 2: Positive control (C2); Lane 3: CSLE; Lane 4: CSLM; Lane 5: CSLA; Lane 6: CSLEA; Lane 7: CSLH; Lane 8: CSSE; Lane 9: CSSM; Lane 10: CSSA; Lane 11: CSSEA; Lane 12: CSSH; Lane 13: CSFE; Lane 14: CSFM; Lane 15: CSFA; Lane 16: CSFEA; Lane 17: CSFH; Lane 18: Quercetin

(B) The % intensity of Form I and Form II in agarose gel electrophoresis image

4. CONCLUSIONS

Five different extracts (aqueous, methanol, ethanol, ethyl acetate, and hexane) of *C. sativum* (leaf, flower, and seed parts) were obtained by using the soxhlet method and the boiling method. The chemical content analysis (total phenol, flavonoid, and anthocyanins), antioxidant activities (total antioxidant, reducing power, metal chelating, lipid peroxidation inhibition, free radical, H_2O_2, $OH^{\cdot}$, and superoxide anion scavenging), enzyme

DOI: http://dx.doi.org/10.32571/ijct.1024979 E-ISSN: 2602-277X

inhibition (AChE, BChE, and urease) and DNA protective activities were applied to *C. sativum* extracts. Although there were studies on the different activities of the *C. sativum* leaf and seed extracts in the literature, no study has been found on *C. sativum* flower extracts. According to their chemical contents and antioxidant activities, results showed that hexane, ethyl acetate, and aqueous extracts have higher chemical content and activity. In enzyme inhibition tests, ethanol extracts had high activity in AChE and BChE inhibition activities, while methanol extracts had high activity in urease inhibition activity. In addition, ethanol extracts are shown to have higher activity than galantamine in AChE inhibition activity. Due to its effects on different forms of plasmid DNA (Form I and Form II), the DNA protection potentials of extracts of *C. sativum* have been demonstrated. Further, by looking at all results, leaf extracts have higher activities than other extracts in general. Flower extracts have high activity in some activity tests but low activity. The different effective properties of this plant in medicine and food can also be examined by applying other methods and techniques, as a medicine or as a food supplement.

ACKNOWLEDGEMENTS

OMÜ-BAP supported this work with the projects numbered PYO.FEN.1904.13.003 and PYO.FEN.1904.13.006.

Conflict of interests

The authors declared no conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.1085417 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Investigation of compatibility of lipase with commercial detergents, surfactants and oxidizing agent as a detergent additive

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Received: 9 March 2022; Revised: 12 April 2022; Accepted: 21 April 2022

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Citation: Gulmez, C.; Atakisi, O.; *Int. J. Chem. Technol.* **2022**, 6 (1), 33-38.

ABSTRACT

Herein, it is aimed to perform some commercial liquid and solid laundry detergents, surfactants, oxidizing agent and pH/temperature studies of lipase from porcine pancreas. For this, optimum pH and temperatures of lipase were investigated in the range of pH 5-13 and temperature 30-80°C. The enzyme stability and compatibility were evaluated in the presence of 8 commercial laundry detergents, triton X-100, tween 20, tween 80, tergitol, sodium deoxycolate, sodium dodecyl sulfate and oxidizing agent H_2O_2 for 1 h at 40°C. Optimum pH and temperatures of enzymes was recorded pH 10 and 40°C, respectively. The findings revealed that lipase was generally stable in detergents and increased by more than 20% in liquid detergent 1 and 3 compared to the control. In addition, enzyme in triton X-100, tween 20, tween 80, and tergitol at 5%(v/v) concentration exhibited about 237%, 281%, 207% and 237% relative activities and activity at 5% H202(v/v) was inhibited by only about 13%. Consequently, pancreatic lipase can be a good choice in enzyme immobilization studies and various biotechnological purposes, especially in detergent applications.

Keywords: Lipase, laundry detergent, surfactant, bleaching agent, detergent industry.

Deterjan katkı maddesi olarak lipazın ticari deterjanlar, yüzey aktif maddeler ve oksitleyici ajan ile uyumluluğunun araştırılması

ÖZ

Bu çalışmada domuz pankreasından elde edilen lipazın ticari sıvı ve katı deterjanlar, yüzey aktif maddeler, oksitleyici ajan ve pH/sıcaklık çalışmalarının gerçekleştirilmesi amaçlanmıştır. Bu amaçla enzimin optimum pH ve sıcaklık çalışmaları sırasıyla pH 5-13 ve 30-80°C sıcaklık aralıklarında araştırılmıştır. Enzimin 8 adet ticari çamaşır deterjanı, triton X-100, tween 20, tween 80, tergitol, sodyum deoksikolat, sodyum dodesil sülfat ve oksitleyici ajan H_2O_2 varlığında 1 saat ve 40°C'de stabilite ve uyumluluğu değerlendirildi. Elde edilen bulgulara göre lipaz deterjanlar içerisinde genel olarak stabildir ve kontrole göre sıvı deterjan 1 ve 3 içerisinde aktivitesini %20'den fazla artırmıştır. Aynı zamanda enzim %5 (v/v) konsantrasyonda triton X-100, tween 20, tween 80, and tergitol varlığında sırasıyla yaklaşık %237, %281, %207 and %237 bağıl aktivite göstermiştir ve aktivite %5 (v/v) H_2O_2 konsantrayonunda sadece yaklaşık %13 inhibe olmuştur. Sonuç olarak; pankreatik lipaz, enzim immobilizasyon çalışmaları ve özellikle deterjan uygulamaları başta olmak üzere çeşitli biyoteknolojik amaçlar için iyi bir seçenek olabilir.

Anahtar Kelimeler: Lipaz, çamaşır deterjanı, yüzey aktif maddeler, ağartıcılar, deterjan endüstrisi.

1. INTRODUCTION

Lipases (E.C.3.1.1.3) are triacylglycerol ester hydrolases that interact with carboxyl ester bonds in triacylglycerols to form fatty acids and glycerol. In addition to their hydrolytic activities on triglycerides, lipases are of the industrial biocatalysts and are involved in catalyzing an array of chemical reactions such as esterification, transesterification, peptide synthesis, and site-selective, chemo-selective stereo-selective transformations.[1,2] Due

DOI: http://dx.doi.org/10.32571/ijct.1085417 E-ISSN: 2602-277X

to their broad substrate specificity and ability to catalyze multiple reactions both in water soluble/insoluble systems, lipases are widely used for various industrial applications. For instance, the relevant enzymes are used in detergent, food, paper, cosmetics, pharmaceutical, leather, waste and various biotechnological processes at industry.[3,4]

Not only lipases but also enzymes such as protease, amylase, and cellulase are used to enhance the efficiency of the detergents.[5] In particular, the main commercial use of hydrolytic lipases includes detergent processing and accounts for one-third of total lipase sales. The relevant enzymes are used in laundry and dishwashing detergents, in which the enzymes provide significant functions in removal of fatty residues.[6] In this regard, recombinant DNA technology and protein engineering in the production of enzymes for various industrial purposes have been great interests of the researchers. So far, enzymes are produced from a single microbial source using recombinant DNA technology in commercial detergents. Since they enable yielding high product, genetic modification opportunity and large amount of protein as well as they are cost-effective, recombinant DNA techniques are widely preferred.[7-9] Commercial detergents use the lipases produced by recombinant method Lipolase from *Humicola lanuginosa*, Lumafast from *Pseudomonas mendocina* and Lipomax of *Pseudomonas glumae*.[10] On the other hand, protein engineering, improves the functions of the enzyme with some modifications in the molecular structure of the enzyme. In this regard, the studies linked to the provide the desired chemical structure that can be applied to different industrial areas are of the recent interests among researchers.[11] Especially, the enzyme immobilization is of the notable purposes. The catalytic activity, reusability, and resistance to various environmental conditions are increased as a result of the physical or chemical binding and capture of the enzyme to a water-insoluble carrier with enzyme immobilization.[12,13] As previously reported, significant advances in the properties of enzymes have been made by using a wide variety of immobilization techniques, recombinant DNA technique and protein engineering. Consequently, their potential for industrial adaptation has been increased.[14] In enzyme immobilization, enzyme selection affects the choice of immobilization strategy. If the enzyme shows low activity initially and has low tolerance to various chemical agents, then an immobilization method that does not affect the enzyme activity is chosen.[15]

The chosen immobilization technique does not always improve the properties of the enzyme available, sometimes it only ensures its reusability.[15] In the case of an enzyme to be used for the detergent industry, it is initially required to construct an immobilization technique with an enzyme whose compatibility is known in detergents. A good detergent enzyme should generally maintain its stability against high temperature-alkaline environment, metal ions, organic solvents, surfactants, inhibitors and other chemical agents.[5,8,9,14] Therefore, as an initial step of the researches, these characteristics of the enzyme should be well- documented in detergent researches. In this study, the characteristics of the free enzyme in detergent chemicals and its suitability for immobilization studies will be evaluated. Based on this, activity of lipase from porcine pancreas were assessed in the presence of some commercial liquid and solid laundry detergents, surfactants and oxidizing agent. Herewith the current report, the compatibility of pancreatic lipase in detergents and its potential for biotechnological applications were investigated.

2. MATERIALS AND METHODS

2.1. Chemicals

The chemicals used for the experiments were procured from Sigma Chemical Co. (St. Louis, MO, USA). The relevant chemicals were as follows: Lipase from porcine pancreas, bovine serum albumin (BSA), p -nitrophenyl palmitate (p-NPP), p-nitrophenol, triton X-100, tween 20, tween 80, tergitol, sodium deoxycolate (DOC), sodium dodecyl sulfate (SDS), HCl, NaOH, acetate, tris, glycine, ethyl alcohol, and acetonitrile. Bradford reagent was purchased Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA, USA). For all experimental stages, double-distilled water (18.2 MΩ; Millipore Co., USA) was used.

2.2. Lipase activity assay

The lipase activity was performed in a spectrophotometrically assay using p-NPP as the substrate. The p-NPP at 10 mM concentration was prepared by mixing ethyl alcohol and 50 mM glycine-NaOH buffer solutions (pH 10) in acetonitrile at a ratio of 1:4:95 (v/v/v). 50 µL of enzyme solution was added to 950 µL of this mixture. The samples were left for incubation at 37°C for 20 min and then, the absorbances regarding lipase activity were recorded at 405 nm. One unit (U ml^{-1}) of alkaline lipase corresponds to the required-amount of enzyme to the release of 1 mol p-nitrophenol per minute under optimum conditions.[16] The protein concentration of enzyme was quantified according to the Bradford method and BSA concentrations (2-12 µg/mL) were used to achieve for standard calibration curve for estimation of protein concentration at 595 nm.[17]

2.3. Determination of optimal pH and temperature for lipase

The lipase activity was assayed for 20 minutes over a range of pH 5-13 at 37°C. Relative activity was measured as described in the lipase activity assays. The buffer solutions (50 mM) of sodium acetate, Tris-HCl, and glycine-NaOH were used for pH 5-6, 7-9, and 10-13, respectively. The effect of temperature on the activity of

DOI: http://dx.doi.org/10.32571/ijct.1085417 E-ISSN: 2602-277X

lipase was evaluated at the temperatures ranging from 30 to 80°C for 20 min. ultimately, the relative activity of lipase was quantified at pH 5-13 at 37°C and the activity of the non-heated enzyme was expressed as 100%.[18]

2.4. Stability and compatibility of lipase with commercial detergents

The lipase stability in presence of commercialized liquid and solid detergents were examined. In this regard, some commercial liquid and solid laundry detergents were used. Of the commercial laundry detergents corresponding to the 1-4 liquid and 5-8 solid detergents, respectively. Firstly, the detergents were diluted to a final-concentrations (7 mg/mL; solid detergents; 10% (v/v); liquid detergents). Then the lipase-added detergents were left for incubation at 40°C for 1 h. Ultimately, the relative activity of lipase was quantified under optimum conditions. The activity of lipase without detergent addition was expressed as 100%.

2.5. Effect of some surfactants and oxidizing agent hydrogen peroxide on lipase activity

The effect of some non-ionic surfactants (5% (v/v) triton X-100, tween 20, tween 80, and tergitol) and ionic surfactants (5% (w/v) sodium dodecyl sulfate (SDS) and 5% (v/v) sodium deoxycolate (DOC) and also the effect of oxidizing agent (5% (v/v) H_2O_2) on lipase activity were assessed after incubation at 40°C for 1 h. Finally, the relative activities of lipase were determined under optimum conditions. The activity of lipase without and any addition was expressed as 100%.

2.6. Statistical analysis

The data were means of three replicates corresponding to the two technical replicates (duplicate measurement per each replicate). The means of the experimental groups were compared using one-way variance analysis (SPSS 16.0).

3. RESULTS AND DISCUSSION

3.1. Determination of optimum pH and temperature of lipase

To determine the optimal catalytic conditions of lipase, the effect of pH and temperature on activities of lipase were evaluated (Figure 1A-B). A range of 5 to 13 of pH was used to reveal the effect of pH on lipase. As shown in the Figure 1A; the optimal pH value of lipase was found to be 10. The enzyme retained more than about 80% of its activity in the pH range of 7-12. Especially, it retained about 95% of its activity, especially at pH 9 and 11. Also, it exhibited 67% and 71% activities in the acidic medium pH 5 and 6, respectively, whilst it retained about 59% of its activity in the highly alkaline medium pH 13.

The optimum temperature of lipase was found to be 40 °C (Figure 1B). The enzyme exhibited very high activity at the temperature range of 30-60°C. It exhibited 93% activity at 50 and 60°C. At higher temperatures, it retained more than 60% of its activity. After the optimum conditions were determined, the activity and specific activities of the lipase were determined as 13.64 U/mL and 10.91 U/mg protein, respectively. However, in the previous reports, lipases have been produced from different sources with a wide range of different pH/temperature activity and stability.[19,20]

The potential uses of the enzymes to be applied for industrial processes are likely dependent on the adaptation of the enzymes to the difficult and harsh reaction conditions.[4,6] For this reason, enzymes are subjected to biotechnological processes such as enzyme immobilization, recombinant DNA technology and nanotechnology in order to increase the adaptation and tolerance of enzymes to industrial applications.[7,11] It can be emphasized once again that lipase might be a good source for biotechnological purposes with the high temperature and acid/alkaline environment stability exhibited by the enzyme.

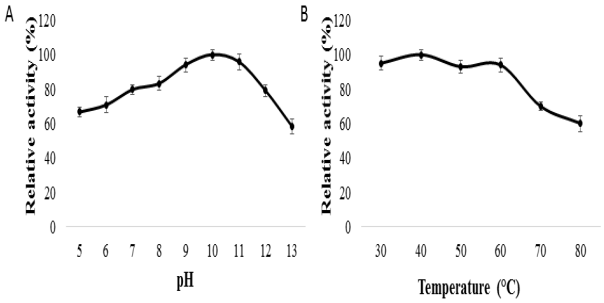

Figure 1. Effects of pH (A) and temperature (B) on the activities of lipase. The activities of the non-heated lipase were taken as 100%.

3.2. Detergent stability and compatibility

To examine suitability and compatibility of the lipase in detergents, lipase was pre-incubated for 1 hours at 40°C with various solid and liquid commercial laundry detergents. The findings of the current revealed that lipase was generally stable (Figure 2). The lipase activity increased significantly in liquid detergent 1, 3 and 4, in comparison to the control ($p<0.001$), while it retained 75% of its initial activity in detergent 2. The enzyme showed more than 120% activity in detergents 1 and 3. The activity in solid detergent 5, 6, 7 and 8 was about 64%, 88%, 89% and 68% respectively.

While biological detergents use enzymes that break down stains, non-biological detergents deal with tough stains with higher temperatures or longer wash times. Therefore, biological detergents are less harmful to the environment and are more economical. In recent years, lipases have been preferred in industrial applications due

DOI: http://dx.doi.org/10.32571/ijct.1085417 E-ISSN: 2602-277X

to their biodegradable, non-toxic, large amount of production and substrate diversity.[21] They are used in detergents to remove oil-based dirt and work by forming a fabric-lipase complex that creates a barrier on clothing. An ideal detergent enzyme is expected to be able to adapt to high washing temperatures and alkaline environment conditions. [5,14] A wide variety of alkaline and thermotolerant lipases have been studied in previous researches and tested in different industrial areas. The lipase from *Geobacillus stearothermophilus* FMR12 had optimum activity at 70 °C and pH 9 and it was stable until 90 °C and pH 11 and in commercial detergents.[9] The lipase from *Aeromonas caviae* LipT51 lipase activity was increased 2.5 times in the presence of laundry detergent and it retained all of initial activity in dish washing and handwashing detergent.[22] The purified lipase from *Bacillus licheniformis* strain NCU CS-5 retained more than 50% of its activity in the presence of commercial detergents at 5 or 25 °C after 3 h incubation.[23] According to findings of the current study, lipase from porcine pancreas is suitable for detergent applications thanks to its high activity and stability in commercial solid and liquid washing detergents. In order to improve the effectiveness of lipase-containing detergents and to understand the deactivation mechanism of lipase under washing conditions, it is necessary to investigate the important kinetic and thermal, hydrodynamic and detergent/surfactant characteristics of the lipase at the oil-water interface.[24]

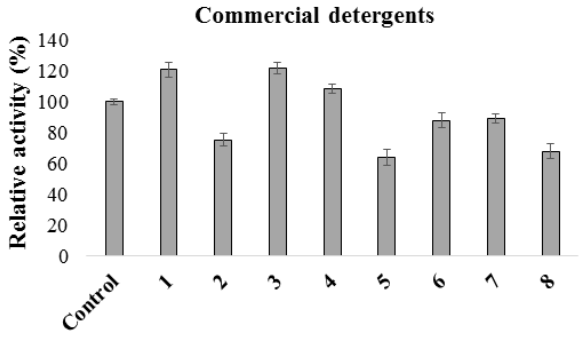

Figure 2. The effect of commercialized liquid and solid detergents on the enzymatic activity. The detergents were used final-concentrations 7 mg/mL for solid detergents and 10% (v/v) for liquid detergents. The activity of lipase without detergent addition was expressed as 100%.

3.3. Effect of some surfactants and oxidizing agent hydrogen peroxide on lipase activity

For determination of the compatibility of enzymes with detergents, their competitiveness and tolerance with detergent chemicals like surfactants, blenching and oxidizing agents are investigated. These chemicals can destroy lipase activity by altering the tertiary structure of lipase. To evaluate the suitability of the lipase in detergent, lipase was tested in presence of some non-ionic surfactants, ionic surfactants and oxidizing agent H_2O_2 by incubating at 40°C for 1 h. As in Figure 3, the enzyme activity increased more than 2-folds in the presence of non-ionic surfactants compared to the control ($p<0.001$). The lipase in triton X-100, tween 20, tween 80, and tergitol at 5% (v/v) concentration displayed about 237%, 281%, 207% and 237% relative activities, respectively. Various lipases produced from different sources exhibited varying characteristics in surfactants. Some reports are available to prove the positive effect of surfactants on the lipase. The lipase from *Haloferax mediterranei* CNCMM 50101 in 10 % (v/v) triton X-100, tween 20, 60 and 80 retained their initial activities 40 °C for 1 h.[20] In another study, lipase isolated from *Bacillus* strain showed about 84%, 103%, 98% and 1% relative activities in 1% (v/v) Triton X-100, Tween 20 and 80, and SDS, respectively.[25] Lutensol XP80 and Triton X-100 strongly was activated the lipase from *Pseudomonas aeruginosa* for 1 h (up to 40 and 30% against the control, respectively).[26] In contrast, lipase from *Staphylococcus* sp. strain CJ3 was strongly inhibited by Triton X-100.[27] In a previous study, it was reported that hydrogen bonds easily form between non-ionic surfactants and protein molecules, increasing conformational flexibility in the active site.[28] In this study, lipase lost about 32% and 25% of its initial activities in the presence of the ionic surfactants DOC and SDS, respectively. Lipase from *Geobacillus stearothermophilus* FMR12 exhibited about 94% and 93% relative activity in the presence of 5% DOC and SDS, while it had %92 and %79 activity at 10% surfactant concentration, respectively.[9] According to the mechanism proposed by Bajpai and Tyagi [29], the negative charges of SDS react with the positive charges of some metal ions such as calcium and magnesium in the washing water and deactivate them. It has been suggested that these ionic surfactants denature the enzyme and reduce the activity by interacting with the forces that preserve the three-dimensional structure of the enzyme.[30] The other mechanism is that ionic interactions between ionic surfactants and enzyme play a role in the inactivation of globular proteins.[31]

Another parameter for detergent applications is the tolerance of enzymes against oxidizing agents. The lipases were generally inhibited in the presence of oxidizing agent H_2O_2 and other bleaching agents at high concentration.[14,32] For instance, while the lipase from *Geobacillus stearothermophilus* showed 65% activity in 5% H_2O_2 (v/v), it lost more than 75% of its activity in the presence of 10% H_2O_2 (v/v).[9] In this study, it was noted that lipase activity at 5% H_2O_2 (v/v) was inhibited by only about 13% after 1 h at 40°C. According to the current findings, it is clear that the tolerance level of the lipase from porcine pancreas against ionic and non-ionic surfactants and oxidizing agent H_2O_2, which is in a

DOI: http://dx.doi.org/10.32571/ijct.1085417 E-ISSN: 2602-277X

concentration equivalent to the washing conditions, is high.

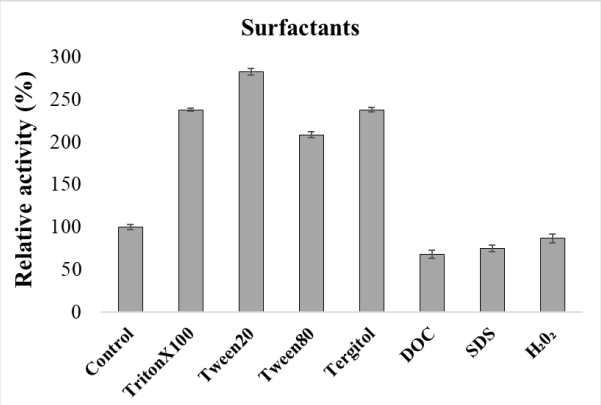

Figure 3. The effect of non-ionic and ionic surfactants on the enzymatic activity. The activity of lipase without any addition was expressed as 100%.

4. Conclusion

As the demand for enzymes with improved characteristics that are resistant to difficult reaction environments in the detergent industry increases, biotechnological researches on detergent enzymes are increasing. In particular, the researchers focus on the properties of enzyme such as kinetics, pH/temperature, and behavior against various chemical agents in washing water. Herewith the findings, it was concluded that lipase exhibited significant stability and compatibility in the presence of various detergents, surfactants and oxidizing agents H_2O_2. Especially for the detergent industry, pancreatic lipase can be a good choice in enzyme immobilization studies and various biotechnological purposes.

