J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg. , 47(1): 220-227, 2023
Doi: 10.33483/jfpau.1122665

ORIGINAL ARTICLE / ÖZGÜN MAKALE

COMPARISON OF ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF SOME POLYPHENOLIC COMPOUNDS AND THEIR BIOTRANSFORMATION EXTRACTS

BAZI POLİFENOLİK BİLEŞİKLERİN VE BİYOTRANSFORMASYON EKSTRELERİNİN ANTİOKSİDAN VE ANTİ-ENFLAMATUVAR AKTİVİTELERİNİN KARŞILAŞTIRILMASI

Damla KIRCI[1,2]*, Betül DEMİRCİ[3]

[1]Selçuk University, Faculty of Pharmacy, Department of Pharmacognosy, 42010, Konya, Turkey

[2]Anadolu University, Graduate School of Health Sciences, 26000, Eskişehir, Turkey

[3]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26000, Eskişehir, Turkey

ABSTRACT

Objective: *Microbial transformations are green biotechnological processes where different microorganisms or enzymes are used to produce new metabolites from defined substrates. Hesperidin, quercetin, and their derivatives have been proven in scientific research to have a variety of biological activities, such as antioxidant, antimicrobial, and anticancer activities.*

Material and Method: *This study performed the microbial transformation of hesperidin and quercetin utilizing 13 different microbial strains. The transformation extracts of hesperidin and quercetin were investigated in antioxidant ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$ methods) and anti-inflammatory effects.*

Result and Discussion: *The biotransformation of hesperidin was observed in 5 of 13 strains. There were Rhizopus stolonifera, Saccharomyces pararoseus, S. cerevisiae, Penicillium claviforme, and Fusarium solani while microbial transformation of quercetin was identified Aspergillus flavus and Penicillium claviforme. The results of this research show that the extracts obtained with the Aspergillus and Penicillium strains were more effective in terms of antioxidant and anti-inflammatory effects. $DPPH^{\cdot}$ antioxidant activity of A. flavus extract (IC_{50}: 22.55±0.32 µg/ml) was higher than the other biotransformation extracts. The IC_{50} value of R. stolonifer from the hesperidin transformation extracts is 25.93±0.36 µg/ml. The microbial transformation of hesperidin by Penicillium strain has not been researched previously.*

Keywords: *Anti-inflammatory activity, antioxidant activity, hesperidin, microbial transformation, quercetin*

* **Corresponding Author / Sorumlu Yazar:** Damla Kırcı
e-mail / e-posta: damla.kirci@selcuk.edu.tr, **Phone / Tel.:** +9003322417978

Submitted / Gönderilme : 28.05.2022
Accepted / Kabul : 07.12.2022
Published / Yayınlanma : 20.01.2023

ÖZ

Amaç: *Mikrobiyal transformasyonlar belirlenen substratlardan yeni metabolitler üretmek için farklı mikroorganizmaların veya onların enzimlerinin kullanıldığı yeşil biyoteknolojik süreçlerdir. Hesperidin, kersetin ve türevlerinin antioksidan, antimikrobiyal ve antikanser özellikleri dahil olmak üzere çeşitli biyolojik aktivitelere sahip olduğu bilimsel araştırmalarda kanıtlanmıştır.*

Gereç ve Yöntem: *Bu çalışmada 13 farklı mikrobiyal suş kullanılarak hesperidin ve kersetin mikrobiyal transformasyonu gerçekleştirilmiştir. Hesperidin ve kersetinin mikrobiyal transformasyonundan elde edilen ekstrelerin antioksidan (DPPH· ve ABTS·+ yöntemleri) ve anti-enflamatuvar aktiviteleri araştırılmıştır.*

Sonuç ve Tartışma: *Hesperidin'in biyotransformasyonu 13 suşdan 5'inde gözlenmiştir. Rhizopus stolonifera, Saccharomyces pararoseus, S. cerevisiae, Penicillium claviforme ve Fusarium solani iken; Kersetinin mikrobiyal transformasyonu Aspergillus flavus ve Penicillium claviforme suşlarında gözlemlenmiştir.*