Conflict of interest

I declare that there is no a conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Determination and comparison of element analysis in the species belonging to different families by inductively coupled plasma-mass spectrometry (ICP-MS)

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Received: 14 March 2022; Revised: 17 June 2022; Accepted: 17 June 2022

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Citation: Umaz, A.; Aydin, F.; Firat, M.; Ertas, A. *Int. J. Chem. Technol.* **2022**, 6 (1), 39-47.

ABSTRACT

The consumption of plants and their use for medicinal purposes are increasing day by day. Therefore, knowing the plant contents is important for human health. In this study, multi-element contents of nineteen species belonging to four different families were determined. The multi-element contents of different species of the same genus belonging to these families were compared. Also, the element contents of different parts of the same genus were compared. The element contents of different species of the same genus and different parts of the same species have been determined that be different from each other. In addition, the Cd and Pb content results of the species were compared with the maximum allowable concentration values in raw plant material of World Health Organization's (WHO). Cd content in all species was found to be higher than the value determined by the WHO. Only the Pb content of the *Sternbergia clusiana* Boiss. (A3) was found to be higher than the value detected by WHO.

Keywords: Plants, macro, micro and toxic elements, ICP-MS

Farklı familyalara ait türlerdeki element analizlerinin indüktif eşleşmiş plazma kütle spektrometrisi (ICP-MS) ile belirlenmesi ve karşılaştırılması

ÖZ

Bitkilerin tüketimi ve tıbbi amaçlı kullanımları her geçen gün artmaktadır. Bu nedenle bitki içeriklerinin bilinmesi insan sağlığı açısından önemlidir. Bu çalışmada dört farklı familyaya ait on dokuz türün çoklu element içerikleri belirlenmiştir. Bu familyalara ait aynı cinsin farklı türlerinin çoklu element içerikleri karşılaştırılmıştır. Ayrıca, aynı cinsin farklı kısımlarının element içerikleri karşılaştırılmıştır. Aynı cinsin farklı türlerinin ve aynı türün farklı kısımlarının element içerikleri birbirinden farklı olduğu tespit edilmiştir. Ayrıca, türlerin Cd ve Pb içerik sonuçları Dünya Sağlık Örgütü'nün (WHO) ham bitki materyalinde izin verilen maksimum konsantrasyon değerleri ile karşılaştırılmıştır. Tüm türlerde Cd içeriği WHO tarafından belirlenen değerden yüksek bulunmuştur. Yalnızca, *Sternbergia clusiana* Boiss'in Pb içeriği. (A3) WHO tarafından tespit edilen değerden daha yüksek olduğu tespit edildi.

Anahtar Kelimeler: Bitkiler, makro, mikro ve toksik elementler, ICP-MS

1. INTRODUCTION

The plants are estimated that there are about one million in the world. About five hundred thousand of these plants have been described and named. Twenty thousand of them were determined to be medicinal plants used for therapeutic purposes as a result of the research conducted by the WHO.[1] There are 10754 plant species in the flora of Türkiye, 3708 of which are endemic.[2] The Amaryllidaceae family is a perennial, flowering structure containing 73 genera and at least 1600 species and is particularly distributed in tropical and subtropical regions

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

of the world. Members of the family; have underground roots, lanceolate leaves, usually three or six bowl-shaped flowers and fruits.[3] Plants belonging to the Amaryllidaceae family are used in folk medicine all over the world, for ornamental purposes in public gardens and parks due to their beautiful flowers. Species of this family have a wide variety of biological effects, including antitumor, antiviral, antibacterial, antifungal, antimalarial, analgesic, and cytotoxic activities.[4] In addition, some species in this family have been used in folk medicine to treat tumors.[5]

Caryophyllaceae is a large family represented worldwide by about 86 genera and more than 2200 species. This family consists of annual or perennial herbaceous plants with swollen nodes, root structure, oblong rims, and diagonally opposite leaves. This family is distributed in the temperate and warm temperate zone of the Northern hemisphere. The distribution center is mainly the Mediterranean region, which is part of Europe and Asia. The Caryophyllaceae family is represented by 32 genera and 500 species in the flora of Türkiye.[6]

Iridaceae is one of the families of flowering plants. This family is known for ornamental genera such as Iris, Gladiolus, and Crocus. The family is a mostly perennial plant family that includes about 80 genera and 1700 species. There are also several shrubs and evergreen herbaceous species of this family. Although the family is distributed almost worldwide, it is most abundant and diverse in Africa. Most species grow in temperate, subtropical, and tropical regions.[7]

Malvaceae, known as hollyhock or mallow, is a family that includes about 243 genera and at least 4225 species of plants, shrubs, and trees. Species of this family are spread all over the world except for the coldest regions of the world, but the spread is greatest in the tropics. Economically important species of this family; Cotton, Cocoa, Linden, Durian, Hibiscus, and Okra.[8]

The World Health Organization (WHO) states that the maximum allowable concentrations of cadmium, arsenic, and lead in raw plant materials are 0.3, 1.0, and 10 mg kg^{-1}, respectively. The determination of macro, micro and toxic element concentrations in plant samples are used various methods such as Atomic Absorption Spectrometry (AAS), Flame Atomic Absorption Spectrometry (FAAS), Graphite Furnace Atomic Absorption Spectrometry (GF-AAS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and X-ray Fluorescence Spectrometry (XRF).[9] In this study; Macro, micro, and toxic element contents of nineteen species belonging to four different families were determined by ICP-MS and the species belonging to these families were compared within themselves. The obtained multi-element content results were compared with the maximum acceptable concentration values in raw plant materials of WHO. In addition, the multi-element contents of the root and aerial parts of some species were determined and the plant parts were compared with each other.

2. MATERIALS AND METHODS

2.1. Plant materials

Species belonging to different families were collected and identified by Mehmet Fırat. The current names of the plants were written by controlling the IPNI (International Plant Name Index). The species were dried with suitable drying methods and stored in VANF (Van Yüzüncü Yıl Faculty of Science Herbayum)

2.2. Reagent and solutions

Analytical purity HNO_3 (70%), H_2O_2 (34.5-36.5%) (Sigma Aldrich, Germany), and ultrapure water (18.2 MΩ) were used in the microwave resolution process. The accuracy and precision of the method were evaluated using the certified reference material NIST 1515 apple leaf (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA).

The mix standard ((100 mg l^{-1}, VHG Labs PN.: VHG ZLGC1813) (Ag, Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn)) was used in ICP-MS measurements. Calibration standard solutions (10-4000 µg l^{-1}) were prepared by appropriate dilution of stock mix standards (1 mg l^{-1}). Limit of detection (LOD) and limit of quantity (LOQ) values for twenty elements were calculated using 10 independent blank solutions.

2.3. Instrument

Macro, micro and toxic element contents in the species were determined using Agilent 7700s model ICP-MS device (Table 2)

2.4. Preparation of plant samples for multi-element analysis

In order to carry out multi-element analysis, the samples dried in the shade were homogenized and ground in a mortar. The samples were then weighed about 0.1 g and placed in microwave teflon tubes. 6 ml of HNO_3 and 2 ml of H_2O_2 were added to the tubes in a 6:2 ratio. In the microwave device, the samples were first heated at 500 W energy for 15 min heated up to 300 °C. Then the samples were kept at 1500 W energy for 15 min at 300 °C throughout.

Finally, samples have gradually reduced the temperature from 300 °C to 90 °C in 500 W energy for 10 min and

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

during this time solubilization was carried out in the microwave closed system. After microwave solubilization, the mixture in teflon tubes was filtered with blue banded filter paper. The obtained filtrate was diluted up to 100ml with ultrapure water by taking 100ml bolon. Then, the diluted samples were put into screw-closed analysis tubes and made ready for analysis.[9,10]

Table 1. Information about of studied the species (plant species, species codes, collection location and collection time)

Plant species	Species Codes	Gathering Places	Harvesting Times	Herbarium Number
Amaryllidaceae familyası				
Ixiolirion tataricum (Pall.) Herb. & Traub	A1	Nevşehir	2014	M. FIRAT 32330 (VANF)
Narcissus tazetta L. subsp. *tazetta.*	A2	Van	2015	M. FIRAT 32469 (VANF)
Sternbergia clusiana Boiss.	A3	Van	2015	M. FIRAT 32625 (VANF)
Sternbergia vernalis (Mill.) Gorer & J.H.Harvey	A4	Van	2015	M. FIRAT 32446(VANF)
Caryophyllaceae familyası				
Gypsophyla nodiflora (Boiss.) Barkoudah	C1	Adıyaman	2015	M. FIRAT 32652 (VANF)
Silene compacta Fisch. ex Hornem.	C2	Bitlis	2015	M. FIRAT 32655 (VANF)
Silene conoidea L.	C3	Erzincan	2017	M. FIRAT 33733 (VANF)
Silene subconica Friv.	C4	Kars	2014	M. FIRAT 31347(VANF)
Vaccaria hispanica (Mill.) Rauschert	C5	Adıyaman	2015	M. FIRAT 32651 (VANF)
Iridaceae familyası				
Gladiolus italicus Mill.	I1	Hakkari	2015	M. FIRAT 32466 (VANF)
Gladiolus kotschyana Boiss.	I2	Van	2014	M. FIRAT 32468(VANF)
Iris pseudocaucasica Grossh.	I3	Van	2014	M. FIRAT 32332(VANF)
Iris sari Schott ex Baker	I4	Adıyaman	2014	M. FIRAT 32420(VANF)
Malvaceae familyası				
Alcea apterocarpa (Fenzl) Boiss.	M1	Van	2014	M. FIRAT 31523(VANF)
Alcea biennis Winterl	M2	Hakkari	2015	M. FIRAT 32527(VANF)
Alcea rosea L.	M3	Diyarbakır	2015	M. FIRAT 32531(VANF)
Alcea setosa (Boiss.) Alef.	M4	Van	2014	M. FIRAT 31327(VANF)
Malva neglecta Wallr.	M5	Van	2015	M. FIRAT 32457(VANF)
Malvella sherardiana (L.) Jaub. & Spach	M6	Diyarbakır	2014	M. FIRAT 30674 (VANF)

Table 2. ICP-MS instrument analytical contitions

Parameters	
RF Power	1550 W
Plasma gas	Argon
Plasma gas flow rate	15 l min^{-1}
Helium gas flow rate	4.3 ml min^{-1}
Nebulizer peristaltic pump intake speed	0.3 rps
Nebulizer peristaltic pump intake time	50 sec
Nebulizer peristaltic pump stabilization time	45 sec
Repeat/example reading	3
Number of scan repetitions	100
Measured element isotopes	^{27}Al, ^{52}Cr, ^{59}Co, ^{63}Cu, ^{7}Li, ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{55}Mn, ^{56}Fe, ^{58}Ni, ^{64}Zn, ^{80}Se, ^{9}Be, ^{112}Cd, ^{5}B, ^{22}Ti, ^{138}Ba ve ^{208}Pb

3. RESULTS AND DISCUSSION

3.1. Calibration studies

The linear calibration chart for elements was generated in the range of 10-4000 µg l^{-1} given in Table 3. Correlation coefficients (r^2) were found to vary between 0.9990 and 0.9999. The LOD and LOQ values were calculated from 3s m^{-1} and 10s m^{-1}. S was the standard deviation of the blank and m was the slope of calibration plot. LOD and LOQ values are given in Table 3.

3.2. Recovery studies for elements

In this study, % recovery values of elements were obtained by using NIST 1515 apple leaf reference material. It was determined that the recovery values ranged between 91-110% (Table 4).

3.3. Determination of multi-element content in species

3.3.1. Multi-element analysis results of species belonging to Amaryllidaceae family

Na, K, Mg, Fe, Al, Ti, Mo, Li, Cu, Cr, Ni, Zn, Se, and Ba contents of the sample of the aerial part of the A1 species

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

were found to be higher than the root. The element contents of the aerial part of this species were determined as 3069, 38162, 2906, 430, 625, 68.89, 4.46, 1.23, 7.59, 2.15, 5.13, 71.23, 49.00 and 83.82 mg kg^{-1}, respectively. In addition, the contents of B, Mn, Co, Cd, and Pb of the root part of this species were determined that were higher than the aerial. The element contents of the root part of this species were determined as 223, 34.70, 0.86, 2.98 and 3.34 mg kg^{-1}, respectively (Table 5).

Table 3. Analytical parameters of the ICP-MS analysis method

Elements	Liner Range (µg l^{-1})	Calibration Equation	r^2	LOD (µg l^{-1})	LOQ (µg l^{-1})
Na	500-4000	y = 5142.84 x + 357838	0.9997	48.500	161.680
K	500-4000	y = 2308.80 x + 315707	0.9997	17.030	56.780
Mg	100-4000	y = 2226.27 x + 12319	0.9994	4.240	14.130
Fe	1000-4000	y = 34007.92 x + 701914	0.9990	5.120	17.080
Al	100-4000	y = 1121.76 x + 1922	0.9995	2.730	9.080
Se	100-4000	y = 829.63 x + 75232	0.9991	0.061	0.202
Li	50-4000	y = 189.10 x + 213	0.9997	0.591	1.970
Ba	10-4000	y = 198949.48 x + 11780	0.9998	0.088	0.290
Be	10-4000	y = 22829.54 x + 709	0.9999	0.730	2.430
Cu	10-4000	y = 31809.14 x + 16409	0.9994	0.330	1.100
Co	10-4000	y = 55754.88 x + 1543	0.9997	0.150	0.490
Cd	10-4000	y = 42086.25 x + 885	0.9996	0.400	1.330
Cr	10-4000	y = 29985.59 x + 4895	0.9994	0.210	0.690
Mn	10-4000	y = 18139.19 x + 5333	0.9995	0.280	0.920
Ni	10-4000	y = 28319.45 x + 6335	0.9995	1.080	3.580
Zn	10-4000	y = 9123.21 x + 7916	0.9994	0.580	1.920
Pb	10-4000	y = 126261.36 x + 6134	0.9996	0.110	0.360
B	10-4000	y = 5768.54 x + 33073	0.9994	0.011	0.035
Mo	10-4000	y = 4155.44x + 196	0.9998	0.078	0.259
Ti	10-4000	y = 260.23 x + 36	0.9994	0.058	0.193

Table 4. Standard reference material (SRM) values and recovery % (mean concentration ± standard deviation, n=3)

Elements	Certificate Value (mg kg^{-1})	Measurement Value (mg kg^{-1})	Recovery %
Na	24.40±2.10	22.15±0.29	91
Mg	2710±120	2467±4	91
Al	285±6	314±2	110
K	16080±210	14478±91	90
Mn	54.10±1.10	50.85±0.19	94
Fe	82.70±2.60	80.77±0.27	98
Ni	0.936±0.094	0.95±0.01	101
Cu	5.69±0.13	5.86±0.10	103
Zn	12.45±0.43	13.7±0.46	110
Ba	48.80±2.30	48.20±0.30	99
Pb	0.470±0.024	0.490±0.002	104
Mo	0.095±0.011	0.089±0.007	94
B	27.60±2.80	28.20±1.57	102

Table 5. Multi-element results of species belonging to Amaryllidaceae family (n = 3)

Elements	A1 (Root)	A1 (Aerial part)	A2	A3	A4
Na	2994±8	3069±19	4015±118	2910±11	2393±19
K	3480±26	38162±227	27639±983	11867±94	14137±18
Mg	1818±15	2906±11	3288±90	10831±42	2280±3
Fe	285±3	430±4	2698±105	16916±42	780±12
Al	368±2	625±2	5716±220	23983±151	971±21
B	223±4	222±3	262±1	243±3	194±3
Ti	52.00±0.57	68.89±0.72	124±1	933±8	72.66±0.86
Mo	2.80±0.01	4.46±0.11	0.65±0.03	2.04±0.12	1.19±0.09
Li	0.68±0.10	1.23±0.31	3.50±0.69	17.43±1.29	1.95±0.24
Cu	3.56±0.07	7.59±0.01	12.04±0.38	17.60±0.18	9.70±0.02
Be	0.01±0.00	0.01±0.00	0.12±0.00	0.91±0.01	0.02±0.00
Cr	1.89±0.02	2.15±0.00	13.05±0.43	77.53±0.74	3.05±0.04
Mn	34.70±0.30	25.49±0.06	148±5	498±5	35.63±0.23
Ni	3.15±0.01	5.13±0.05	21.74±0.73	104±1	8.22±0.07
Co	0.86±0.02	0.67±0.04	1.84±0.07	7.69±0.07	0.71±0.03
Zn	38.73±0.35	71.23±0.49	62.43±1.64	109±1	48.81±0.37
Se	48.01±0.29	49.00±0.47	50.36±0.54	49.64±0.51	50.95±0.23
Cd	2.98±0.02	2.52±0.02	0.95±0.04	1.94±0.01	1.89±0.02
Ba	9.68±0.02	83.82±0.11	25.07±0.37	68.37±0.37	9.60±0.11
Pb	3.34±0.03	1.77±0.01	1.14±0.02	13.76±0.02	1.53±0.01

Measured value = mean concentration ± SD. (mg kg^{-1}). **A1:** *Ixiolirion tataricum*, **A2:** *Narcissus tazetta* subsp. *tazetta*, **A3:** *Sternbergia clusiana*, **A4:** *Sternbergia vernalis*

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

When the element contents of different species of the same genus are evaluated; it was determined that Na, Mg, Fe, Al, B, Ti, Mo, Li, Cu, Be, Cr, Mn, Ni, Co, Zn, Cd, Ba and Pb contents of A3 species were higher than A4. K and Se contents of A4 species were found to be higher than that of A3 (Table 5). The different element content of samples belonging to different species of the same genus can be said that the species changes depending on genetic factors, geographical location, climatic factors, vegetation period, air pollution, and environmental factors.

When the Cd and Pb contents in species belonging to the Amaryllidaceae family were compared with the maximum allowable concentration amount in raw plant materials of WHO; Cd content of all species belonging to this family was found to be higher than WHO values. In addition, the Pb content of only the A3 sample from this family was found to be higher than WHO values.

3.3.2. Multi-element analysis results of species belonging to Caryophyllaceae family

K, Mg, Fe, Al, B, Ti, Mo, Li, Cu, Be, Cr, Mn, Ni, Co, Zn, Se, Ba, and Pb contents of samples belonging to the aerial part of the C1 species were detected to be higher than the root. The element contents of the aerial part of this species were determined as 18808, 14443, 3556, 4143, 199, 147, 3.21, 2.06, 23.41, 0.04, 17.19, 205, 34.50, 10.55, 43.41, 39.62, 16.96 and 3.37 mg kg^{-1}, respectively. In addition, the element contents of Na and Cd of the root part of this species were determined that were higher than the aerial part and element contents were determined as 2488 and 3.50 mg kg^{-1}, respectively (Table 6).

In the study of determination of the macro and micronutrient content in the leaf samples of some shrub plants; element contents of *Gypsophila sphaerocephala* plant were determined by AAS device. Macro elements in the leaf part of the species were determined as N; 1.52%, P; 0.14%, K; 0.96%, Ca; 3.71% and Mg; 0.47%. Concentrations of micro elements were determined as Fe; 367.23, Zn; 11.40, Mn; 42.10 and Cu; 2.82 mg kg^{-1}.[11] Na, K, Mg, Fe, Al, B, Ti, Mo, Li, Cu, Be, Cr, Mn, Ni, Co, Zn, and Pb contents in samples belonging to the aerial part of the C4 species were found to be higher than the root. The element contents of the aerial part of this species were determined as 2329, 45449, 8469, 1805, 2743, 205, 120, 14.62, 2.45, 9.07, 0.12, 6.99, 105, 15.81, 1.34, 50.90 and 1.11 mg kg^{-1}, respectively. The contents of Ba and Cd of the root part of species were determined that were higher than the aerial part and the element contents were determined as 146 and 1.69 mg kg^{-1}. In addition, Se content could not be detected in both parts of the species (Table 6). The contents of B, Mo, Mn, Zn, Se, and Cd of the aerial part of the C5 species were determined that were higher than the root. The element contents of the aerial part of this species were determined as 224, 8.30, 69.66, 57.88, 52.84, and 4.68 mg kg^{-1}, respectively. Na, K, Mg, Fe, Al, Ti, Li, Cu, Be, Cr, Ni, Co, Ba, and Pb contents of the root part of the species were found to be higher than the aerial. The element contents of the root part of this species were determined as 2485, 55420, 3575, 2399, 3816, 199, 2.99, 6.59, 0.08, 9.33, 15.89, 1.33, 51.19 and 0.66 mg kg^{-1}, respectively. In addition, Se content could not be detected in both parts of the species (Table 6).

In the study of the determination of element concentrations in plant ash samples of *Silene compacta* species; the element concentrations of the vegetable ash sample were determined by the ICP-AES device. It was determined as Fe; 0.9, Ag; 2.0, As; 39.4, Cd; 8.0, Co; 10.4, Cu; 568, Mo; 10.3, Ni; 17.3, Pb; 79.1, Zn; 607 and V; 11.3 mg kg^{-1}.[12]

In the study of the determination of trace elements in soil-plant systems of copper mine areas in the area where the Murgul Copper mine is from the Black Sea Region of Türkiye; macro and micro element concentrations of *S. compacta* plant were determined as N; 1.24%, P; 0.24%, K; 2.60%, Ca; 1.00%, Mg; 0.48%, Fe; 9320, Cd; 0.5, Zn; 221.0, Pb; 46.0, Cu; 125.5, Co; 5.5, Ni; 16.5 and Al; 8985 mg kg^{-1}.[13]

In the study of heavy metal uptake in some plant species in Metalliferous regions in Northern Greece; heavy metal concentrations in the structure of *S. conica* plant in the Vouves area were determined by AAS device. The heavy metal concentrations of the plant were determined as Zn: 860, Pb: 450, Cd: 5, Ni: 20, Fe: 146, Mn: 35, Ca: 20850, Mg: 2550, N: 11250 and Na: 435 µg g^{-1}.[14]

In the study of the determination of the element concentrations of three different plant species in the Sari Gunay gold layer of Northwest Iran; element concentrations of 3 different plant species were determined by the ICP-MS device. Element concentrations of *S. conoidea* plant were determined as Au; 260.05, Ace; 57.1, C5; 3.7, Ag; 0.9, Mo; 1.16, Sb; 64.77, Hg; 0.10, Cu;9.7, Pb;50.6 and T1; 2.73 mg kg^{-1}.[15]

When the element contents of different species of the same genus are evaluated; it was determined that the Na, Fe, B, Li, Be, Cr, Co and Pb contents of the C2 species were higher than the other species. K, Mg, Al, Ti, Mo, Cu, Mn, Ni and Zn contents of the aerial part of the C4 species were found to be higher than the other species. In addition, the Cd and Ba contents of the root part of the species were found to be higher than the other species (Table 6). When the Cd and Pb contents in species belonging to the Caryophyllaceae family are compared with the maximum allowable concentration amount in raw plant materials of WHO; it was determined that the Cd content of all species belonging to this family was higher than the WHO values, and the Pb content was much lower than the WHO values.

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

Table 6. Multi-element results of species belonging to the Caryophyllaceae family (n = 3)

Elements	C1 (Root)	C1 (Aerial part)	C2	C3	C4 (Root)	C4 (Aerial part)	C5 (Root)	C5 (Aerial part)
Na	2488±24	2401±21	3127±28	2218±9	2176±31	2329±27	2485±21	2246±29
K	9580±116	18808±91	27373±183	21871±197	25917±47	45449±152	55420±387	29925±69
Mg	5237±5	14443±71	5018±13	3924±28	2597±20	8469±50	3575±39	3477±19
Fe	1280±5	3556±24	2266±10	914±7	1130±4	1805±10	2399±8	384±3
Al	2181±11	4143±6	2482±9	1365±6	1871±27	2743±7	3816±25	473±3
B	194±2	199±2	443±9	172±7	159±1	205±6	155±8	224±4
Ti	68.51±0.21	147±1	94.53±1.46	66.53±0.99	74.69±0.22	120±1	199±1	42.49±0.17
Mo	1.56±0.10	3.21±0.08	0.85±0.09	5.54±0.09	4.26±0.07	14.62±0.11	1.90±0.11	8.30±0.09
Li	1.30±0.38	2.06±0.57	2.49±0.66	1.01±0.24	1.06±0.23	2.45±0.35	2.99±0.30	1.01±0.08
Cu	6.80±0.02	23.41±0.08	5.90±0.06	6.56±0.04	5.81±0.03	9.07±0.07	6.59±0.08	4.94±0.03
Be	0.01±0.00	0.04±0.00	0.16±0.01	0.09±0.00	0.08±0.00	0.12±0.01	0.08±0.00	0.01±0.00
Cr	9.76±0.02	17.19±0.09	8.36±0.02	3.83±0.01	4.64±0.03	6.99±0.04	9.33±0.06	2.53±0.01
Mn	46.41±0.46	205±1	47.21±0.24	40.42±0.15	27.61±0.12	105±1	49.72±0.28	69.66±0.54
Ni	13.47±0.01	34.50±0.09	12.09±0.03	8.37±0.02	8.67±0.01	15.81±0.05	15.89±0.09	4.04±0.02
Co	1.81±0.03	10.55±0.06	1.36±0.00	0.74±0.03	0.80±0.01	1.34±0.02	1.33±0.01	0.34±0.01
Zn	19.83±0.25	43.41±0.13	37.09±0.12	35.33±0.13	26.99±0.24	50.90±0.46	36.17±0.56	57.88±0.07
Se	36.55±0.36	39.62±0.39	N.D.	N.D.	N.D.	N.D.	49.75±0.2	52.84±0.13
Cd	3.50±0.01	3.07±0.01	2.93±0.02	3.57±0.02	3.96±0.24	1.69±0.01	2.61±0.02	4.68±0.01
Ba	7.15±0.04	16.96±0.28	10.47±0.07	148±1	197±11	146±1	51.19±0.48	11.97±0.09
Pb	0.68±0.01	3.37±0.02	1.54±0.01	0.64±0.00	0.58±0.04	1.11±0.01	0.66±0.01	0.43±0.00

N.D.: No Detected, **Measured value** = mean concentration ± SD. (mg kg^{-1}). **C1:** *Gypsophyla nodiflora*, **C2:** *Silene compacta*, **C3:** *Silene conoidea*, **C4:** *Silene subconica*, **C5:** ***Vaccaria hispanica***

3.3.3. Multi-element analysis results of species belonging to Iridaceae family

When the element contents of different species of the same genus are evaluated; it was determined that Na, Mg, Fe, Al, B, Ti, Li, Cu, Be, Cr, Mn, Ni, Co, Zn, Cd and Pb contents of I1 species were higher than I2. It was determined that K, Mo, Se, and Ba contents of I2 species were higher than I1. Na, Mg, Fe, Al, Ti, Be, Cr, Mn, Ni, Co, Zn, Cd, Ba, and Pb contents of I3 species were determined that be higher than I4. K, B, Li, Cu, Mo, and Se contents of the I4 species were found to be higher than the I3 (Table 7). In the study of the accumulation and distribution of heavy metals in aquatic plants; concentrations of heavy metals accumulated in the above-ground and subsoil parts of *Iris pseudocaucasica* were determined by ICP-MS device. Heavy metal concentrations accumulated in the above-ground part of the plant were determined as Cr; 8.34, Fe; 8.01, Cu 0.43, and Zn; 4.43 mg g^{-1}. Heavy metal concentrations accumulated in the subsoil part of the plant were determined as Cr; 39.98, Fe; 134.10, Cu 1.43 and Zn; 10.45 mg g^{-1}.[16]

When the Cd and Pb contents in the species belonging to the Iridaceae family are compared with the WHO's maximum allowable concentration in raw plant materials; it was determined that the Cd content of all species belonging to this family was higher than the WHO values, and the Pb content was much lower than the WHO values.

Table 7. Multi-element results of species belonging to the Iridaceae family (n = 3)

Elements	I1	I2	I3	I4
Na	2571±20	2471±4	2695±19	2417±8
K	36054±212	37561±250	10320±42	45227±292
Mg	3869±15	2709±19	3029±21	2815±25
Fe	1053±10	140±2	1143±11	307±1
Al	1410±6	203±1	1653±7	496±2
B	236±2	216±7	236±3	243±6
Ti	126±1	34.70±0.77	138±1	33.19±0.43
Mo	1.70±0.01	1.82±0.07	0.69±0.03	2.46±0.04
Li	1.72±0.33	0.73±0.10	1.32±0.26	1.65±0.51
Cu	9.31±0.08	5.14±0.06	4.37±0.03	5.88±0.03
Be	0.03±0.00	0.01±0.00	0.04±0.00	0.01±0.00
Cr	5.21±0.01	1.31±0.01	13.69±0.04	1.88±0.00
Mn	76.15±0.56	34.86±0.06	35.68±0.19	14.23±0.11
Ni	9.25±0.01	1.95±0.02	8.04±0.05	3.32±0.03
Co	1.44±0.02	0.50±0.01	0.87±0.03	0.37±0.03
Zn	63.98±0.51	52.67±0.65	22.62±0.25	22.03±0.03
Se	42.44±0.2	43.84±0.57	42.78±0.52	42.88±0.66
Cd	3.55±0.03	1.38±0.02	3.41±0.03	1.59±0.01
Ba	13.97±0.13	30.14±0.17	61.26±0.7	35.05±0.12
Pb	0.87±0.02	0.63±0.00	1.12±0.01	1.03±0.01

Measured value = mean concentration ± SD. (mg kg^{-1}).
I1: *Gladiolus italicus*, **I2:** *Gladiolus kotschyana*, **I3:** *Iris pseudocaucasica*, **I4:** *Iris sari*

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

3.3.4. Multi-element analysis results of species belonging to Malvaceae family

Fe, Al, Ti, Li, Cr, Mn, Ni, Co, Cd, and Pb contents of the root part of M4 species were determined to be higher than the aerial. The element contents of the root part of this species were determined as 660, 1050, 47.59, 1.10, 1.66, 61.20, 4.46, 0.63, 4.08 and 0.91 mg kg^{-1}, respectively. Na, K, Mg, B, Mo, Cu, Zn, and Ba contents of the aerial part of M4 species were found to be higher than the root. The element contents of the aerial part of this species were determined as 2129, 25042, 3979, 286, 2.81, 7.42, 37.18, and 71.35 mg kg^{-1}, respectively. In addition, Be and Se contents could not be detected in the samples belonging to both parts of this species (Table 8). In the study to determine the heavy metal content of *Alcea rosea* species growing on the roadside soils of a Turkish Lake Basin; the heavy metal content of the species belonging to different locations was determined by AAS device. Metal concentrations of Fe, Mn, Cu, Zn, Cd, Cr, Ni, and Pb were found to be 300.62, 42.94, 17.20, 21.73, 11.26, 3.25, 2.63, and 2.59 mg kg^{-1}, respectively.[17]

In another study of the determination of metal concentration of this species; the metal analysis of the species was determined by the ICP-OES device and Al, As, B, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Se concentrations were detected as 30, 0.34, 9.50, 0.08, 0.15, 6.10, 15, 5.40, 0.68, 0.22 and 0.09 mg kg^{-1}, respectively.[18]

The Na, K, Li and Se contents of the aerial part of M5 species were determined that be higher than the root. The element contents of the aerial part of this species were determined as 4688, 60744, 5.26, and 5219 mg kg^{-1}, respectively. In addition, the contents of Mg, Fe, Al, B, Ti, Mo, Cu, Be, Cr, Mn, Ni, Co, Zn, Cd, Ba, and Pb of the root part of the species were determined to be higher than the aerial. The element contents of the root part of this species were determined as 10800, 4779, 7332, 250, 335, 3.16, 11.51, 0.18, 17.36, 182, 30.00, 2.62, 53.21, 2.90, 58.04 and 1.43 mg kg^{-1}, respectively (Table 8). In the study carried out to determine the mineral composition of *Malva neglecta*, which is consumed as a vegetable in the Central Black Sea Region of Türkiye; the concentrations of the macro and micro element content of the species were determined with the AAS device. Element concentrations of the species were determined as K; 2828.6, Mg; 408.5, Fe; 19.2, Zn; 8.1, Mn; 3.0, Cu; 8.6, P; 610.8; Na; 610.2 and Ca; 1519.9 µg g^{-1}.[19] Mg, Fe, Al, B, Ti, Mo, Li, Cu, Be, Cr, Mn, Ni, Co, Zn, Se, Cd, Ba, and Pb contents of the aerial part of M6 species were detected that be higher than the root. The element contents of the aerial part of this species were determined as 7412, 6294, 5456, 274, 765, 10.78, 3.41, 20.23, 0.12, 18.32, 218, 53.11, 5.09, 62.33, 51.06, 1.94, 44.66 and 1.58 mg kg^{-1}, respectively. In addition, it was determined that the Na and K contents of the sample of the root part of this species were higher than the aerial. The element contents of the root part of this species were determined as 2295 and 28375 mg kg^{-1}, respectively (Table 8).

Table 8. Multi-element results of species belonging to the Malvaceae family (n = 3)

Elements	M1	M2	M3	M4 (Root)	M4 (Aerial part)	M5 (Root)	M5 (Aerial part)	M6 (Root)	M6 (Aerial part)
Na	2106±26	3705±30	2264±14	2113±10	2129±9	2353±7	4688±65	2295±19	2243±5
K	33256±165	23178±122	33877±30	21110±300	25042±241	49974±465	60744±362	28375±181	24215±169
Mg	4081±14	4352±42	5439±21	1817±10	3979±22	10800±70	3303±5	4908±19	7412±51
Fe	163±1	519±2	318±4	660±5	294±1	4779±12	1464±12	893±6	6294±12
Al	173±1	407±2	331±1	1050±11	380±1	7332±30	2428±25	1434±13	5456±43
B	282±11	330±10	329±8	181±7	286±5	250±3	229±3	225±5	274±4
Ti	10.20±0.21	14.45±0.50	25.82±0.35	47.59±1.30	25.75±0.38	335±2	90.15±0.33	79.83±0.98	765±10
Mo	1.74±0.10	4.10±0.08	4.43±0.06	0.75±0.05	2.81±0.12	3.16±0.11	0.88±0.04	1.05±0.08	10.78±0.05
Li	0.02±0.27	1.28±0.36	0.24±0.32	1.10±0.13	0.21±0.14	4.59±0.12	5.26±0.47	1.56±0.30	3.41±0.45
Cu	13.81±0.06	53.95±0.81	9.80±0.10	7.08±0.05	7.42±0.04	11.51±0.08	6.72±0.03	7.96±0.03	20.23±0.06
Be	N.D.	N.D.	N.D.	N.D.	N.D.	0.18±0.01	0.06±0.00	0.02±0.00	0.12±0.00
Cr	1.05±0.01	9.72±0.04	1.59±0.01	1.66±0.01	1.49±0.01	17.36±0.07	6.02±0.02	4.11±0.01	18.32±0.07
Mn	24.43±0.07	36.37±0.24	35.72±0.13	61.20±0.50	40.33±0.06	182±1	32.10±0.27	42.93±0.36	218±0.57
Ni	3.16±0.01	8.06±0.04	3.95±0.02	4.46±0.01	3.18±0.02	30.00±0.09	11.87±0.06	7.82±0.02	53.11±0.06
Co	0.36±0.01	1.08±0.02	0.32±0.01	0.63±0.01	0.42±0.01	2.62±0.02	1.02±0.01	0.66±0.01	5.09±0.02
Zn	47.41±0.18	107±1	35.33±0.23	20.26±0.13	37.18±0.18	53.21±0.52	24.88±0.51	62.09±0.31	62.33±0.21
Se	N.D.	N.D.	N.D.	N.D.	N.D.	50.90±0.39	52.19±0.45	50.77±0.62	51.06±0.40
Cd	1.67±0.01	3.02±0.02	1.47±0.01	4.08±0.01	2.87±0.02	2.90±0.02	0.39±0.00	0.72±0.01	1.94±0.01
Ba	88.08±0.56	64.28±0.25	179±1	9.77±0.03	71.35±0.61	58.04±0.65	37.06±0.10	43.85±0.22	44.66±0.41
Pb	0.78±0.01	3.39±0.04	0.98±0.01	0.91±0.01	0.83±0.01	1.43±0.02	0.75±0.02	0.69±0.00	1.58±0.01

N.D.: No Detected, **Measured value** = mean concentration ± SD. (mg kg^{-1}).
M1: ***Alcea apterocarpa*****, M2:** ***Alcea biennis*****, M3:** ***Alcea rosea*****, M4:** ***Alcea setosa*****, M5:** ***Malva neglecta*****, M6:** ***Malvella sherardiana***

In the study conducted to determine the mineral composition of the *Malvella sherardiana* plant, which is consumed as a vegetable in the Central Black Sea Region of Türkiye; the macro and micro element content of the species was determined by AAS device. Element contents of the species were determined as K; 3660.8, Mg; 416.1, Fe; 22.4, Zn; 10.1, Mn; 5.3, Cu; 9.7, P; 701.7; Na; 687.7 and Ca; 1730.0 µg g^{-1}.[19] When the element contents of different species of the same genus are evaluated; it was determined that the Na, B, Li, Cu, Cr, Ni, Co, Zn, and Pb contents of M2 species were higher than the other species. K, Mg, Mo and Ba contents of M3 species were determined that be higher than other species. Fe, Al, Ti, Mn, and Cd contents of the root part

DOI: http://dx.doi.org/10.32571/ijct.1087834 E-ISSN: 2602-277X

of M4 species were determined to be higher than other species. In addition, Be and Se contents could not be detected in samples of all species (Table 8).When the Cd and Pb contents in the species belonging to the Malvaceae family are compared with the WHO's maximum allowable concentration in raw plant materials; it was determined that the Cd content of all species belonging to this family was higher than the WHO values, and the Pb content was much lower than the WHO values.

4. CONCLUSIONS

Although the mineral elements in the structure of plants have positive effects on human health, toxic elements pose a danger. Therefore, it is very important to determine the toxic and mineral element content in plants. In this study, nineteen different species used for purposes as edible, ornamental, and medicinal plants were investigated in terms of multi-element content. The results obtained were compared among different species of the same genus and samples belonging to different parts of the same species. The aerial parts of A1, C1, C4 and M6 samples were determined that had more multi-element content than the root. Many element contents of the root parts of the C5, M4, and M6 samples were determined to be higher than the aerial part. The element contents of different species of the same genus were determined that were different from each other and some element contents were higher than the other species. Thus, it has been determined that the multi-element content of the samples belonging to different species of the same genus will vary depending on geographical location, climatic factors, air pollution, and environmental factors. The K and Na contents of M5 species can be said that be higher than the other species, and the mineral elements of this species are more beneficial to human health. The element contents of A3 (Fe, Al, Zn), C1 (Mg), and M2 (Cu) species are higher than other species and it can be said the macro and micro element contents of these species should be used carefully considering the negative relationship of the elements above the threshold concentration levels in terms of health. The Pb content of the A3 species was determined that be higher than the value determined by the WHO, and if this species is used, the toxic dose relationship should be considered.

Conflict of interests

The authors declare that there is no conflict of interest.

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DOI: http://dx.doi.org/10.32571/ijct.1087834 **E-ISSN: 2602-277X**

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DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Antimicrobial, antioxidant and mutagenic effect potential of red pepper (*Capsicum annum*)

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Received: 14 March 2022; Revised: 20 June 2022; Accepted: 26 June 2022

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Citation: Akkoç, Y.; Arslan, M.; Erbil, N.; Murathan, Z. T., O. *Int. J. Chem. Technol.* **2022**, 6 (1), 48-55.

ABSTRACT

In this study, the biochemical, microbiological, mutagenic and antioxidant properties of red pepper (*Capsicum annum*) grown in 6 districts of Adıyaman province (Kahta, Gerger, Sincik, Samsat, Tut, Gölbaşı) were investigated. The bioactive compounds of Gerger samples, which also exhibited higher antioxidant activity. The results of antimicrobial activity showed that Kahta pepper extract exhibited antibacterial activity against *Staphylococcus aureus* ATCC 6538 with an MIC (minimum inhibition concentration) value of 0.715 mg/ml. The lowest MIC values in the pepper extracts were obtained in Kahta pepper extract (0.715-1.43 mg ml^{-1}), and the highest MIC values were recorded in Tut pepper extract (1.1525-4.61 mg ml^{-1}). The mutagenic activity results of peppers indicated no mutagenic effect at the doses tests and in the studies carried out on TA 100 strains and Salmonella typhimurium TA 98 and in the absence of S9. Dose dependent increase was recorded on *Salmonella typhimurium* TA 98 strain of Samsat pepper samples. But, the rising was not statistically importent.

Keywords: Phenolics compounds, bioactive compounds, medicinal plants, flavonoids.

Kırmızı biberin (*Capsicum annum*) antimikrobiyal, antioksidan ve mutajenik etki potansiyeli

ÖZ

Bu çalışmada Adıyaman ilinin 6 ilçesinde (Kahta, Gerger, Sincik, Samsat, Tut, Gölbaşı) yetiştirilen kırmızı biberin (*Capsicum annum*) biyokimyasal, mikrobiyolojik, mutajenik ve antioksidan özellikleri araştırılmıştır. Gerger örneğinin biyoaktif bileşikleri en yüksek antiaoksidan aktivite gösterdi. Antimikrobiyal aktivitenin sonuçları, Kahta biber ekstraktının, 0.715 mg/ml MİK (minimum inhibisyon konsantrasyonu) değeri ile *Staphylococcus aureus* ATCC 6538'e karşı antibakteriyel aktivite sergilediğini gösterdi. Biber ekstraktlarında en düşük MİK değerleri Kahta biber ekstraktında (0.715-1.43 mg ml-1), en yüksek MİK değerleri Tut biber ekstraktında (1.1525-4,61 mg ml^{-1}) elde edilmiştir. Biberlerin mutajenik aktivite sonuçları, doz testlerinde ve TA 100 suşları ve *Salmonella typhimurium* TA 98 üzerinde yapılan çalışmalarda ve S9 yokluğunda mutajenik etki göstermedi. Samsat biber örneklerinde *Salmonella typhimurium* TA 98 suşunda doza bağlı artış kaydedilmiştir. Ancak, yükseliş istatistiksel olarak önemli değildir.

Anahtar Kelimeler: Fenolik bileşikler, biyoaktif bileşikler, şifalı bitkiler, flavonoidler.

1. INTRODUCTION

Pepper is cultivated under cover or in the open field in various countries of the world, and is an important crop for the consumer, producer and processing industry.[1] The pepper is an annually cultivated crop in temperate climates. The species cultivated in the Southeastern Anatolia and the Eastern Mediterranean Regions of Türkiye is *Capsicum annuum* L.[2] The carotenoid and vitamin C content of red pepper has significant effects on anti-cardiovascular diseases, some types of cancer, atherosclerosis and anti-aging. A plethora of researchers have been addressed on the carotenoids due to the benefits on human health and attractive color in foods.[3] The pepper has been used in medicine since ancient times due to the diuretic effect and activation of the stomach

DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

and glands.[1] Positive effects of red pepper on lipid metabolism, diabetic neuropathy, gallstones and inflammatory diseases, and accelerating effect on digestion have been reported.[4] The pepper has been used as a medicine for stomachache, arthritis, rash, snake bites and dog bites and for healing wounds in China and America.[5]

Previous studies have been conducted to define biochemical, antimicrobial and antimutagenic properties of red pepper grown in different regions of Türkiye. "We herein examined the biochemical, antimicrobial and antimutagenic properties of red peppers grown in Adıyaman province. The goal of this study was to determine the biochemical, antimicrobial and mutagenic properties of red peppers grown in Adıyaman province of Türkiye.

2. MATERIALS AND METHODS

2.1. Plant Materials

The red pepper samples were collected from 6 different districts of Adıyaman province (Kahta, Gerger, Sincik, Samsat, Tut, Gölbaşı) and stored at -20 ºC in the laboratory until analysis.

2.2. Extraction

Leaves, stems and seeds were removed from pepper samples and 20 ml of methanol was added to 2 gram of red pepper sample, and homogenized. The mixture was held ed at 4 ºC for 24 hours in a shaking oven, and centrifuged at 5000 rpm for 10 min. The supernatant was utilized for total flavonoid, phenolic substance and antioxidant capacity analysis. Oxalic acid was used as the solvent for total vitamin C analysis, and the same extraction method was used. Biochemical analyzes were carried out immediately after the extraction.

40 g of red pepper samples were weighed for the extracts used in mutagenic and antimicrobial tests, and the pepper samples were homogenized by adding 200 ml of distilled water. The mix was extracted at 190 rpm and room temperature for 72 h. Later on, the mix was centrifuged at 5000 rpm for 10 min. The supernatants were concentrated using a rotary evaporator. The concentrated extracts were sterilized with a 0.22 µm microfilter and kept at -20 ºC until further analysis.