Bu çalışmanın sonuçları Aspergillus ve Penicillium suşları ile yapılan çalışmada elde edilen ekstrelerin antioksidan ve anti-enflamatuvar aktiviteler açısından daha etkili olduğunu göstermektedir. A. flavus ekstresi (IC_{50}: 22,55±0,32 µg/ml) DPPH· antioksidan aktivitesi diğer biyotransformasyon ekstrelerinden daha yüksek bulunmuştur. Hesperidin transformasyon ekstrelerinden R. stolonifer'in IC_{50} değeri 25,93±0,36 µg/ml'dir. Hesperidinin Penicillium suşu tarafından mikrobiyal dönüşümü daha önce çalışılmamıştır.

Anahtar Kelimeler: *Anti-enflamatuvar aktivite,, antioksidan aktivite, hesperidin, kersetin, mikrobiyal transformasyon,*

INTRODUCTION

Biotransformation is an alternate technique for producing new bioactive secondary metabolites with much promise. Microbial factories show benefits, for example, multiplying, ease of large-scale production, environment-friendly, and solvent-free. Also, microbial transformation improves the selectivity of natural products without any toxic chemicals. Microbial biotransformation, enzyme engineering, and plant cell co-transformation are some biological techniques for producing bioactive flavonoids. Biotechnological processes for flavonoid production have attracted interest since they allow the production of new flavonoids that do not exist in nature. The utilization of microorganisms to carry out reactions in the asymmetric synthesis of bioactive flavonoids is noteworthy.

The significant reactions during microbial biotransformation are dihydroxylation, hydroxylation, *O*-demethylation, *O*-methylation, deglycosylation, C ring cleavage of the benzo-γ-pyrone system, cyclization [1,2].

Citrus species have significant levels of flavonoids, which have antioxidant properties; additionally, nutritional citrus has no adverse effects or cytotoxicity in healthy individuals [3]. Hesperidin (hesperetin-7-*O*-rutinoside) belongs to the flavanone group of glycoside flavonoids composed of disaccharide (rutinose) and aglycone unit (hesperetin) [4].

Hesperidin contains a broad spectrum of biological activities, including antioxidant and anti-inflammatory effects, antibacterial and antifungal properties, antidiabetic, Alzheimer's anticancer, anti-HIV, anticoagulant, antiplatelet, and immunomodulatory effects [3,5-8].

Quercetin is a flavonoid and, more specifically, a flavonol, which is a potent antioxidant flavonoid. Onions, grapes, berries, cherries, broccoli, and citrus fruits are high in this plant pigment. Quercetin has been associated to a number of health benefits, such as anti-inflammatory, antiviral, and anticancer effects, and the ability to treat a variety of cardiac diseases. [9,10].

Hesperidin and quercetin have all been found to be limited by its water solubility, particularly following oral administration. In this situation, hesperidin causes significant changes in the physiological effects *in-vitro* and *in-vivo* [9,11,12].

Within the scope of the study, hesperidin and quercetin have been subjected to microbial transformation via different microorganisms and conversion took place. The metabolites have detected with Thin Layer Chromatography (TLC) plate in 12 days. The substrates (hesperidin and quercetin) and

the transformation extracts have been evaluated for their *in vitro* antioxidant activity (DPPH· and $ABTS^{\cdot+}$ methods) and *in vitro* anti-inflammatory effects.

MATERIAL AND METHOD

Chemicals and Microorganism

Hesperidin and Quercetin were purchased from Sigma-Aldrich, Germany.