2.3. Catalase activity analysis

Catalase activity was measured using the modified method described by Lartillot and co-workers[6] and Bergmeyer.[7] A substrate solution was prepared adding 10 mmol L^{-1} H_2O_2 in 50 mmol L^{-1} phosphate buffer. Then, 2.5 mL of substrate solution was added onto 20 mL of enzyme-containing solution (prepared by diluting with 1/2000 buffer) and incubated at 37 °C for 2 minutes. In order to stop the reaction, 0.5 mL of 1N HCl was added to the solution, and the absorbance was measured at 240 nm. For blank (Ar), 2.5 mL buffer (50 mmol L^{-1} phosphate buffer with pH 6.8) and 0.5 mL 1 N HCl were used. The absorbance (As) of the reaction mix was measured with a mixture of 2.5 mL of substrate and 0.5 mL of 1 N HCl. The absorbance (At) caused by the protein was measured in the mixture of 20 mL of the test solution, 2.5 mL of buffer and 0.5 mL of 1 N HCl. The change in absorbance due to enzymatic activity was calculated using the following equivalence (Eq. 1).

$$A = (As + At) - Ar \quad \text{(Eq. 1)}$$

In the equation; Ar is absorbance in the presence of enzyme and substrate; As is the initial absorbance (absorbance of the reaction mixture at the time zero); At is the absorbance of the mixture (in the presence of enzyme and the lack of substrate). The activity of catalyze was calculated using the following equation (Eq. 2).

$$Activity\ (IU\ mL^{-1}) = A.V_{total} / \hat{I}.t.\ V_{sample} \quad \text{(Eq. 2)}$$

In the equation; Î is the specific absorbance coefficient of hydrogen peroxide is about 0.0396 $cm^2/\neq mol$, the value is 0.068 $cm^2/\neq mol$ for tertiary hydrogen peroxide. t is the reaction time.

2.4. Total phenolic content

Total phenolic content was defined using the Folin-Ciocalteu method proposed by Spanos and Wrolstad.[8] The 1000 µl of Folin-Ciocalteu and 800 µl (7.5%) sodium carbonate were added to the 200 µl extract. After the incubation for 2 h. at room temperature, the absorbance at 765 nm was measured using a spectrophotometer. Total phenolic content of red pepper samples was calculated as mg gallic acid equivalent (GAE) 100 g^{-1} extract using gallic acid standard. The analysis was reiterated three times.

2.5. Total flavonoid content

Total flavonoid substance of red pepper samples was defined using the method suggested by Quettier and co-workers.[9] 1 ml of 2% aluminum chloride was added to 1 ml of extract and held in the dark for 1 hour at room temperature. The total flavonoid content of the red peppers was measured in a spectrophotometer at 415 nm. The equivalent of mg quercetin was calculated as mg quercetin 100 g^{-1} extract using the calibration curve prepared by the routine standard. The analysis was reiterated three times.

2.6. Total ascorbic acid content

Total ascorbic acid was defined spectrophotometrically according to the AOAC[10] method. 400 µL of 0.4%

DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

$C_2H_2O_4$ and 4.5 ml of 2.6-$C1_2H_7NCl_2O_2$ solutions were added into 100 µl of supernatant, and the absorbance was measured at 520 nm. The amount of vitamin C in red pepper samples was calculated as mg 100 g^{-1} using the calibration curve obtained with pure ascorbic acid.

2.7. Antioxidant activity assays

2.7.1. DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of red pepper extracts was determined using the method proposed by Bakhshi and Arakawa.[11] 4 ml of DPPH solution (0.1 M) was combined with 1 ml of extract and kept in a shaker at room temperature for 30 min. in a dark environment. The absorbance of the solution at 515 nm was determined in a spectrophotometer. The antioxidant capacity of red pepper was calculated using the following equivalence (Eq. 3)

%DPPH=(AcontrolAsample)/Acontrolx100 (Eq. 3)

2.7.2. ABTS radical scavenging activity

The 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging activity was defined using the method proposed by Re and co-workers.[12] A 1:1 stock solution was prepared with 2.45 mM potassium per sulfate and 7 mM ABTS and incubated at room temperature for 16 hours in the dark. For the measurements, the stock solution was diluted with C_2H_4O until the absorbance of 0.7±0.05 at 734 nm. 150 µl of sample was mixed with 2.85 ml ABTS solution and incubated for 6 min. at room temperature. The absorbance was measured at 734 nm, and CH_3OH solution was used as the blank. The percent ABTS was computed using the following equivalence (Eq. 4). The analysis was reiterated three times.

%ABTS inhibition = (AcontrolAsample)/Acontrolx100.

2.7.3. FRAP Assay activity

FRAP (ferric reducing ability of plasma) analysis was implemented using the method introduced by Benzie and Strain.[13] The solution of the method was prepared by stiring 25 ml of $C_2H_3NaO_2$ buffer (300 mM, pH 3.6), 2.5 ml of TPZT solution (10 mM in 40 mM HCl) and 2.5 ml $FeCl_3.6H_2O$ (20 mM). The mixture was heated at 37 °C in a water bath and 900 µl of the solution was taken into a cuvette and the first absorbance was determined. 100 µl of the diluted (1:4 v/v water) sample was taken into the cuvette and 3 ml of FRAP solution was added. After 4 minutes, absorbance was measured at 593 nm. A standard curve (100-1000 µl) was prepared using $FeSO_4$ solution, and the results were calculated in µmol Fe (II)g^{-1}. The analysis was reiterated three times.

2.8. Antimicrobial activity

The antimicrobial activity of the aqueous extracts obtained from the Gerger, Sincik, Gölbaşı, Kahta, Samsat and Tut pepper samples was tested by the Broth Microdilution Method.[14,15] The test bacteria used in the study were *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 33495, *Bacillus licheniformis* ATCC 14580 and *Staphylococcus aureus* ATCC 6538. Bacterial cultures incubated for 16 hours were set to McFarland 0.5 turbidity standard, and Mueller Hinton Agar was used as the medium.

The amount of dilution for Sincik pepper extract was between 6.89-0.0269 mg/ml, 4.61-0.0180 mg/ml for Tut pepper extract, 4.14-0.1567 mg/ml for Gölbaşı pepper extract, 5.72-0.0223 mg/ml for Kahta pepper extract, 10.65-0.0416 mg/ml for Samsat pepper extract and 6.12-0.0239 mg/ml for Gerger pepper extract. Plates were incubated at 37 ºC for 18 hours. After the incubation, 0.5% TTC solution was added to the wells and incubated for another 30 min. The wells with no color change at the last of the incubation were determined as MICs (minimum inhibition concentration).

2.9. Genotoxicity tests

2.9.1. *Salmonella typhimurium* reverse mutation assay

The mutagenic activity of aqueous extracts obtained from red pepper samples were determined using the plaque incorporation method developed by Maron and Ames.[16] The mutagen activity analyzes were performed on Salmonella typhimurium TA 100 and Salmonella typhimurium TA 98 strains. 4-Nitro-Ophenylenediamine (4-NPD; Product Number: 108898-5G, Sigma Aldrich, St. Louis, MO, USA) was used as a positive control for TA 98 (10µg/plate). Sodium azide (SA; Cat. No. S 2002, Sigma Aldrich) was used as a positive control for TA 100 (100µg/plate). Plates were incubated at 37°C for 48-72 hours, then his+ revertant bacterial colonies were counted on the plates. The analyzes were used in the tests without the metabolic activation system S9 mix using the S. typhimurium TA 98 and TA 100 strains. The analysis was reiterated three times.

2.10. Statistical analyses

Statistical analysis of the datum was implemented using SPSS (version 20) statistical software. The number of colonies that returned with the effect of red pepper samples were determined. One-way analysis of variance (ANOVA) was used to define the statistical difference among the control and the dissimilar concentrations of red pepper samples. The mean values of different pepper cultivars were grouped using the Dunnett test.

DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

3. RESULTS AND DISCUSSION

3.1. Results

The catalase activities of red pepper samples are given in Table 1. The highest catalase activity was recorded in Gerger (1106.7 U/mg) and the lowest was in Kahta (763.3) pepper samples.

Table 1. Catalase activities of red pepper samples.

SAMPLES	ACTIVITY (U/mg Protein)
Kahta	763.3
Tut	899.4
Gölbaşı	8651
Gerger	1106.7
Sincik	764.5
Samsat	941.9

The total phenolic, total ascorbic acid, total flavonoid contens and antioxidant capacities of the red pepper samples are given in Table 2. The Gerger samples had the highest total ascorbic acid content (259.8 mg 100^{-1}). The total ascorbic acid content of Sincik, Samsat and Tut samples was not importantly different from each other. The lowest total ascorbic acid content was determined in Gölbaşı samples (209.8 mg/100g). The total phenolic content in methanolic extracts of pepper samples was determined spectrophotometrically using the Folin Ciocalteu method. The maximum total phenolic acid content was obtained in Gerger sample (235.9 mg/100g), and the lowest content was in Kahta samples (73.4 mg/100g). The maximum total flavonoid content was recorded in Samsat samples (139.6 mg/100g), and the minimum content was in Sincik and Kahta samples. The highest FRAP content was determined in Gerger and Tut samples, and the lowest value was in Sincik samples. The maximum ABTS radical scavenging activity was in Gerger (35.3%) and the minimum activity was in Kahta (8.5%) sample. The maximum DPPH radical scavenging activity was obtained in Gerger and Samsat (52, 50%) sample, while the lowest value was obtained in Kahta (22.5%) sample. The results showed that bioactive compounds and antioxidant activities were high in Gerger sample.

Table 2. Total ascorbic acid, phenolic substance, flavonoid substance and antioxidant capacity in red pepper samples.

SAMPLES	TAC (mg/100g)	Total phenolic substance (mg/100g)	Total flavonoid substance (mg/100g)	FRAP (µmol FeII/g)	ABTS (%)	DPPH (%)
Kahta	232.3±3.6^{bc}	73.4±2.4^{e}	66.1±3.5^{d}	144.4±3.9^{c}	8.5±0.3^{d}	22.5±2.7^{c}
Tut	246.5±2.5^{b}	191.2±3.5^{b}	102.3±7.4^{b}	253.8±16.1^{a}	28±0.2^{b}	36±3.8^{b}
Gölbaşı	209.8±34.1^{c}	126.7±2.9^{d}	72.8±12.6^{c}	149±7.4^{c}	21.3±0.7^{bc}	33±1.2^{b}
Gerger	259.8±3.8^{a}	235.9±12.5^{a}	109.3±16.2^{b}	251.7±6.3^{a}	35.3±2.2^{a}	52±3.3^{a}
Sincik	247.3±5.2^{b}	147.2±2.3^{c}	67.3±5.3^{d}	141.1±1.4^{c}	14.5±0.3^{c}	28±0.9^{bc}
Samsat	245.7±3.8^{b}	184.2±4.9^{b}	139.6±12.3^{a}	243.8±17.3^{b}	32.6±3.2^{a}	50±2.9^{a}

The antimicrobial activity of the samples is given in Table 3. The antimicrobial activity results revealed that pepper extracts exhibited a significant antibacterial activity, and *Staphylococcus aureus* ATCC 6538 is more sensitive to pepper extracts. Kahta pepper extract exhibits antibacterial activity against *Staphylococcus aureus* ATCC 6538 with a MIC value of 0.715 mg/ml. Kahta pepper extract had the lowest MIC values (0.715-1.43 mg/ml), while Tut pepper extract (1.1525-4.61 mg/ml) had the highest MIC values.

Table 3. MIC values of red pepper extracts.

Bacteria	Kahta (mg/ml)	Tut (mg/ml)	Gölbaşı (mg/ml)	Sincik (mg/ml)	Gerger (mg/ml)	Samsat (mg/ml)
Klebsiella pneumoniae ATCC 33495	1.43	4.61	2.007	1.7225	3.06	2.6625
Bacillus licheniformis ATCC 14580	1.43	4.61	2.007	1.7225	1.53	1.3312
Enterobacter aerogenes ATCC 13048	1.43	4.61	4.14	3.445	3.06	2.6625
Staphylococcus aureus ATCC 6538	0.715	1.1525	1.0035	1.7225	1.53	2.6625

The mutagenic activities of the samples are shown in Tables 4 and 5. The mutagenicity tests of pepper extracts used in this study were investigated on *Salmonella typhimurium* TA 98 and TA 100 strains. Four different doses of pepper samples (12.5, 25, 50 and 100 µl/plate) were used in the mutagenicity experiments. The mutagenic effect of pepper samples on *Salmonella typhimurium* TA 98 and TA 100 strains was not detected at the doses tested and in the absence of S9 (Tables 4, Table 5). The mutagenic effect on Salmonella typhimurium TA 98 strain of Samsat pepper samples was increased with the rise in the treatment dose. However, the increase in mutagenic effect was not statistically significant.

3.2. DISCUSSION

Many different studies have been implemented on red pepper; however, the antioxidant capacity, genotoxicity, antimicrobial and mutagenic activity of Adıyaman red

DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

peppers have not been investigated. The maximum total phenolic content was acquired in Gerger sample (235.9 mg/100g), and the lowest content was in Kahta sample (3.4 mg/100g). The highest total flavonoid content was defined in Samsat sample (139.6 mg/100g), and the minimum value was in Sincik and Kahta samples. Ergün[17] reported that the total phenolic content in the methanol extracts of Cemele pepper grown in Kırşehir province was 27.03 µg/ml and the total flavonoid content was 39.67 µg/ml. The highest FRAP contents were obtained in the Gerger and Tut pepper samples, and the minimum value was in the Sincik sample. The FRAP content ranged from 141.1 to 253.8 µmol Fe II/g. The minimum ABTS radical scavenging activity was obtained in Gerger (35.3%), and the minimum value was in Kahta (8.5%) samples. The maximum DPPH radical scavenging activity was recorded in Gerger and Samsat (52, 50%) samples, and the minimum value was in Kahta (22.5%) samples. The bioactive compounds and the antioxidant activity of

Table 4. Mutagenic effect of red pepper extracts on *Salmonella typhimurium* TA 98.

Samples	Concentration	Number of colonies returned
		Mean±Sd**
Kahta	Control	16.67±1.45
	Positive control (4-NPD)*	2086±109
	12.5 µl/plate	19.33±4.67
	25 µl/plate	10.33±1.2
	50 µl/plate	28.33±4.48
	100 µl/plate	20.00±1.00
Tut	Control	16.67±1.45
	Positive control (4-NPD)*	2989±109
	12.5 µl/plate	13.00±3.51
	25 µl/plate	13.33±1.76
	50 µl/plate	28.67±6.69
	100 µl/plate	19.33±0.882
Gölbaşı	Control	16.67±1.45
	Positive control (4-NPD)*	2889±109
	12.5 µl/plate	23.00±1.53
	25 µl/plate	16.00±0.577
	50 µl/plate	13.67±2.4
	100 µl/plate	39.3±15.2
Gerger	Control	23.00±6.43
	Positive control (4-NPD)*	1754±588
	12.5 µl/plate	16.00±2.89
	25 µl/plate	15.67±1.86
	50 µl/plate	18.67±1.20
	100 µl/plate	15,33 ± 2,40
Sincik	Control	23,00 ± 6,43
	Positive control (4-NPD)*	1754 ±588
	12.5 µl/plate	14,667±0,882
	25 µl/plate	17,00 ±4,51
	50 µl/plate	16,00±1,00
	100 µl/plate	18,00± 4,36
Samsat	Control	12,67 ± 2,60
	Positive control (4-NPD)*	1262,0±57,6
	12.5 µl/plate	12,333±0,882
	25 µl/plate	19,33 ±1,67
	50 µl/plate	31,33±6,84
	100 µl/plate	34,00±4,16

DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

Table 5. Mutagenic effect of red pepper extracts on *Salmonella typhimurium* TA 100.

Samples	Concentration	Number of colonies returned Mean±Sd**
Kahta	Control	232.00±2.31
	Positive control (4-SA)*	2326±183
	12.5 µl/plate	360.0±30.1
	25 µl/plate	252.0±18.3
	50 µl/plate	258.3±14.9
	100 µl/plate	290.0±23.4
Tut	Control	232.00±2.31
	Positive control (4-SA)*	2326±183
	12.5 µl/plate	591±151
	25 µl/plate	394.0±51.8
	50 µl/plate	340.0±54.5
	100 µl/plate	396.7±11.7
Gölbaşı	Control	232.00±2.31
	Positive control (4-NPD)*	2326±183
	12.5 µl/plate	407.3±66.8
	25 µl/plate	551±114
	50 µl/plate	520.7 ±35.8
	100 µl/plate	366.7±22.8
Gerger	Control	98.00±11.2
	Positive control (4-NPD)*	5677±1138
	12.5 µl/plate	65.33± 4.33
	25 µl/plate	97.33±6.36
	50 µl/plate	81.3±14.4
	100 µl/plate	96.3±12.8
Sincik	Control	98.0±11.2
	Positive control (4-NPD)*	5677±1138
	12.5 µl/plate	146.00±5.13
	25 µl/plate	129.3±19.2
	50 µl/plate	80.00 ±5.69
	100 µl/plate	71.67±1.67
Samsat	Control	128.0±23.4
	Positive control (4-NPD)*	2059 ±152
	12.5 µl/plate	128.0± 34.1
	25 µl/plate	141.3±39.8
	50 µl/plate	136.0±13.7
	100 µl/plate	115.67±3.84

Gerger pepper sample were high. Arın [18] reported that the DPPH radical scavenging activity of hot red pepper was 35%. Shan and co-workers[19] determined the total antioxidant capacity in hot pepper samples as 6.05 mmol/100 g. Keçeli[20] determined the antioxidant levels of bell pepper, green pepper and paste pepper as 1.264, 0.562 and 0.386 mmol/100 g fresh matter, respectively. Akça[21] reported the antioxidant values of Kundu F1, Bafra F1, Abide F1 and Istek F1 pepper varieties as 5.09, 8.88, 6.26 and 6.90 mmol/100 g dry matter, respectively. The differences between studies may be based on to the differences in climatic characteristics, soil structure and cultural practices, and the differences in varieties and cultivars may also affect the results.

The antimicrobial activity analysis indicated that all pepper samples had varying degrees of antimicrobial activity. The lowest MIC values were determined in Kahta red pepper extract. The most sensitive test bacteria

DOI: http://dx.doi.org/10.32571/ijct.1087514 E-ISSN: 2602-277X

was *Staphylococcus aureus* ATCC 6538. Previous studies on *Capsicum annum* show that extracts of *Capsicum annum* generally exhibit antimicrobial activity, which is in conformity with the results acquired in this study. Ali and co-workers[22] reported the inhibitory effect of ethyl acetate extract among the different extracts (hexane, acetone, dichloromethane, ethyl acetate and ethanol) obtained from *Capsicum annum* on *Bacillus subtilis* ATCC 6663, *Aspergillus flavus* ATCC 3261 and *Trichophyton longifusus* ATCC 22397 (73, 77 and 70%, respectively).

In another study, the effects of different varieties of *Capsicum annum* on foodborne pathogenic microorganisms were investigated. The results showed that all pepper extracts used inhibited the growth of *Staphylococcus aureus* and *Listeria monocytogenes*, and Effix and Fantasia varieties of peppers were considered the most effective varieties in terms of antimicrobial activity.[23]

Gebara and co-workers[24] reported that *Capsicum annum* fractions exhibited the highest inhibitory activity especially against *Candida* and *Mycobacterium tuberculosis* species. The results of this working consistent with those previously reported show that pepper has varying degrees of antimicrobial effect on the test microorganisms.

The mutagenicity studies carried out are generally related to capsaicin, which is obtained from the cayenne pepper. Previous studies have shown that capsaicin may have anticarcinogenic and antimutagenic activity.[25,26] In addition, the capsaicin selectively induces apoptosis in cancerous cells and may have a tumor stimulating effect.[26,27] Oğuzhan and co-workers[28] reported that capsaicin is one of the most important secondary metabolites in peppers, and capsaicin has antimutagenic and anticarcinogenic potential. In addition, the researchers indicated that the extract in methanol showed a better protective effect in terms of DNA protection at the highest concentrations, while the dichloromethane extract of capsaicin provided a higher protective effect at low concentrations.

The mutagenicity was not reported in two different studies using the *Salmoonella-microsome* assay to detect capsaicin and pepper extract mutagenicity.[29,30] Another study was revealed that capsaicin was mutagenic with metabolic activation in test strains of TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with *Salmonella typhimurium* histidine deficiency.[31] The intraperitoneal application of capsaicin to male mice caused an increase in micro nucleated normochromic erythrocytes in peripheral blood and sister chromatid exchanges in bone marrow cells.[32] Similarly, the intraperitoneal application of alcoholic extracts of *Capsicum frutescens* pepper fruits to mice led to positive results in mouse bone marrow micronucleus test.[33]

The mutagenic activity results revealed that different pepper extracts of Adıyaman province do not have a mutagenic effect on *Salmonella typhimurium* TA 98 and TA 100 strains in the absence of S9 and at the doses tested in this working. These results are coherent with the findings of Rockwell and Raw,[29] Buchmann and co-workers.[30]

The antioxidant capacity, genotoxicity and antimicrobial catalase enzyme activities of red peppers collected from different regions of Adıyaman province were different. The difference can be associated with soil type, climate and growing conditions of pepper plants.

4. CONCLUSIONS

Red pepper is an important vegetable for many countries. In this study, the biochemical, antimicrobial mutagenicity and antioxidant properties of red peppers collected from different regions of Adıyaman province of Türkiye were investigated. Mutagenic activities on *Salmonella typhimurium* TA 98 and TA 100 strains were not detected in the absence of S9 and at the doses tested.

The results revealed that the catalase activity of pepper samples was high. Bioactive compounds and antioxidant activity were high in Gerger samples. The antimicrobial activity results showed that the lowest MIC values were in Kahta pepper extract, and the highest MIC values were in Tut pepper extract.

Conflict of interests

I declare that there is no a conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.1107128 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Automatic soil ph level detection using extreme learning machine via image processing

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Received: 21 April 2022; Revised: 4 June 2022; Accepted: 5June 2022

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Citation: Turhal, C. U.; Turhal, K. *Int. J. Chem. Technol.* **2022**, 6 (1), 56-60.

ABSTRACT

The pH values in the soil, that is, the acid or basic structure of the soil, affects the amounts of nutrients that the plant receives from the soil. For the plant to take the main nutrients in the soil and grow is only possible at suitable pH values. In this paper a novel soil pH level detection method based on optical imaging is proposed. As the level detection algorithm an Extreme Learning Machine (ELM) is used. In the constructed model while the RGB values of the true color soil images and pH index are used as the inputs of ELM the pH level of soil images are used as the output of ELM. In the experimental studies fifty soil sample images obtained from the literature are used. And a significantly high pH level detection performance of 97.5 % is obtained. This result reveals that the proposed method is a significantly important method to determine the pH levels of soil samples and could be a strong alternative to the traditional methods.

Keywords: Soil pH, optical imaging, extreme learning machine, data classification.

Görüntü işleme yoluyla aşiri öğrenme makinesi kullanilarak otomatik toprak ph seviye tespiti

ÖZ

Topraktaki pH değerleri yani toprağın asit veya bazik yapısı bitkinin topraktan aldığı besin maddelerinin miktarlarını etkiler. Bitkinin topraktaki temel besin maddelerini alması ve büyümesi ancak uygun pH değerlerinde mümkündür. Bu makalede optik görüntülemeye dayalı yeni bir toprak pH seviyesi tespit yöntemi önerilmiştir. Seviye tespit algoritması olarak bir Aşırı Öğrenme Makinesi (AÖM) kullanılmıştır. Oluşturulan modelde AÖM girdisi olarak gerçek renkli toprak görüntülerinin RGB değerleri ve pH indeksi kullanılırken, AÖM çıktısı olarak toprak görüntülerinin pH seviyesi kullanılmıştır. Deneysel çalışmalarda literatürden elde edilen 50 adet toprak örneği görüntüsü kullanılmıştır. Ve % 97.5 değerinde önemli ölçüde yüksek bir pH seviyesi algılama performansı elde edilmiştir. Bu sonuç, önerilen yöntemin toprak örneklerinin pH seviyelerini belirlemede oldukça önemli bir yöntem olduğunu ve geleneksel yöntemlere güçlü bir alternatif olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Toprak pH'ı, optik görüntüleme, aşırı öğrenme makinesi, veri sınıflandırma.

1. INTRODUCTION

Soil is a substance that consists of the decomposition products of rocks and organic materials in various scales that contains a wide world of living things and valuable life source for plants.[1,2] In agricultural applications the chemical contents of the soil have to be known before the cultivation in order to increase the product yield. By systematic analysis of soil its type and general structure could be determined.[3] Among the contents, the pH value, which is used to define the acidity or basicity degree of the soil, is an important factor indicating soil health. If the pH value in the soil is less than 4.5, it is defined as strong acid, between 4.6-5.5, medium acid, between 5.6-6.5, neutral, between 6.6-7.5, slightly alkaline, and above 8.5 as strongly alkaline.[4] Plants could grow in the soil that has appropriate pH values by means of taking the main nutrients. The fact that the pH value in the soil is at its limit values may cause the plant not to receive the nutritions in the soil or it may become toxic for the plant due to excessive dissolution of the nutritionas.[5] Even if there are enough main nutrients (nitrogen (N),

DOI: http://dx.doi.org/10.32571/ijct.1107128 E-ISSN: 2602-277X

phosphorus (P), potassium (P)) in a soil that is too acidic or too basic, the plant cannot absorb these elements or has difficulty taking them.[6] The pH value of soil directly affects the growth of the plant. When it is between 6.0 and 7.5, the plant can easily take up the nutrients in the soil. If the pH value in the soil is too acidic, some nutrients such as nitrogen, phosphorus, and potassium in the soil are less. On the contrary, although it is strongly alkaline, nutrients such as iron, manganese, and phosphorus are scarce in the soil.[6] When the soil pH value is 8 and above, plants cannot be grown in that soil.[7] The color of the soil also gives us information about the structure of the soil. Organic matter, presence of water, oxidation and pH are the factors that determine the color association in soil.[8]

Traditionally colorimetric and electrometric methods are used to determine soil pH. Although the colorimetric method using dyes or indicators whose colors can change according to the H ion activity in the environment is practical, it is not sensitive. Except for the test kits used to approximate soil pH in the field, indicators are not preferred for pH determination in soil. Today, pH determinations in soil are generally made using electrometric methods using a pH meter. The principle of the method is to measure the hydrogen ion activity of the soil, which is mixed with water or saturated with water in certain proportions, with a pH meter.[9] However these methods have some disadvantages such that they require expertise and long time requirements. They also are not automatic and some results are not purely objective, they are expert dependent. In the study given in the authors presented a literature survey related to automatic soil nutrients detection methods applied to eliminate the disadvantages of the traditional methods.[10] Accordingly, Chen and co-workers,[11] presented a study that determined the soil potassium level by using a machine learning algorithm that is Support Vector Machines (SVM) algorithm on plant leaf images. The starting point of this study was the visual changes in the plant leaf caused by the potassium level in the soil. Li and co-workers,[12] on the other hand, carried out the determination of soil nitrogen content using the Uniform variable elimination – extreme learning machine (UVE-ELM) method on a hyperspectral image. Similarly, Aitkenhead and co-workers,[13] carried out a study on hyperspectral images to detect NPK values of soil. However hyperspectral imaging is unpractiable in terms of obtaining images. In this study we proposed a novel optical imaging based soil pH level detection method using ELM. This method only performs soil pH level detection using the RGB images of soil samples acquired with a camera or with a smartphone on the working area. In the constructed ELM, inputs are the RGB values and the pH indices of soil images and the output is the measured pH values of soil samples. It automatically determines the pH level of the soil sample only using the soil image and doesn't require any measurements or any expertise as in the traditional methods. The contributions of the proposed method can be summarized as follows: As far as we know from the literature:

- It is the first time in the literature an automatic soil pH level detection algorithm is proposed.
- Detection of soil pH level has been performed without using any measurements in contrast to the traditional methods.
- Extreme Learning Machine is used for the first time in the literature to detect the pH level for soil samples.
- It does not require additional equipment as it uses the image data captured in the farming area, so it is cost-effective, time-effective and also user friendly which allows farmers usage ease.
- Traditional methods are based on some kind of measurement. However, since the proposed method is based on only a computer aided system, it is a precious alternative method for determining the pH level of the soil samples.
- It can also be extended to the other soil properties detection.

The rest of the paper is organized as follows: Materials and methods are given in the second section, then results and discussion and conclusion are given.

2. MATERIALS AND METHODS

2.1. Materials

Fifty soil sample images as given in Figure 1 collected from the Nathnagar block of Bhagalpur district are used as the soil dataset.[14] The images in JPEG format were captured using a digital camera. A true color digital image with the size of mxn has three color components as Red (R), Green (G) and Blue (B). The true colors are the composition of these three components in various values. In Figure 2, RGB color components of the soil sample images given in Figure 1 and their pH values measured by traditional methods are given.

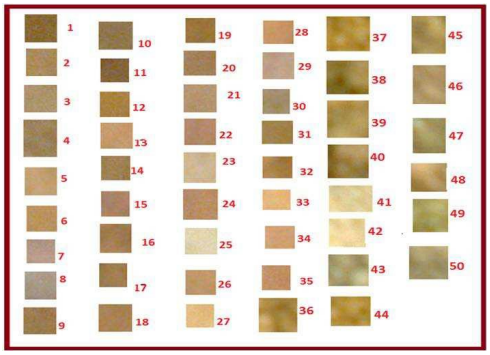


Figure 1. The soil samples data set.[14]

DOI: http://dx.doi.org/10.32571/ijct.1107128 E-ISSN: 2602-277X

2.2. Methods

Extreme Learning Machine (ELM) is a kind of neural network and is proposed for single hidden layer feedforward Neural Networks originally.[15] In contrast to traditional neural networks, hidden nodes parameters, input biases and weights are randomly selected while the output weights are analytically determined in ELM. A number of hidden nodes is the only parameter needed to be defined. So the learning process of the network is performed fastly. It has three layers: the input layer, the hidden layer and the output layer as can be seen in Figure 3. It has a good generalization performance and a fast learning speed, due to direct learning.

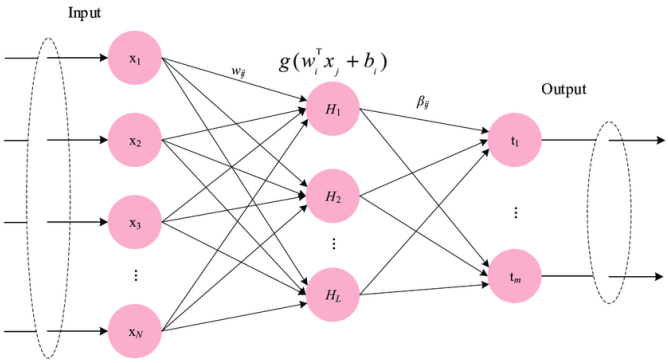


Figure 3. Extreme Learning Machine.[16]

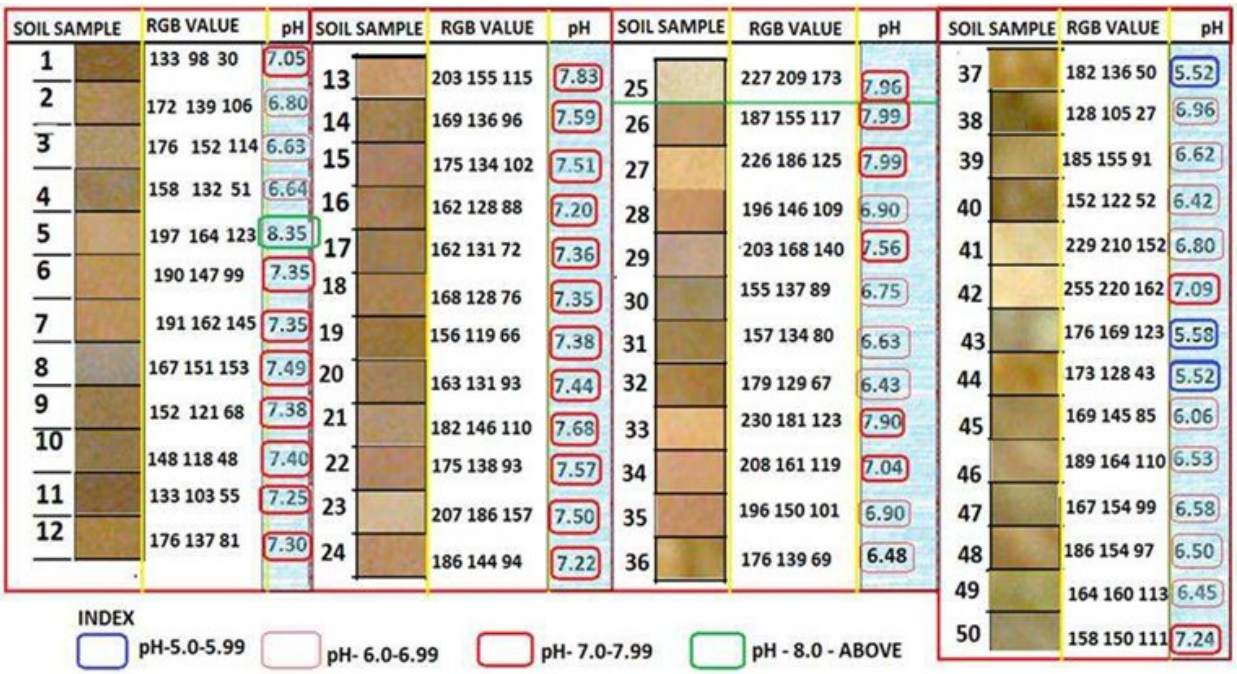


Figure 2. The soil samples data set with RGB values and corresponding measured pH values. [14]

In the proposed study the pH level detection is performed according to the RGB values of soil image pixels. In [10] authors reveal the relationship between the RGB values of different soil samples images and their measured pH values using traditional methods. They also defined a pH index via the RGB values as it is given in Equation 1.

$$pH\ index = (\frac{R}{G})/B \quad (1)$$

In our study based on this idea we created an ELM network whose inputs are the RGB values of image pixels and the pH indices and the output is the pH level of that soil sample which is determined by the measured pH value using the traditional methods. This method is basically a classification namely a supervised learning algorithm so it includes a training step and a testing step. At first the dataset given in Table 1 is splitted into two as the training set and the test set. While in the training step using the training data set a classification model is constructed, in the testing step using the test data set classification performance of the model is evaluated. The created model detects the pH level of soil samples without any physical measurements but only using the soil image R, G, B values and the corresponding pH indices. Once the model is constructed and the accuracy performance is revealed then by applying it for a new soil sample the pH level of that sample can be determined. In the testing step the test data attributes (R, G, B values and pH indices) are given to the constructed classification model as the inputs. Then the class labels (pH levels) are predicted using the model. The performance of the proposed model is performed by the comparison of each testing object's predicted class label with the actual class labels in the data set. Thus using this classification algorithm, the unknown sample class label (the pH level) is detected using the known attribute values (the R, G, B and the pH index).

DOI: http://dx.doi.org/10.32571/ijct.1107128 E-ISSN: 2602-277X

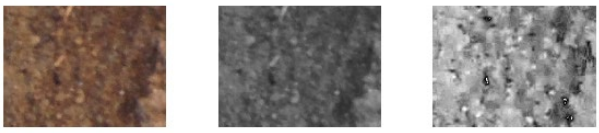
Table 1. A sample of soil data set used in this study.

	ELM Inputs				ELM Output	ELM Output
No	R	G	B	pH index	**Measured pH**	**Discrete pH**
1	133	98	30	0,0452	**7,05**	**Positive**
2	172	139	106	0,0116	**6,8**	**Positive**
3	176	152	114	0,0101	**6,63**	**Positive**
4	158	132	51	0,0234	**6,64**	**Positive**
5	197	164	123	0,0097	**8,35**	**Negative**
6	190	147	99	0,013	**7,35**	**Positive**
7	43	176	169	123	**5,58**	**Negative**
8	44	173	128	43	**5,52**	**Negative**
9	152	121	68	0,0184	**7,38**	**Positive**
10	148	118	48	0,0261	**7,4**	**Positive**

In this study besides the RGB images different color spaces such as gray scale image and HSV color space are also evaluated. In order to obtain a gray scale image from a RGB image the formula given in Equation 2 is used. The intensity value of pixels of grayscale image varies between 0-255. While 0 is corresponding to black color 255 corresponds to white color. And the values between 0 and 255 correspond to the gray colors between the black and white. The HSV color space has three components that are Hue (H), Saturation (S) and Value (V) respectively. In this color space, Hue represents the color component while Saturation controls the amount of color used and Value controls the brightness of the color. Thus for the studies related to the color information only the Hue component of HSV color space is used. In Figure 4 a soil sample image and its corresponding gray scale image and Hue component image are given.

$$Gray\ Scale\ İntensity = 0.2989.R + 0.5870.G + 0.1140.B \quad (2)$$

$$Hue = \left(60.\frac{G-B}{R-B}\right) + 360 \quad (3)$$



a b c

Figure 4. Different color space representations of a soil sample image. a) Original image, b) Gray scale image of the original sample, c) Hue component of the HSV color space image of the original sample.

3. RESULTS AND DISCUSSION

The confusion matrix belonging to the experimental studies performed on the testing data set is as given in Figure 5. A preprocessing step is performed before the experimental studies in the data set. In the preprocessing step the measured continuous pH values in Table 1 are converted to discrete pH values to be the Positive and the Negative two class. In this conversion while the Positive class indicates the pH values between 6.00 and 7.50 which is appropriate for plant growing the Negative class indicates the other pH values which does not allow the plant to grow. In the experimental studies 10 fold cross validation is used and 97.5 % pH level detection accuracy is obtained in the test data set which is a significantly high performance. Same experimental study is performed using both the gray scale images and HSV color space of images. In both it is obtained 97.5 % accuracy as it is the case using the RGB values. Same experimental study is also performed using another machine learning algorithm named decision trees (J48) and using the J48 algorithm 72% accuracy is obtained in the experimental studies.

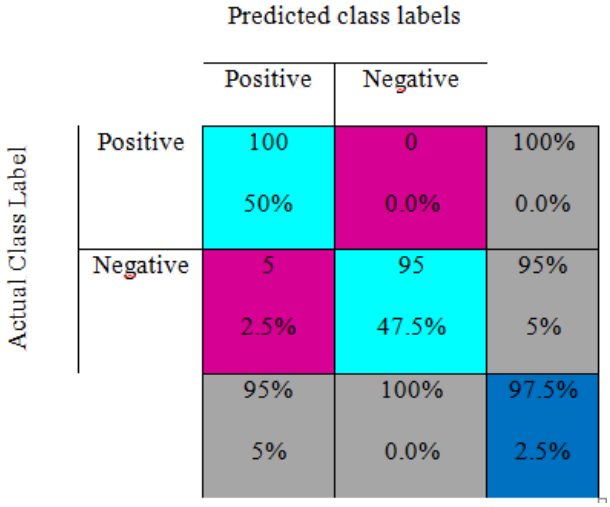


Figure 5. Confusion matrix of the test data set.

4. CONCLUSIONS

In precision agricultural cultivation applications the use of machine learning algorithms are important alternatives to the traditional methods. Because while they are providing an automatic, time effective and cost effective they also don't require any expertise. Besides, in the proposed method it does not need to transport soil samples to a laboratory or another place, and it does not require any technical sensing equipment. The only need is a camera or simply a smartphone. According to the experimental studies performed in this study it is shown that the proposed pH level detection method only using the soil images provides significantly high pH level detection performance for soil samples. The accuracy results obtained in this study reveals that the proposed method is a significantly important method to determine the pH levels of soil samples and could be a strong alternative to the traditional methods. It is thought that the proposed study will be an important guide for researchers studying in this field. We are planning to transform this study, which is presented in our future studies, into a larger-scale research in this field on a larger data set that we will construct ourselves in terms of the

DOI: http://dx.doi.org/10.32571/ijct.1107128 E-ISSN: 2602-277X

machine learning algorithms that are used and the soil properties.

Conflict of interests

The authors declare that they have no known competing financial interests or conflicts that could have appeared to influence the work reported in this paper.

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DOI: http://dx.doi.org/10.32571/ijct.1130734 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Copper catalyzed C–N bond formation and synthesis of imidazopyridinone derivatives

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Received: 14 June 2022; Revised: 21 June 2022; Accepted: 30 June 2022

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Citation: Doğan, Ş. D.; Ongun, M. *Int. J. Chem. Technol.* **2022**, 6 (1), 61-65.

ABSTRACT

The formation of the C–N bond is critical because it allows for the incorporation of nitrogen into organic molecules. Despite significant advances in this area, the formation of the C–N bond continues to be a challenge for organic chemists owing to the need of severe reaction conditions or costly catalysts in many cases. As a result, developing alternate, milder, and less expensive C–N bonding techniques is a challenge. Herein, a series of novel imidazopyridinone derivatives (**8a-8e**) were synthesized via copper-mediated C–N bond-forming reaction. This reaction takes place under mild conditions with high efficiency, step economy, and tolerance for a wide range of functional groups. All synthesized new compounds were analyzed by ^{1}H NMR, ^{13}C NMR and mass spectrometry.

Keywords: Synthesis, imidazopyridinones, Copper-catalyzed, C–N bond formation, urea.

Bakır katalizörlüğünde C–N bağı oluşumu ve imidazopiridinon türevlerinin sentezi

ÖZ

C–N bağının oluşumu, azot atomunun organik moleküllere katılmasında açısından oldukça önemlidir. C–N bağı oluşumu ile ilgili önemli gelişmeler kaydedilmesine rağmen zorlayıcı reaksiyon koşulları veya pahalı katalizörlere ihtiyaç duyulmasından dolayı organik kimyacılar için bu alan güncelliğini korumaktadır. Sonuç olarak alternatif olabilecek daha kolay ve daha ucuz yollu C–N bağı oluşturma tekniklerinin geliştirilmesi çalışmaları güncelliğini korumaktadır. Bu çalışmada imidazopiridinon türevleri (**8a-8e**) bakır katalizörlüğünde C–N bağı oluşturma reaksiyonu kullanılarak sentezlenmiştir. Bu C–N bağı oluşumu reaksiyonu oldukça kolay uygulanabilir koşullarda, yüksek verimlerle ve az basamaklı olarak farklı türevlerin sentezine uygulanabilmiştir. Sentezlenen yeni bileşiklerin yapı tayininde ^{1}H NMR, ^{13}C NMR ve kütle spektrumları kullanılmıştır.

Anahtar Kelimeler: Sentez, imidazopiridinon, bakır katalizörlüğü, C–N bağı oluşumu, üre.

1. INTRODUCTION

Imidazopyridinones (**1**) with a pyrido-fused cyclic urea framework are useful heterocyclic building blocks which are common structural element of compounds with a wide range of intriguing biochemical and pharmacological properties.[1,2] Imidazopyridinones and related cyclic urea derivatives were revealed to have antipyretic, antiulcer, antiviral, cytostatic, antimicrobial, cardiovascular properties, p38 MAPK and TNF-α inhibitory activities (Figure 1).[3,4]

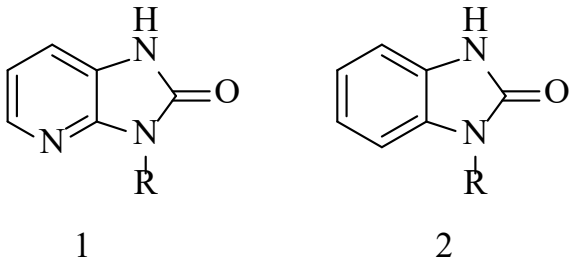

Figure 1. Important cyclic urea containing compounds

DOI: http://dx.doi.org/10.32571/ijct.1130734 E-ISSN: 2602-277X

As a consequence, the development of effective methods for the preparation of imidazopyridinones and formation of cyclic urea frame have received much attention. Due to the high demand, numerous synthetic routes to these interesting compounds have been developed, the most of which include 1,2-diaminopyridine as key intermediates.[5-7] Because their cyclization reactions have involved toxic chemicals such as phosgene, triphosgene and carbonyldiimidazole, limited availability of diversely substituted 1,2-diaminopyridine and harsh reaction condition, alternative protocols such as transition-metal-catalyzed intramolecular C−N bond formation have been developed.

Transition-metal-catalyzed C-N bond-forming reactions are of continuing interest. Several studies have revealed the generation of C-N bond formation by nitrogen nucleophilic displacement of aryl halogen catalyzed by transition metals.[8, 9] The transition-metal-catalyzed C−N cross-coupling methodologies for the synthesis of benzimidazole derivatives have also been reported.[8, 10] Liu and coworkers synthesized via a copper-catalyzed one-pot process N-substituted 1,3-dihydrobenzimidazol-2-ones (**2**) from N′-substituted N-(2-halophenyl)ureas under microwave heating.[11] But there are limited studies on its application to the synthesis of imidazopyridinones derivatives. Using the copper as a transition metal for the formation of C-N bond formation has received significant interest in the past two decades due to their effectiveness, low cost and more environmentally friendly compared to other metals.[12, 13]

In light of these considerations, we reported the protocol for the synthesis of imidazopyridinones, from *N*-(4-phenyl)-*N*'-(2-bromo-3-pyridinyl)-urea derivatives, via a copper (I)-catalyzed intramolecular cyclization process from N′-substituted N-(2-halopyridinyl)ureas using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base in dimethyl sulfoxide (DMSO) under microwave heating.