The following microorganisms are utilized in microbial transformation reactions: *Rhizopus stolonifer* (MF461023), *Aspergillus parasiticus* (NRRL 2999), *A. terreus* var. *africanus* (isolate), *A. alliaceus* (NRRL 317), *A. flavus* (ATCC 9807), *Penicillium valentinum* (Isolate), *P. chrysogenum* (NRRL 792), *P. claviforme* (MR 376), *Fusarium solani* (ATCC 1284), *F. culmorum* (Isolate), *Saccharomyces cerevisiae* (ATCC 9763), *S. pararoseus* (ATCC 11385), and *Phanerachaete chrysosporium* (E 446).

Cultivation and Transformational Conditions

At 5°C and 25°C, the culture was maintained and precultured on potato dextrose agar (PDA) slants, respectively. α-Medium was used in the microbial transformation assay with fungi prepared, whereas Mueller Hinton Broth was used for the yeast and bacteria. Microorganisms were grown aerobically at 150 rpm on a shaker incubator at 24°C. After two days of growth, hesperidin and quercetin were added, and the fermentations were maintained for another 12 days [13]. Samples were collected from the fermentation medium in the 12^{th} day and the samples were extracted with ethyl acetate three times. The ethyl acetate phases were concentrated with a rotary evaporator to dryness under reduced pressure at 40°C. The dried extracts were kept at -20°C until there were tested for antioxidant and enzyme inhibition properties.

DPPH· Radical Scavenging Antioxidant Activity

The capacity of different fractions to scavenge the stable DPPH· free radicals was measured in the method of Clarke et al. [14]. Following the incubation period and at 517 nm, the absorbance was measured. The control was made by replacing distilled water with the extract, and the standard was ascorbic acid. The percentage of the extract that scavenges DPPH radicals was measured using the formula:

$$\text{DPPH}\cdot \text{ scavenging activity (\%)} = (Ac-As)/Ac \times 100$$

Ac: the absorbance of control

As: the absorbance of sample/standard

$ABTS^{\cdot+}$ Radical Scavenging Antioxidant Activity

The activity determination was performed via the $ABTS^{\cdot+}$ cation radical decolorization with minor modifications [15]. The $ABTS^{\cdot+}$ was prepared by reacting 15 ml of 7 mM aqueous solution of $ABTS^{\cdot+}$ with 264 µl of 140 mM potassium persulphate. Before using the mixture was allowed to rest for 12 hours in the dark at room temperature. The $ABTS^{\cdot+}$ working solution was diluted with methanol to give an absorbance of 0.70±0.02 at 734 nm before the test. In a 96-well plate. The reaction mixtures consisted of 50 µl sample and 100 µL $ABTS^{\cdot+}$ working solution. After allowing the mixture to sit in the dark for 10 min. the absorbance was estimeted at 734 nm. All tests were carried out in triplicate. The percentage scavenging effect was calculated same as the DPPH· assay.

In vitro Anti-inflammatory Activity

According to previous research, inhibition levels of Lipoxygenase (1.13.11.12, Type I-B, 7.9 Unit/mg) enzyme activity were measured spectrophotometrically on a 96-well quartz plate [16]. For 10 minutes at 25°C, potassium phosphate buffer (pH: 8.80), 40 µl test compounds, and 20 µl lipoxygenase enzyme were incubated. Each well received 300 µl of this mixture. The reaction was then started by adding linoleic acid solution and measuring the change in absorbance at 234 nm for 10 minutes. The assays were repeated four times. As a positive control, Nordihydroguaiatic acid (NDGA) was used.

Calculation of the % inhibition was shown below:

% Inhibition=(E-S)/E*100
E: The absorbance of the enzyme without a sample
S: The absorbance of the enzyme with the test sample

Statistical Analysis

GraphPad Prism Software Version 8.0 was used to perform statistical analysis to compare differences in data between the standard and experimental groups. The average and standard deviation were used to express the findings (S.D.). Statistically significant results were compared using a two-way ANOVA with Tukey Multiple Comparison Test, and IBM SPSS Statistics 22 assessed *p*-values of less than 0.05 to be statistically meaningful.