2. MATERIALS AND METHODS

2.1. General procedure for the synthesis of urea derivatives 7a-7e

7a-7e were synthesized according to the reported literature procedure. Briefly, they are obtained by the reaction of refluxing the acyl azide **6** in dry benzene with corresponding amine to produce the urea derivatives. The resulting residue obtained upon the completion of the reaction was purified by crystallization from ethanol to afford the compounds **7a-7e**.[14, 15]

2.2. General procedure for the synthesis of imidazopyridinones derivatives (8a-8e)

A mixture of urea derivative **7a-7e** in 1 mL DMSO, CuI (0.2 equiv) and DBU (2 equiv) were taken in a glass vial and heated under microwave at a power of 60 W for 30 min (TLC). After completion of the reaction monitored by TLC, the reaction mixture was extracted with EtOAc (3x25 mL). The combined EtOAc layers were then dried over anhydrous $MgSO_4$ and removed under reduced pressure. The crude product was purified by column chromatography to afford pure compounds **8a-8e.**

2.2.1. 3-Phenyl- 1,3-dihydro- 2H-imidazo [4,5-b] pyridin-2-one (8a)

Brown solid, yield: 53%. mp 233-234 °C; R_f (50% Hexane/EtOAc):0.23; IR (ATR) 3662, 2987, 2900, 1686, 1594, 1393, 1229, 1065. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H, -NH), 7.92 (dd, J = 5.2, 1.5 Hz, 1H), 7.66 – 7.62 (m, 2H), 7.52 (t, J = 7.9 Hz, 2H), 7.43 – 7.37 (m, 2H), 7.09 (dd, J = 7.7, 5.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 153.06, 144.23, 140.28, 133.91, 129.25, 127.67, 126.65, 123.24, 118.52, 115.90. HRMS (EI): $[M+H]^+$, found 212.0835. $C_{12}H_{10}N_3O$ calculated 212.0824.

2.2.2. 3-(4-Methoxyphenyl)- 1,3-dihydro- 2H-imidazo[4,5-b] pyridin-2-one (8b)

White solid, yield: 50%. mp 252-254 °C; R_f (50% Hexane/EtOAc):0.14; IR (ATR) 3663, 2988, 2906, 1708, 1623, 1517, 1386, 1231, 1066. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H), 7.90 (d, J = 5.2 Hz, 1H), 7.49 (d, J = 8.6 Hz, 2H), 7.37 (d, J = 7.7 Hz, 1H), 7.17 – 6.97 (m,3H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 158.69, 153.31, 144.55, 140.28, 128.26, 126.48, 123.15, 118.30, 115.77, 114.52, 55.84. HRMS (EI): $[M+H]^+$, found 242.0925. $C_{13}H_{12}N_3O_2$ calculated 242.0930

2.2.3. 3-(4-Chlorophenyl)-1,3-dihydro-2H-imidazo[4,5-b]pyridin-2-one (8c)

White solid, yield: 67%. mp 225-226 °C; R_f (50% Hexane/EtOAc):0.28; IR (ATR) 3662, 2985, 2887, 1713, 1496, 1384, 1229, 1066, 891. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H, -NH), 7.94 (dd, J = 5.3, 1.5 Hz, 1H), 7.74 (dd, J = 9.1, 2.5 Hz, 2H), 7.64 – 7.55 (m, 2H), 7.41 (dd, J = 7.7, 1.4 Hz, 1H), 7.11 (dd, J = 7.7, 5.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 152.85, 143.90, 140.29, 132.89, 131.76, 129.23, 128.04, 123.30, 118.75, 116.09. HRMS (EI): $[M+H]^+$, found 246.0431. $C_{12}H_9ClN_3O$ calculated 246.0434.

2.2.4. 3-(4-Nitrophenyl)-1,3-dihydro-2H-imidazo[4,5-b]pyridin-2-one (8d)

Yelow solid, yield: 31%. mp decomposition above 290°C; R_f (50% Hexane/EtOAc):0.26; IR (ATR) 366, 2987, 2875, 1729, 1592, 1504, 1383, 1348, 1229, 1066. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44-11.03 (brs, 1H, -NH), 8.40 (d, J = 8.5 Hz, 2H), 8.16 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 5.2 Hz, 1H), 7.46 (d, J = 7.7 Hz, 1H), 7.25 – 7.13 (m, 1H). ^{13}C NMR (100 MHz, DMSO) δ 152.54, 145.45, 143.38, 140.36, 140.18, 125.90, 124.67, 123.57,

DOI: http://dx.doi.org/10.32571/ijct.1130734 E-ISSN: 2602-277X

119.42, 116.55. HRMS (EI): $[M+H]^+$, found 257.0662. $C_{12}H_9N_4O3$ calculated 257.0675.

2.2.5. 3-(2-Aminophenyl)-1,3- dihydro- 2H-imidazo [4,5-b]pyridin-2-one (8e)

Brown solid, yield: 33%. mp 248-250°C; R_f (50% Hexane/EtOAc):0.34; IR (ATR). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H, -NH), 7.82 (d, J = 5.2 Hz, 1H), 7.32 (dd, J = 7.7, 1.6 Hz, 1H), 7.19 – 7.10 (m, 1H), 7.01 (dd, J = 7.6, 5.3 Hz, 2H), 6.84 – 6.78 (m, 1H), 6.66 – 6.54 (m, 1H), 5.03 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 153.33, 146.47, 145.11, 139.98, 130.31, 129.76, 123.94, 120.89, 117.81, 116.28, 115.26, 108.95. HRMS (EI): $[M+H]^+$, found 227.0933. $C_{12}H_{11}N_4O$ calculated 227.0923.

3. RESULTS AND DISCUSSION

In order to apply the C-N bond formation procedure and synthesis of imidazopyridinones derivatives, corresponding N-(4-phenyl)-N'-(2-bromo-3-pyridinyl)-urea derivatives (**7a-7e**) were synthesized as a starting compounds (Figure 2). The starting scaffold N-(4-phenyl)-N'-(2-bromo-3-pyridinyl)-urea derivatives (**7**) was obtained in accordance with previously published protocols. The bromination reaction of **3** was performed in hydrobromic acid at °0 C, gave the bromination product **4**. [16] The oxidation of bromination product **4** to 2-bromonicotinic acid (**5**) was carried out in water using $KMnO_4$.[17] Urea derivatives **(7a-7e)** were achieved from corresponding acyl azides **6** which were obtained by the treatment of 2-bromonicotinic acid (**5**) with ethyl chloroformate in the presence of Et_3N followed by the addition of NaN_3 aqueous solution. Refluxing the acyl azide in dry aprotic solvent (benzene) initiated rearrangement (Curtius rearrangement) to the corresponding isocyanate which was then reacted with corresponding amine to produce the urea derivatives.[14] In order to reveal the effects of electron donating and electron withdrawing substituents in the C-N bond formation procedure, five different urea derivatives (**7a-7e**) were synthesized.

For the synthesis of imidazopyridinones derivatives via C-N bond formation, we adapted the procedure applied to the synthesis of dihydrobenzimidazol-2-one derivatives known in the literature to our own system [11]. The target compounds, imidazopyridinones derivatives **8a-8e**, were synthesized via C-N bond formation using copper iodide as catalyst and DBU as a base. Treatment of **7** with CuI in DMSO in the presence of DBU afforded the desired cyclized compound **8a-8e** (Figure 3) via intramolecular C-N bond formation reaction but in a modest yield (31-67%). In all cases, the final products were purified by silica gel chromatography.

Postulated structures of the newly synthesized imidazopyridinones derivatives (**8a-8e**) were in full agreement with their spectral data. Although ^{1}H NMR spectra of pyridine derivatives (**7a-7e**) revealed the presence of two D_2O exchangeable singlet signals attributable to the -NH groups of the urea group in the regions δ 10.30 – 8.39 and 8.54 – 8.29 ppm, imidazopyridinones derivatives (**8a-8e**) showed only one D_2O exchangeable singlet signals attributable to the -NH groups of the cyclic urea group in the regions δ 12.44 – 11.03 ppm. On the other hand, ^{13}C-NMR spectra of compounds **8a-8e** confirmed with presence of C=O peak of cyclic urea motif at 158.69 – 152.54 ppm. The experimental and supplementary information sections require a detailed procedures and spectral data for the target compounds.

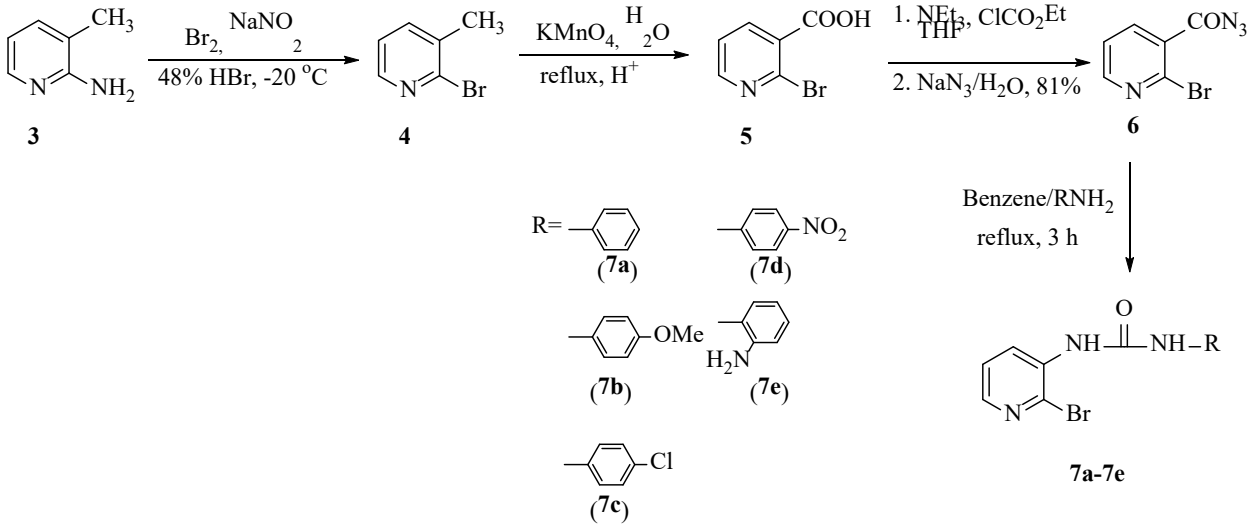

Figure 2. Synthesis of starting compound **7a-7e**

DOI: http://dx.doi.org/10.32571/ijct.1130734 E-ISSN: 2602-277X

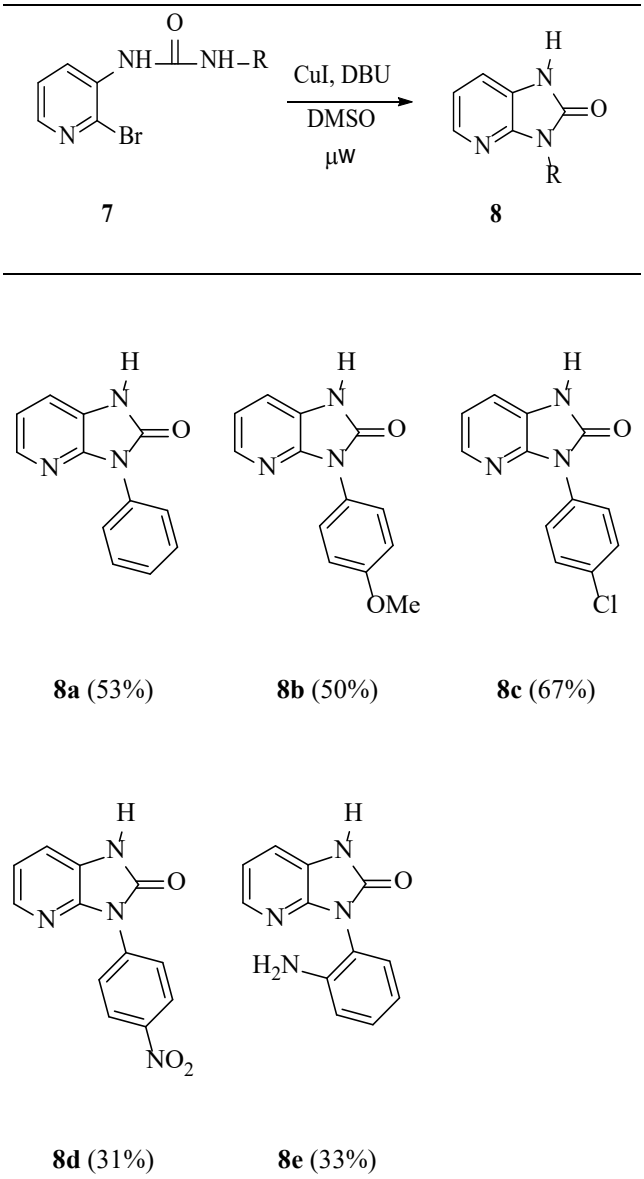

Figure 3. Synthesis of imidazopyridinones derivatives **8a-8e**

4. CONCLUSIONS

In this paper, a new series of imidazopyridinone compounds has been successfully designed and synthesized (**8a-8e**) via Cu(I)-mediated/MW-assisted C-N bond formation of N-(4-phenyl)-N'-(2-bromo-3-pyridinyl)-urea derivatives **7a-7e**. This synthetic strategy allows the synthesis of libraries rich in biological active compounds.

ACKNOWLEDGEMENTS

The author is indebted to the Faculty of Pharmacy at Erciyes University for the use of faculty facilities. This study was published as Melike Ongun's graduation project thesis (2018).

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DOI: http://dx.doi.org/10.32571/ijct.1130734 **E-ISSN: 2602-277X**

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DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

The usage of palm *(Washingtonia filifera)* fibers for the removal of crystal violet from synthetic dye solution by adsorption

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Received: 16 June 2022; Revised: 26 June 2022; Accepted: 27 June 2022

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Citation: Keskinkan, O.; Karabaş, B.; Sarı, B.; Yeşiltaş, H. K.; Ersü, Ç. B. *Int. J. Chem. Technol.* **2022**, 6 (1), 66-75.

ABSTRACT

The objective to obtain cheap and easily synthesized adsorbents from natural materials is gaining importance day by day. Adsorbents should be environmentally friendly, non-toxic, easily produced, insoluble in water, have a porous structure, have a large surface area, and be scientifically accepted. In this study, the removal of crystal violet dye from synthetic dyestuff solution was investigated using palm tree *(Washingtonia filifera)* fibers. In order to determine the contact time, the first set of experiments employed 0.5 g of palm fibers and initial dye concentrations between 2.5-160 mg/L. As a result of the study, it was determined that the crystal violet removal was 87.96% at the end of the 180-minute contact time at equilibrium, and the removal complied with the pseudo-second-order kinetic model type 1. The equilibrium time for the highest initial adsorbate concentration (160 mg/L) was 2880 minutes (2 days) in stationary phase systems while it was 180 minutes (3 hours) in mobile phase systems. It was also understood that palm fiber, which is an environmentally advantageous material, can be used in the removal of crystal violet dyestuff.

Keywords: *Washingtonia filifera*, dyestuff removal, crystal violet, chemical kinetic.

Sentetik boyar madde çözeltisinden kristal viyolenin adsorpsiyonla gideriminde palmiye (*Washingtonia filifera*) liflerinin kullanılması

ÖZ

Doğal malzemelerden düşük maliyetli ve kolay sentezlenen adsorbanlar elde etme hedefi her geçen gün önem kazanmaktadır. Adsorbanlar çevre dostu, toksik olmayan, kolayca üretilebilen, suda çözünmeyen, gözenekli bir yapıya sahip, geniş bir yüzey alanına sahip ve bilimsel olarak kabul görmüş olmalıdır. Bu çalışmada, palmiye ağacı *(Washingtonia filifera)* lifleri kullanılarak sentetik boyarmadde çözeltisinden kristal viyole boyanın giderimi araştırılmıştır. Temas süresini belirlemek için, ilk deney grubu 0,5 g palmiye lifi ve 2,5-160 mg/L arasındaki başlangıç boya konsantrasyonları kullanılmıştır. Araştırma sonucunda ise dengeye 180 dakikalık temas süresi sonunda kristal viyole gideriminin %87,96 olduğu ve giderimin yalancı ikinci dereceden kinetik model tip 1'e uygun olduğu belirlenmiştir. En yüksek başlangıç adsorbat konsantrasyonu (160 mg/L) için sabit fazlı sistemlerde 2880 dakika (2 gün), mobil fazlı sistemlerde ise 180 dakikada (3 saat) dengeye ulaşılmıştır. Çevre açısından avantajlı bir malzeme olan palmiye lifinin de kristal viyole boyar maddelerin uzaklaştırılmasında kullanılabileceği tespit edilmiştir.

Anahtar Kelimeler: *Washingtonia filifera*, boyarmadde giderimi, kristal viyole, kimyasal kinetik.

1. INTRODUCTION

Today, dyestuffs are used as auxiliary substances in the production activities of industries that include printing and dyeing processes such as paper, cosmetics, leather, pharmaceuticals, especially the textile industry. The amount and type of dyestuffs used in the relevant production processes are highly variable according to the production process and product variety.[1-3] It is estimated that the amount of dyestuff produced annually in the world is more than 70,000 tons and that more than 10,000 types of dyestuffs are introduced to the market every year.[4] All of the dyestuffs produced for industrial utilization are not used efficiently in the process, and their residues pass into the wastewater of the process.[1,5] In the case of dyestuff containing wastewater discharges

DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

to receiving environments, the dyestuff reduces the light transmittance of the system. On the other hand, the photosynthetic ability of the system with reduced light transmittance decreases and the amount of dissolved oxygen originating from photosynthesis decreases.[4] As a result of this undesirable situation, the organisms living in the water are adversely affected and the efficiency obtained from the system decreases. As a result of such discharges to the receiving environment, the ecosystem of the receiving environment is adversely affected and an aesthetically undesirable appearance occurs. In addition, dyestuffs can cause various acute and chronic health problems for living things due to the impurities in their structure. Therefore, it is important for the environment and public health to treat and monitor wastewater containing dyestuffs regularly before they are discharged to receiving environments.[3,6]

Various treatment processes, including biological, chemical and physical methods, are used in the removal of dyestuffs from wastewater. Biological treatment methods are evaluated under aerobic and anaerobic systems. Biological treatment methods are systems utilizing living organisms and cannot be operated efficiently at high dyestuff concentrations. In addition, due to the living organisms it contains during the operation of the biological system, biological treatment methods are more sensitive to contents of the wastewater than chemical and physical processes.[4] Coagulation-flocculation and precipitation method, Fenton process, and various oxidation processes are among the chemical methods used in dyestuff removal. Various operational problems such as energy consumption, use of chemicals and formation of chemical waste sludge are among the disadvantages of these processes.[5] Membrane filtration methods, ion exchange, and adsorption methods can be given as examples of physical treatment methods. Physical treatment methods have been preferred more than biological and chemical treatment methods in recent years in terms of high removal efficiency and ease of operation in dyestuff removal.[4,5]

Membrane filtration systems, which are among the physical treatment methods, are pressurized systems and the protection of the membranes is important for the efficiency of the system. In addition, the disposal of the concentrated waste formed within the membrane is a disadvantage of this system. In the ion exchange process, not only the dyestuff is removed, but also the anions and cations present in the wastewater are removed. In this case, the operating time of the system will decrease and the regeneration will be needed in shorter periods. The adsorption method, on the other hand, is more popular among physical treatment methods and is widely preferred because it can be used for high dyestuff concentrations. In addition, obtaining high removal efficiencies by the adsorption method is also an important criterion in the preference of the process.[4,5,7]

The adsorbents to be used in the adsorption process should primarily be of low cost and sufficient efficiency. Nowadays, due to the increasing environmental pollution, adsorbents produced from wastes instead of conventional activated carbon or materials prepared by carbonization attract more attention today. Adsorbents should also be environmentally friendly, non-toxic, easily produced, insoluble in water, have a porous structure, large surface area, and scientifically accepted.[5,7,8] Adsorption is similar to an equilibrium reaction and continues until a dynamic equilibrium is reached between the solute concentration remaining in the solution and the solute concentration attached onto the adsorbent surface. In order to indicate the adsorption equilibrium, the amount of adsorbate in unit weight of the solid adsorbent against the solute concentration remaining in the solution at constant temperature is plotted. Generally, these curves are non-linear and are called adsorption isotherms.[9]

Adsorption is an effective way to obtain high quality effluent that does not produce any harmful waste in the treatment of high-flow wastewater.[10] Activated carbon columns are used in many industries for the treatment of toxic, non-biodegradable wastes and also as a final treatment step after biological oxidation processes.[11] Activated carbon is an effective adsorbent widely used in dye removal, and adsorption is still the leading treatment method. However, some problems arise in the use of activated carbon. Activated carbon is an expensive product and the higher the quality, the higher the price. From this point of view, the interest for a cheap and easily available adsorbent for dye removal is increasing day by day.[12] There are many researchers doing various studies to find such an adsorbent. Among these researches, it is possible to come across various biological materials, both dry and wet.[13] These include lichen,[14] algae,[15] tree and fruit residues,[16-19] seeds,[20-23] and aquatic plants.[12,24]

Although some adsorption studies with palm species were found among previous studies, no adsorption studies with *Washingtonia filifera* fibers were found. In this study, palm (*Washingtonia filifera*) tree fibers were used to remove the crystal violet dyestuff. In addition, the removal values obtained in the removal study were used as data in the zero-order, first-order, second-order, four types of false-second-order, and Elovich kinetic models, and to determine which kinetic model could explain the removal better.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Adsorbent

The palm tree *(Washingtonia filifera)* used in this study is widely distributed in the Mediterranean region. During

DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

the preparation of the adsorbent, *Washingtonia filifera* fibers were first soaked in water until the woody layer on it was peeled off. Following the soaking process, the brown woody layer on the palm fibers was peeled off and then the fibers were washed and left to dry at room temperature. Following the drying process, the fibers were cut to 1 cm in length and the adsorbent preparation process was completed.

2.1.2. Preparation of dyestuff (Crystal Violet) solution

Crystal violet dyestuff ($C_{25}H_{30}N_3Cl$) was obtained from Merck and the dyestuff solution to be used in this research was prepared synthetically. Synthetic wastewater solutions were prepared to contain 1.25, 2.5, 5, 10, 20, 40, 80, and 160 mg/L crystal violet concentration. The general structure of the crystal violet dyestuff is given in Figure 1.

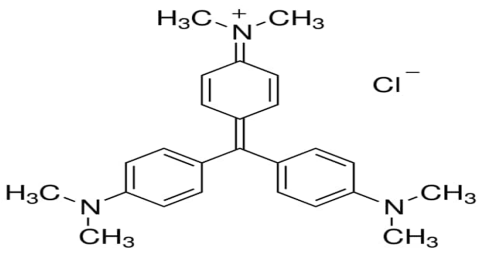


Figure 1. General structure of crystal violet dyestuff. [25]

2.2. Method

2.2.1. Adsorption experiments

Dye removal studies were carried out separately at 8 different dye concentrations (1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg/L) under stationary and mobile phase (300 rpm). The amount of adsorbent to be used in the study was chosen as 0.5 g, and the study was carried out at 25°C ambient temperature with the initial pH value of 6.5. The removal study was carried out with a contact time of 4 day in the absence of the mixing and was limited to 4 hours in the reactor where the mixing was maintained. In addition, the sampling was done for 9 (60, 120, 180, 240, 300, 1440, 2880, 4320 and 5760 minutes) and 13 (5, 10, 15, 30, 45, 60, 80, 100, 120, 150, 180, 210 and 240 minutes) different time periods in stationary and mobile reactors, respectively. The amount of adsorption at equilibrium, q_e (mg/g), was calculated by Equation 1.

$$q_e = \frac{(C_0 - C_e)xV}{W} \quad \text{(Equation 1)}$$

In Equation 1, C_0 and C_e are the liquid-phase concentrations of dye at initial and after a certain time (mg/L), V (L) ise the volume of the solution, and W (g) is the dry mass of sorbent used.

The crystal violet removal was observed after specified time periods. The dyestuff measurements in this study were carried out at 590 nm, where the crystal violet dyestuff demonstrates the highest adsorbance value using Perkin Elmer brand Lambda 35 UV/VIS model spectrophotometer.

2.2.2. Kinetic analysis

Data obtained in adsorption experiments were applied to zero-order, first-order, second-order, four types of pseudo-second-order and Elovich kinetic models. The equations, linear expressions, indices, and kinetic parameters of the related kinetic models are given in Table 1. The calculation of the q_t value shown in Table 1 is done using Equation 2.[26]

Table 1. Kinetic Models and Linear Expressions (KN= Intercept, E= Slope).

Model	Equation	Lineer Description	Plot	Parameters
Zero Order	$q_t = q_e - k_0 t$	$q_t = q_e - k_0 t$	q_t *and* t	$q_e = KN$ $k_0 = -E$
First Order	$q_t = q_e \exp(k_1 t)$	$\ln\frac{q_e}{q_t} = k_1 t$	*ln* q_t *and* t	$q_e = KN$ $k_1 = -E$
Second Order	$q_t = q_e/(1 + q_e k_2 t)$	$q_t^{-1} = q_e^{-1} + k_2 t$	q_t^{-1} *and* t	$q_e = KN^{-1}$ $k_2 = E$
Elovich	$q_t = \beta \ln(\alpha\beta t)$	$q_t = \beta \ln(\alpha\beta) + \beta \ln t$	q_t *and ln* t	$\beta = E$ $\alpha = \frac{1}{E} e^{(\frac{KN}{E})}$
Pseudo Second Order	$q_t = \frac{k_{2p} q_e^2 t}{(1 + q_e k_{2p} t)}$	Type (1) $t/q_t = 1/k_{2p}q_e^2 + t/q_e$	t/q_t *and* t	$q_e = E^{-1}$ $k_{2p} = E^2/KN$
		Type (II) $\frac{1}{q_t} = \left(\frac{1}{k_{2p}q_e^2}\right)\left(\frac{1}{t}\right) + \left(\frac{1}{q_e}\right)$	$1/q_t$ *and* $1/t$	$q_e = KN^{-1}$ $k_{2p} = KN^2/E$
		Type (III) $q_t = q_e - (1/k_{2p}q_e)q_t/t$	q_t *and* q_t/t	$q_e = KN$ k_{2p}=1/(E*KN)
		Type (IV) $\frac{q_t}{t} = k_{2p}q_e^2 - k_{2p}q_e q_t$	q_t/t *and* q_t	$q_e = -KN/E$ $k_{2p} = E^2/KN$

DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

$$q_t = \frac{(C_0 - C_t)xV}{W} \quad \text{(Equation 2)}$$

The term, q_t, expressed in Equation 1 is the amount of adsorbate (dyestuff) retained per unit adsorbent (mg/g), C_0 and C_t values are the concentrations of dyestuff in the solution at the beginning and measured at time t, respectively, V is the solution volume in the reactor, and W expresses the amount of adsorbent.[26]

3. RESULTS AND DISCUSSION

3.1. Equilibrium Time

The expression of the amount of substance removed over time (q_e) is a measure of the dyestuff capacity of the present material. The q_e values of the values determined as a result of the adsorption study are given in Figures 2 and 3 for the stationary and mobile phases, respectively.

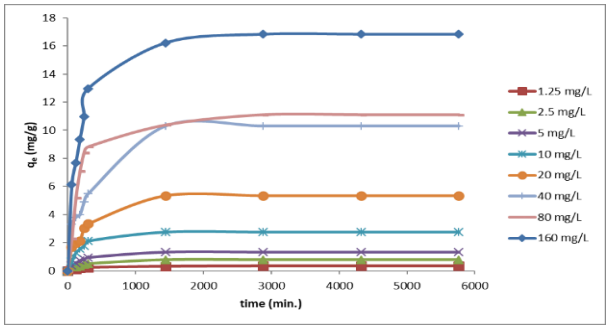


Figure 2. Stationary phase q_e plot.

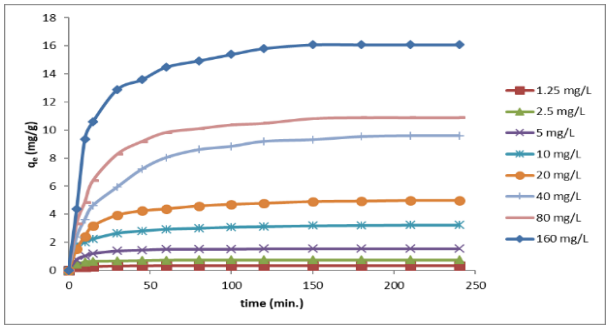


Figure 3. Mobile phase q_e plot.

Due to the empty surfaces of the material at the beginning of the adsorption and the high concentration of the dyestuff in the solution, the adhesion to the surface is fast at the beginning. As a result of the saturation of the surface with the dyestuff, the amount of the removed substance decreases as time progresses. In Figure 2 and Figure 3, it is observed that the dyestuff is removed more quickly at the beginning and it is understood that the amount of substance retained in the material decreases as time progresses. The equilibrium time for the highest initial adsorbate concentration (160 mg/L) was 2880 minutes (2 days) in stationary phase system while it was 180 minutes (3 hours) in mobile phase system. Sulyman et al. studied crystal violet removal with date palm (L.) Dead leaflets (*Phoenix dactylifera*) obtained an equilibrium capacity value of 65.55 mg/g using 500 mg/L initial crystal violet solution.[27] El-Sayed et al. studied crystal violet removal with palm kernel (*Phoenix dactylifera*) fibers. The researchers determined the adsorption equilibrium capacity to be 52.9 mg/g in the study they carried out with an initial dye concentration of 160 mg/L.[28] In this study, the capacity values reached at the moment of equilibrium in stationary and mobile phases (160 mg/L initial crystal violet solution) are 16.82 mg/g and 16.08 mg/g, respectively.

3.2. Results of Adsorption Experiments

In this study, the highest removal efficiencies obtained in stationary and mobile phase studies were determined as 75.17% and 88.73%, respectively. The removal efficiencies and initial solution concentrations determined in all study sets of the crystal violet removal study are given in Table 2 and Table 3 for stationary and mobile conditions, respectively. Sultana et al. studied crystal violet removal with powder adsorbent obtained from coconut shell. The researchers achieved 91% crystal violet removal after 150 minutes of contact time. They also found that the adsorption process slowed down after the 150th minute.[29] Rani et al., on the other hand, studied the removal of crystal violet dye from wastewater with adsorbent material prepared from citrus peel (*Citrus limetta*).

Table 2. Removal efficiencies and initial crystal violet concentrations under stationary operating conditions.

Time (t, minute)	Initial crystal violet concentration (C_0, mg/L)							
	1.25	2.50	5	10	20	40	80	160
	Removal, %							
60	17.08	24.19	20.16	18.35	20.84	20.14	7.39	9.42
120	20.10	25.82	28.55	34.56	24.30	23.19	16.77	11.79
180	31.39	32.04	38.47	42.22	26.36	24.34	22.96	14.32
240	39.64	46.26	42.33	47.95	37.12	29.79	27.24	16.79
300	46.46	48.86	49.46	57.76	41.04	33.47	28.61	19.87
1440	61.26	75.14	69.65	75.17	65.67	62.83	33.65	24.84
2880	67.23	75.14	69.65	75.17	65.67	62.83	36.01	25.77
4320	67.23	75.14	69.65	75.17	65.67	62.83	36.01	25.77
5760	67.23	75.14	69.65	75.17	65.67	62.83	36.01	25.77

DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

As a result of the study, they achieved 89.87% crystal violet removal.[30] Kumbhar et al. obtained 98.7% crystal violet removal with the composite adsorbent they synthesized using tea waste and iron oxide (Fe_3O_4). In addition, the found that the efficiency of crystal violet removal decreased when the pH value was more or less than 7.[31]

Table 3. Removal efficiencies and initial crystal violet concentrations under mobile operating conditions.

Time (t, minute)	Initial crystal violet concentration (C_0, mg/L)							
	1.25	2.50	5	10	20	40	80	160
	Removal, %							
5	51.72	47.81	43.34	47.59	20.11	15.67	11.39	7.12
10	54.31	66.67	57.79	55.36	31.97	23.85	16.60	15.24
15	57.76	71.49	68.17	61.38	42.23	30.41	21.87	17.30
3	67.24	74.56	78.56	72.87	52.60	39.13	28.35	21.06
45	68.97	77.63	81.94	77.13	56.61	47.47	31.41	22.19
60	72.41	81.14	85.55	80.63	58.58	52.95	33.63	23.66
80	72.41	81.58	85.78	82.60	61.36	56.69	34.55	24.40
100	72.41	81.58	86.00	84.68	62.86	58.21	35.45	25.13
120	72.41	82.46	87.13	85.78	63.93	60.56	35.85	25.83
150	72.41	82.46	87.13	87.31	65.53	61.37	36.99	26.26
180	72.41	82.46	87.58	87.96	65.96	62.82	37.23	26.26
210	72.41	82.46	87.58	88.73	66.60	63.22	37.23	26.26
240	72.41	82.46	87.58	88.73	66.60	63.22	37.23	26.26

As a result of their study for crystal violet removal with processed date palm *(Phoenix dactylifera)* at pH 6.5, Sulyman et al. determined the highest removal value as 96% .[27] Alshabanat et al., with a similar study, obtained approximately 98.5% removal in mobile medium and at pH value of 2.[32] El-Sayed performed a crystal violet removal study with non-processed (raw) palm kernel *(Phoenix dactylifera)* fibers and reached approximately 85% removal at pH 7.2 using 0.05 g adsorbent.[28]

In this study, 88.73% removal efficiency was obtained using 0.5 g *Washingtonia filifera* fibers without any physico-chemical treatment. The summary chart of related studies and this study are given in Table 4. In their study regarding crystal violet removal with *Carpobrotus edulis* plant, Dabagh et al., reported that the increase or decrease of the pH value did not have a significant effect on the dye removal.[33]

Table 4. Previous studies summarizing the highest crystal violet removal rates.

Researchers	Adsorbant	Study Conditions	Removal, %
Sultana et al., 2022[29]	Coconut shell	200 rpm mixing speed 150 minute contact time 0,1 g amount of adsorbent pH 5 36 mg/L crystal violet initial concentration Synthetic wastewater	91
Rani et al., 2022[30]	Citrus peel *(Citrus limetta)*	200 rpm mixing speed 120 minute contact time 0.5 g amount of adsorbent pH 7 20 mg/L crystal violet initial concentration Synthetic wastewater	89.87
Kumbhar et al, 2022[31]	Tea waste /Fe_3O_4	Mobile reactor 90 minute contact time 2 g/L amount of adsorbent 100 mg/L crystal violet initial concentration pH 7 Synthetic wastewater	98.71
Sulyman et al, 2016[27]	Date palm (L.) dead leaflets *(Phoenix dactylifera)*	200 rpm mixing speed 30 minute contact time 50 mg/L amount of adsorbent 5 mg/L crystal violet initial concentration pH 6,5 Synthetic wastewater	96
Alshabanat et al., 2013[32]	Date palm fiber *(Phoenix dactylifera)*	100 rpm mixing speed 0,25 g amount of adsorbent $3*10^{-5}$ mol/L crystal violet initial concentration pH 2 Synthetic wastewater	≅ 98.5

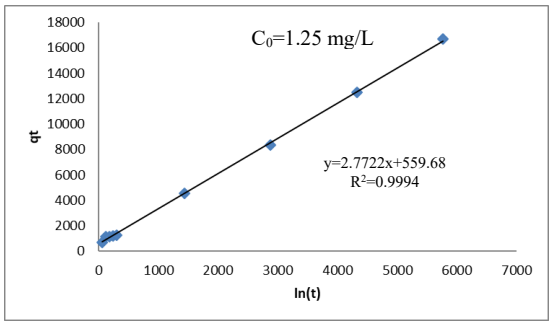
DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

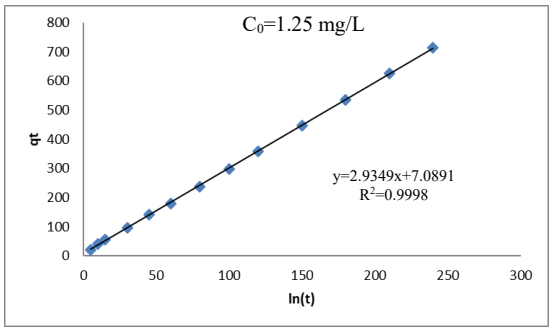
Table 4. Continued

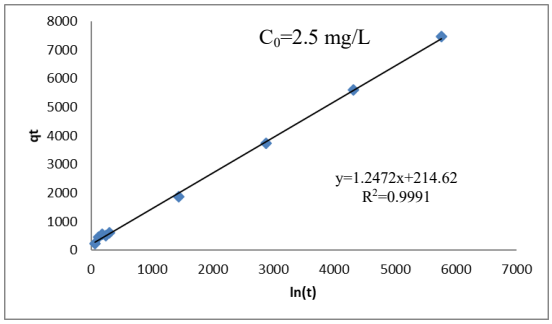
El-Sayed, 2011[28]	Palm kernel fiber *(Phoenix dactylifera)*	200 rpm mixing speed 60 minute contact time 0,05 g amount of adsorbent 0,15 mg/L crystal violet initial concentration pH 7.2 Synthetic wastewater	≅ 85
Current Study	**Palm fiber *(Washingtonia filifera)***	**300 rpm mixing speed** **210 minute contact time** **0.5 g adsorban miktarı** **pH 8.5** **10 mg/L crystal violet initial concentration** **synthetic wastewater**	**88.73**

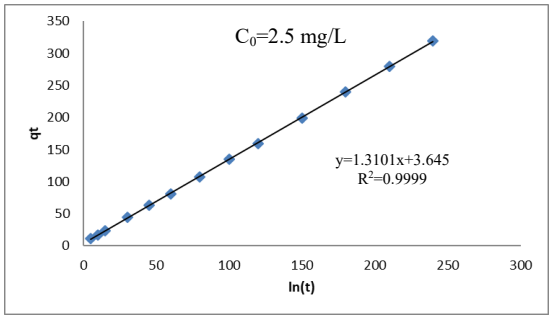
3.3. Kinetic Analysis

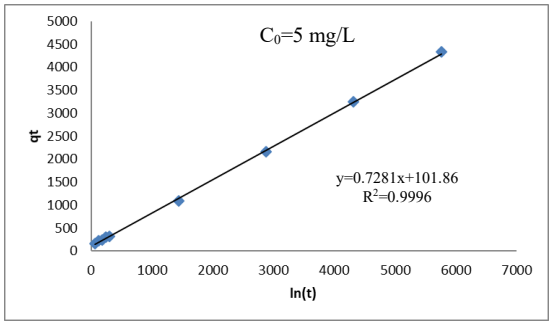
While performing the adsorption, the expression of speed is important in terms of understanding the relationship between the adsorbent and the adsorbate.[27,30,31] The removal data obtained under stationary and mobile test conditions were used as data in the kinetic models as shown in Table 1. As a result of the kinetic analyses, it was determined that the crystal violet removal was more suitable for the pseudo-second order kinetic equation type I for all study sets. In Table 5, the kinetic coefficients of all kinetic models are given, whereas in Figure 4, the graphs of pseudo-second-order kinetic equation type I are provided for stationary and mobile experimental sets.

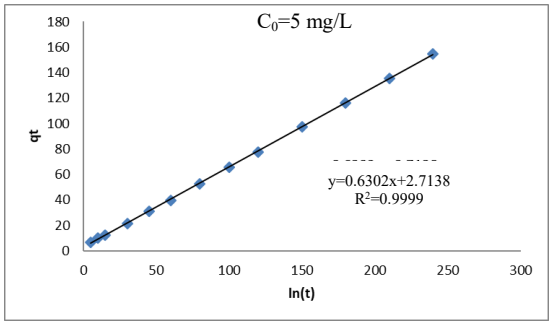




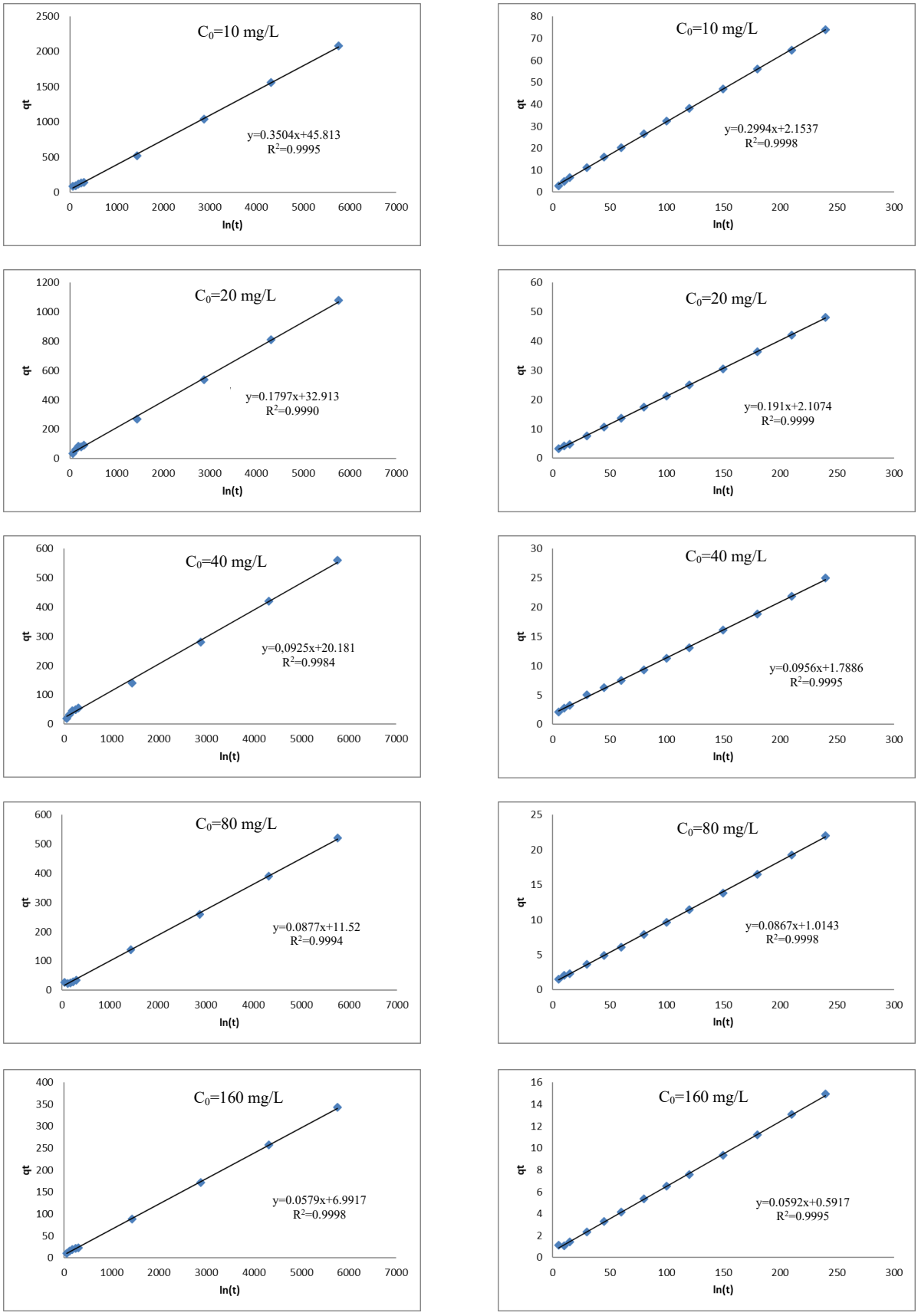








DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X



(a) **(b)**

Figure 4. Pseudo-second order type I plots of stationary (a) and mobile (b) test sets removal of crystal violate using palm (*Washingtonia filifera*) fibers from synthetic dye solition.

DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

Table 5. Coefficients of all kinetic models.

Kinetic Model		Parameter	C_0, mg/L							
			1.25	2.5	5	10	20	40	80	160
Stationary Phase										
Zero Order		q_e, mg/g	0.1708	0.3982	0.7379	1.5863	2.6818	4.8133	6.6301	9.9593
		k, mg/g.min	-0.00004	-0.00009	-0.0001	-0.0003	-0.0006	-0.0013	-0.001	-0.0016
		R^2	0.6537	0.638	0.6106	0.5621	0.6607	0.6975	0.5053	0.6219
First Order		q_e, mg/g	-1.8528	-0.9881	0.3697	0.3784	0.9245	1.5163	1.7863	2.2544
		k, mg/g.min	-0.0002	-0.0002	-0.0002	-0.0001	-0.0002	0.0002	0.0001	-0.0001
		R^2	0.5345	0.5709	0.517	0.4392	0.6046	0.668	0.3481	0.544
Second Order		q_e, mg/g	0.1435	0.3505	0.6452	1.3220	2.3906	4.3706	5.1466	9.1157
		k, mg/g.min	-0.0009	-0.0004	-0.0002	-0.00009	-0.00005	-0.00003	0.00002	-0.00001
		R^2	0.4072	0.4873	0.3976	0.2981	0.5268	0.6148	0.2148	0.4532
Pseudo Second Order	**Type I**	Q, mg/g	0.3607	0.8017	1.3734	2.8538	5.5648	10.8108	11.4025	17.2711
		k, mg/g.min	0.0137	0.0072	0.0052	0.0026	0.0009	0.0004	0.0006	0.0004
		R^2	0.9994	0.9991	0.9996	0.9995	0.999	0.9984	0.9994	0.9998
	Type II	q_e, mg/g	0.3475	0.7129	1.3852	3.2637	4.8239	8.6206	15.5038	16.5562
		k, mg/g.min	0.0146	0.0103	0.0044	0.0014	0.0015	0.0009	0.0002	0.0005
		R^2	0.9288	0.8587	0.9904	0.982	0.8715	0.8195	0.9306	0.9602
	Type III	q_e, mg/g	0.3584	1.2934	1.4054	2.9803	5.3151	9.8538	11.599	17.116
		k, mg/g.min	0.0138	-0.0037	0.0042	0.0019	0.0011	0.0006	0.0005	0.0004
		R^2	0.8916	0.8213	0.9764	0.9322	0.8275	0.7456	0.7121	0.9384
	Type IV	q_e, mg/g	0.3636	0.8269	1.4067	3.0363	5.6470	10.9565	12.9183	17.4545
		k, mg/g.min	0.0121	0.0062	0.0041	0.0018	0.0009	0.0004	0.0003	0.0004
		R^2	0.8916	0.8213	0.9764	0.9322	0.8275	0.7456	0.7121	0.9384
Elovich		β, g/mg	0.0602	1.2472	0.2134	0.4377	0.9216	1.847	-1.6575	-0.0077
		α, mg/g.min	1.4693	4.35E+74	0.7437	12.5035	0.1037	0.0392	-2.3090	0.0000034
		R^2	0.9375	0.9252	0.9276	0.8963	0.9361	0.9389	0.8341	0.9267
Mobile Phase										
Zero Order		q_e, mg/g	0.2835	0.6256	1.1907	2.3202	3.0884	4.9113	6.5859	10.344
		k, mg/g.min	-0.0003	-0.0008	-0.0021	-0.0052	-0.0107	-0.0263	-0.0248	-0.0332
		R^2	0.4919	0.4275	0.4697	0.6568	0.5906	0.6947	0.6003	0.5559
First Order		q_e, mg/g	-1.2687	-0.4839	0.1479	0.8275	1.0663	1.5219	1.8214	2.2664
		k, mg/g.min	-0.0011	-0.0012	-0.0017	-0.002	-0.0031	-0.0043	-0.0033	-0.003
		R^2	0.478	0.3767	0.4181	0.5991	0.4779	0.577	0.5017	0.4197
Second Order		q_e, mg/g	0.2789	0.6059	1.1254	2.2527	2.6939	4.2194	5.7438	8.7565
		k, mg/g.min	-0.0037	-0.0019	-0.0015	-0.0008	-0.001	-0.0008	-0.0005	-0.0003
		R^2	0.4638	0.3274	0.3648	0.5397	0.3656	0.4422	0.3989	0.2929
Pseudo Second Order	**Type I**	q_e, mg/g	-0.3407	0.7633	1.5867	3.3400	5.2356	10.4602	11.5340	16.8918
		k, mg/g.min	1.2150	0.4708	0.1463	0.0416	0.0173	0.0051	0.0074	0.0059
		R^2	0.9998	0.9999	0.9999	0.9998	0.9999	0.9995	0.9998	0.9995
	Type II	q_e, mg/g	0.3377	2.3359	1.6168	3.1826	5.3879	10.0704	11.6550	18.3823
		k, mg/g.min	1.2262	2.7559	0.1120	0.0679	0.0147	0.0059	0.0067	0.0037
		R^2	0.8883	0.8064	0.9968	0.9509	0.9954	0.9947	0.9966	0.9512
	Type III	q_e, mg/g	0.339	0.7712	1.6137	3.219	5.2926	10.3390	11.688	17.039
		k, mg/g.min	1.1856	0.3824	0.1139	-0.0628	0.0160	0.0053	0.0066	0.0055
		R^2	0.8732	0.952	0.991	0.9313	0.9837	0.9819	0.9920	0.8782
	Type IV	q_e, mg/g	0.3441	0.7749	1.6158	3.2484	5.2990	10.3923	0.9137	17.5151
		k, mg/g.min	1.0198	0.3623	0.1127	0.0579	0.0157	0.0052	13.9980	0.0047
		R^2	0.8732	0.952	0.991	0.9313	0.9837	0.9819	0.992	0.8782
Elovich		β, g/mg	0.0271	0.0665	0.1865	0.4023	0.868	2.0007	-0.776	2.7242
		α, mg/g.min	68337.01	9397.015	165.4741	47.0353	2.2891	0.3544	-0.0001	0.9089
		R^2	0.8714		0.8628	0.9685	0.9368	0.9779	0.9448	0.9108

When Table 5 is examined, it is seen that the R^2 values obtained among all kinetic models are closest to the R^2=1 value in pseudo-second-order type 1. Therefore, it was determined that the removal of crystal violet from palm fibers was suitable for pseudo-second-order type 1. Various researchers who have studied crystal violet removal have reported that adsorption is suitable for pseudo-second-order type 1 as a result of their research studies.[28-34] The pseudo-second order kinetic model expresses chemical adsorption and indicates the electron sharing between the adsorbate.[35]

Conflict of interests

I declare that there is no a conflict of interest with any person, institute, company, etc.

4. CONCLUSION

In this study, it is understood that the adsorbent material synthesized from palm fibers can be prepared without requiring any heat treatment and can be used for dyestuff removal. In the study, the highest crystal violet removal

DOI: http://dx.doi.org/10.32571/ijct.1131313 E-ISSN: 2602-277X

efficiency was obtained with 88.73% in the experiment with stirring. The capacity values reached at the moment of equilibrium in stationary and mobile phases (with 160 mg/L initial crystal violet concentration) are 16.82 mg/g and 16.08 mg/g, respectively. As the ultimate result, dye removal can be achieved with this natural adsorbent material obtained from palm trees, which are widely grown in the Mediterranean region. Palm fiber, which is a natural and inexpensive material, can be disposed of by burning after its use for dystuff removal or can be used as raw materials in various industries such as the construction industry.

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DOI: http://dx.doi.org/10.32571/ijct.1060235 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Developing new polymeric nanoparticles for controlled release of quercetin as an alternative material protecting from COVID-19

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Received: 19 January 2022; Revised: 5 July 2022; Accepted: 5 July 2022

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Citation: Türkcan, C. *Int. J. Chem. Technol.* **2022**, 6 (1), 76-80.

ABSTRACT

Research of drugs for COVID-19, the most striking studies include the ACE-2 receptors used by COVID-19 by binding to the lung cells for entry. In the researchers conducted, it was determined by the results of the docking studies performed on the agents that block the receptor by binding to the receptor, such as COVID-19, several molecules have the interest to bind to this receptor. One of these numbered molecules is herbal flavonoid called "Quercetin". In this study, quercetin imprinted polymeric materials were designed, synthesized and characterized. For synthesis, emulsion polymerization technique was used for obtaining quercetin imprinted polymeric materials. SEM and Zeta-Size analysis were used as preliminary characterization. After a week release experiment, quercetin imprinted polymeric nanoparticles were released by 14%. In the light of these results, it is predicted that quercetin printed polymeric material can be used for protection from COVID-19 and treatment of COVID-19 on the inhaler route.

Keywords: COVID-19, quercetin, flavonoids, nanoparticles.

COVID-19'dan korunmada alternatif materyal olarak kersetin kontrollü salımı için yeni polimerik nanopartikül geliştirilmesi

ÖZ

COVID-19 için ilaç araştırmalarında en dikkat çekici çalışmalar COVID-19'un akciğer hücrelerine giriş için bağlanarak kullandığı ACE-2 reseptörlerini içeriyor. Yapılan araştırmalarda, COVID-19 gibi reseptöre bağlanarak reseptörü bloke eden ajanlar üzerinde yapılan docking çalışmalarının sonuçlarıyla, birçok molekülün bu reseptöre bağlanma ilgisi olduğu belirlendi. Bu numaralandırılmış moleküllerden biri de "Quercetin" adı verilen bitkisel flavanoiddir. Bu çalışmada, kuersetin baskılı polimerik malzemeler tasarlanmış, sentezlenmiş ve karakterize edilmiştir. Sentez için, kersetin baskılı polimerik malzemelerin elde edilmesinde emülsiyon polimerizasyon tekniği kullanılmıştır. Ön karakterizasyon olarak SEM ve Zeta-Size analizi kullanıldı. Bir haftalık salım deneyinden sonra, kuersetin baskılı polimerik nanopartiküller %14 oranında salındı. Bu sonuçlar ışığında, kuersetin baskılı polimerik materyalin inhaler yolunda COVID-19'dan korunma ve COVID-19 tedavisinde kullanılabileceği tahmin ediliyor.

Anahtar Kelimeler: COVID-19, kersetin, flavonoidler, nanopartiküller.

1. INTRODUCTION

Being confined to the plant kingdom, quercetin as a plant-derived flavonoid is widely found in herbal sources, *viz.* apple, onion, pomegranate greens. Owing to high antioxidant properties of the relevant compound, a plethora of biological activities such antiviral, antihistaminic, anti-inflammatory, antibacterial properties have been attributed to the quercetin. Those remarkable activities have been revealed to be linked to its structure. Corresponding to aforementioned properties, the compound is used in the treatment of diabetic foot wounds, acne treatment, skin blemishes, and the elimination of allergic reactions. Critically, as reported in the previous studies, the active substance quercetin used in the nanopolymer has found promising properties in the treatment of COVID-19. In a docking study, the largest protein of COVID-19 was purified and molecules with similar properties were listed as binding affinity to the ACE-2 receptor. The binding energies and 3-dimensional structures of molecules have been investigated and quercetin is included in these molecules.[1] In another publication, it is mentioned as one of the two active agents prescribed for treatment.[2] There

DOI: http://dx.doi.org/10.32571/ijct.1060235 E-ISSN: 2602-277X

are studies mentioned as inhibitors for SARS-CoV-2 infection. It is stated that it inhibits the virus by changing gene expression activities at the genetic level.[3,4]

The most important reason why the molecular suppression technique is preferred in controlled release systems is that the existing interactions can be controlled. This provides the opportunity to control emission levels. Thus, the release time and quantity of your active ingredient can be designed to suit the conditions.[5]

In current studies, it interacts with the active site of the ACE-2 receptor, closing the interaction site of COVID-19 and preventing the virus from entering the cell. It blocks the virus through this way and is predicted to have a protective effect.[6] Apart from this, the virus has effects in terms of blocking protein synthesis and it is stated that it is effective in SARS viruses. Apart from this feature, the anti-inflammatory feature of quercetin enables it to eliminate the side effects that may occur during virus infection.[7] As mentioned in the docking studies in the literature, the quercetin molecule ranks first among the molecules that are predicted to be effective in terms of binding to the receptor with similar morphological structure by isolating the largest protein group of COVID-19.[1]

In this study; the active substance, as clearly revealed to be effective against COVID-19, is combined with the nanomaterial and developed as a controlled release. Within the scope of the project, a controlled release drug that can affect COVID-19 has innovative aspects. The nanopolymer to be used in the study has a biocompatible structure. The active substance of quercetin was imprinted into the nanopolymer by molecular printing method and then controlled release studies were carried out. Scanning electron microscope (SEM) images were obtained for characterization of quercetin imprinted polymeric nanoparticle.

2. MATERIALS AND METHODS

2.1. Materials

Quercetin was provided from Sigma Chemical Co (St. Louis, USA). 2-Hydroxyethyl Metacrylate (HEMA), poly Vinyl Alcohol (PVA), and ethylene glycol dimethacrylate (EGDMA) were obtained from Aldrich (Steinheim, Germany). Potassium persulfate (KPS) was supplied from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

2.2. Methods

The absorbance values of Azure A and Pb (II)–Azure A complex were determined by using Shimadzu UV–160A ultraviolet/visible spectrophotometer. The atomic absorbance values of Pb (II) ions were measured by using Shimadzu AA 7000 flame atomic absorption spectrometer and a lead hollow cathode lamp.

2.2.1. Synthesis of quercetin imprinted polymeric nanoparticles

Polymeric material was synthesized with molecular imprinted technique for obtaining controlled release material. For this, 0.25 mg/mL Quercetin dissolved 200 microliters of isopropyl alcohol and 25 microliters of AA (Acrylic Acid) was added to solution. After then pre-complex mixture was mixed for 2 hours at room conditions using a magnetic stirrer. 275 mg Poly (vinyl alcohol), the stabilizer was dissolved in 25 mL distilled water. Pre-complex of Quercetin and AA was stopped and added onto PVA solution. 60 microliters of HEMA as monomer and 30 microliters of EGDMA were added into reactor as crosslinker. The last chemical, 45 mL KPS (0.44 mg/mL) was added onto reactor as an initiator. Final reaction mixture was treated with nitrogen gas and mixed in a water bath at 70˚C for 5 hours. At the end of the polymerization, synthesized polymeric material was rinsed two times with distilled water (100 mL) and ethanol to remove the unreacted quercetin imprinted polymeric nanoparticles, initiators and other used chemicals. After that, polymeric polymeric material was dried at 37˚C and then stored at +4 °C until further analysis.[8,9]

2.2.2. Characterization of quercetin imprinted polymeric nanoparticle

Overall structure and the surface morphology of quercetin imprinted polymeric nanoparticles were studied by using a SEM device (Quanta 250 S FEG). For this, surface of the membrane was covered with thin film of gold and was analyzed with different magnifications. Quercetin imprinted polymeric nanoparticles were analyzed with Zeta-Sizer (NanoS, Malvern Instruments, London, UK) for obtaining nanoparticle's size.

2.2.3. Controlled release of quercetin

While the polymeric material with quercetin imprinted was synthesized, washing with water and ethanol was performed after the synthesis to remove chemicals that did not participate in polymerization. During the washing process, the polymeric material suppressed with quercetin is precipitated by centrifuge and the upper phase is discarded. Then, the solvent was added on the precipitate and mixed in an ultrasonic bath. After the mixing process, chemicals that do not react are removed from the environment by precipitation again.

Spectrophotometry was used to determine quercetin. Determining final release concentrations of quercetin imprinted polymeric nanoparticles spectrophotometrically at 372 nm by using a UV-VIS spectrophotometer (Shimadzu, 1601, Japan).

DOI: http://dx.doi.org/10.32571/ijct.1060235 E-ISSN: 2602-277X

The polymeric material suppressed with quercetin for controlled release was kept in pH 7.4 phosphate buffer at 37 degrees at body temperature for a period of one week. The quercetin release amount was determined by taking samples at regular intervals. For this, the polymeric part was precipitated by taking the sample from the solution and centrifuged and analyzed in the upper phase.

3. RESULTS AND DISCUSSION

Herewith the present study, a polymeric material was synthesized using molecularly imprinting technique using quercetin as a target molecule for controlled release system. The novel quercetin imprinted polymeric nanoparticle was characterized through two characterization methods.

3.1. SEM Results

The findings of SEM images revealed that diameter of polymeric materials was about 45-65 nm with a spherical structure (Figure. 1).

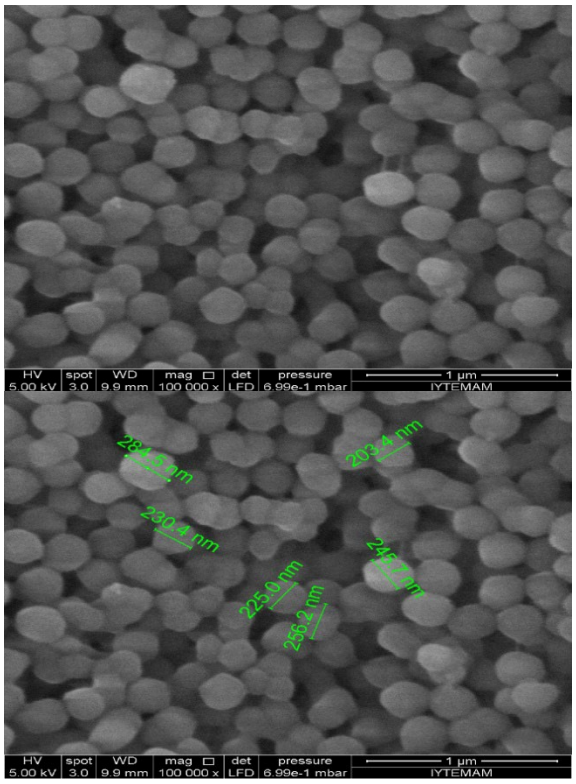

Figure 1. SEM images of quercetin imprinted polymeric nanoparticles.

With respect to the structure of the novel nanoparticle, it is seen that more than one intertwined global structure is together. SEM images are generally not included in the printing studies in the literature.[10-12] It is seen that agglomeration occurs in another polymeric structure.[13,14] When the chemical characteristics of the monomers and target molecules used in the study are considered, aggregation is actually an expected result.

3.2. Zeta-Size Analysis Results

Dimensional analysis of the obtained quercetin imprinted polymeric nanoparticles are given in Figure 2 below.

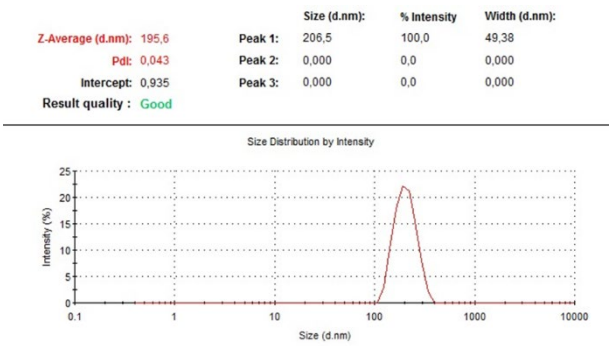

Figure 2. Zeta-Size Analysis of Quercetin imprinted polymeric nanoparticles.

Particle sizes may vary depending on the area of use. As previously reported, the size of another polymeric structure was in the range of 140-210 nm [15]. As mentioned in another publication, particle sizes ranged from 48 nm to 96 nm [16]. Regarding findings of the current study, the quercetin imprinted polymeric nanoparticle shows a scale similar to those in the literature in terms of size. The particles obtained in the study show compatibility with the findings of SEM analysis.

3.3. Controlled Release Results of Quercetin

For the characterization of the quercetin printed polymeric material; after SEM and Zeta-Size analyzes were performed, release studies were carried out to be compatible with the body. For release studies, trials were carried out for one week at body temperature and using pH 7.4 phosphate buffer. Trial results are given in Figure 3.

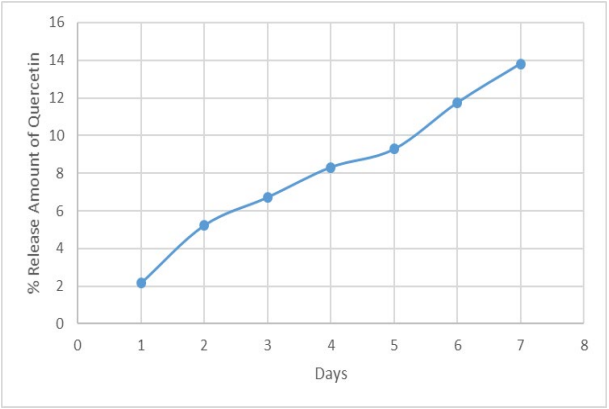

Figure 3. Controlled release results of quercetin from imprinted polymeric material.

DOI: http://dx.doi.org/10.32571/ijct.1060235 E-ISSN: 2602-277X

According to the trial results, there is a certain amount of active substance release every day within a week. According to the 7th day result, it is seen that the material releases the target molecule by 14 %. This shows that the release time may be longer. In a study in the literature, 30 % of the compound was released in a two-hour period. In another study in the literature, there are materials that emit 56-65 % within 24 hours.[17] In another study, it released approximately 70 % in a 7-hour period.[18] However, the emission amount of the quercetin imprinted polymeric nanoparticle developed in the study is very low in comparison to the former reports. Those findings might exert either advantage or disadvantage. Since the release time is long, it can be applied at more intermittent periods. If the amount of active ingredient is low, the application intervals can be more frequent.[19]

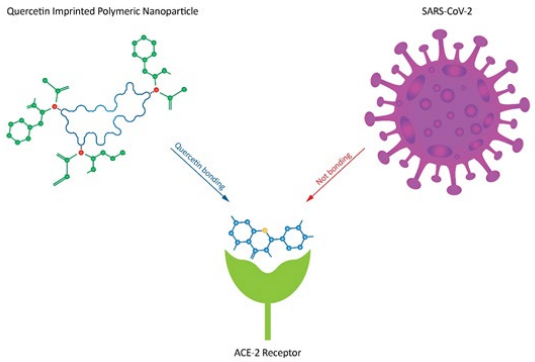

Figure 4. Blocking of ACE-2 receptor with Quercetin imprinted polymeric nanoparticles to avoiding SARS-CoV-2.

As seen in Fig-4; quercetin is released from quercetin imprinted polymeric nanoparticles and ACE-2 receptor is blocked. SARS-CoV-2 virus could not bond with the ACE-2 receptor.

4. CONCLUSIONS

This study includes preliminary trials of the material developed to protect against COVID-19 and treat caught people. The active ingredient quercetin, which is included in the material developed from the study, prevents the binding of the virus by blocking the region where COVID-19 binds in the lungs in literature studies. In addition, since the active ingredient of quercetin has anti-inflammatory effects, it is predicted that it will alleviate the symptoms after the disease. In the study, quercetin printed polymeric material was synthesized by emulsion polymerization method using molecular printing technique. In order to characterize the synthesized quercetin printed polymeric material, SEM images were taken and Zeta-Size analyzes were performed. The SEM images revealed the spherical structure of the particle and Zeta-Size analysis displayed the diameter as 200 nm. Both SEM and Zeta-Size analysis displayed are consistent and compatible with each other. Following characterization analysis, the release studies were made by selecting the values that could be optimum for the body. In the release studies of pH 7.4 phosphate buffer and 37° C body temperature for a week, it is seen that quercetin releases approximately 14% of the printed polymeric material at the end of seven days. It is predicted that the quercetin imprinted polymeric nanoparticle developed can be used practically by inhaler and will reach the lungs and block the binding of the virus to the receptors by interacting with ACE-2 receptors. Since the developed quercetin printed polymeric material has the feature of releasing, it will also be present in the lungs in case of contact with the virus, and can prevent transmission during the day. In addition, it is predicted that in case of contamination of quercetin imprinted polymeric nanoparticle, when the inhaler is used on the way, it can eliminate the inflammation occurring in this area.

ACKNOWLEDGEMENTS

I thank Zafer Zeren for the schematic drawings and graphic design.

Conflict of interests

I declare that there is no a conflict of interest with any person, institute, company, etc.

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DOI: http://dx.doi.org/10.32571/ijct.1060235 **E-ISSN: 2602-277X**

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