RESULT AND DISCUSSION

Microbial transformation of Hesperidin and quercetin were carried out using 13 microorganisms. The metabolites were detected using thin-layer chromatography (TLC).

The biotransformation of Hesperidin was observed in 2 of 13 strains. Extracts obtained from biotransformation of quercetin with *Aspergillus flavus* (E1) and *Penicillium claviforme* were shown to be effective (E2). While the metabolite of *P. claviforme* is more polar than quercetin; the metabolite of *A. flavus* are more nonpolar than quercetin.

The biotransformation of Hesperidin was observed in 5 of 13 strains. There are *Rhizopus stolonifera* (E3), *Saccharomyces pararoseus* (E4), *Penicillium claviforme* (E5), *S. cerevisiae* (E6), and *Fusarium solani* (E7). According to the TLC plate, the metabolites of F. solani, S. pararoseus, R. stolonifera and S. cerevisiae are more nonpolar than hesperidin. One of the P. claviforme metabolites is more polar than hesperidin.

In vitro Antioxidant Activity

The substrates (hesperidin and quercetin) and the transformation extracts were tested for their *in vitro* antioxidant activity by $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ methods.

The antioxidant activity involves different mechanisms such as radical scavenging, iron chelation, and peroxidation delay. In this research, antioxidant mechanisms of quercetin, hesperidin and the transformation extracts were elucidated by $DPPH^{\cdot}$, and $ABTS^{\cdot+}$ methods. *In vitro* antioxidant activities results (%) of the $DPPH^{\cdot}$, and $ABTS^{\cdot+}$ methods were given in Tables 1 and 2, respectively. The IC_{50} value of the $DPPH^{\cdot}$, and $ABTS^{\cdot+}$ methods of transformation extracts, positive control and quercetin are illustrated in Figures 1 and 2, respectively.

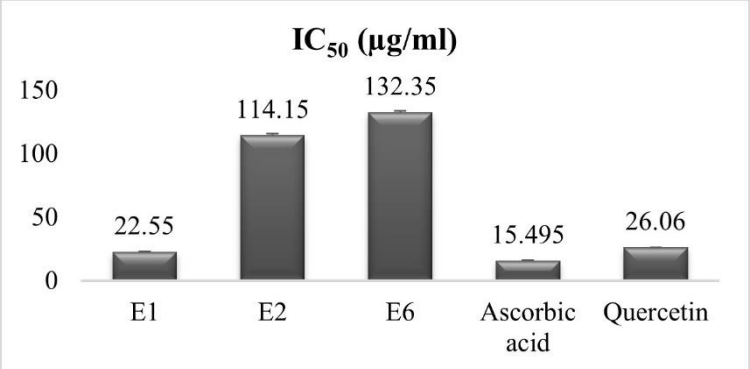

Figure 1. $DPPH^{\cdot}$ IC_{50} values of the extracts, ascorbic acid and quercetin
E1: *Aspergillus flavus*; **E2:** *Penicillium claviforme;* **E6:** *Saccharomyces cerevisiae*

The transformation extracts of quercetin were observed the highest antioxidant activity in both methods. The biotransformation extract of Fusarium solani (E7) (18.681 ± 0.14%, 200 µg/ml) was quite low. The transformation extract of Saccharomyces cerevisiae (E6) (58.93 ± 2.81%, 200 µg/ml) has more antioxidant potential than other transformation extracts of hesperidin. In this study, the $DPPH^{\cdot}$ antioxidant activity of *Aspergillus flavus* extract (E1) (IC_{50}: 22.55±0.32 µg/ml) was higher than the other biotransformation extracts and their substrate. The positive control of the method is ascorbic acid (IC_{50}: 15.50±0.62 µg/ml). As a result, the polar components of transformation extracts of quercetin may bemainly responsible for their free radical scavenging capability.

Table 1. $DPPH^{\cdot}$ Antioxidant activity (%) of the substrates and transformation extracts

	Concentration				F	*P*
	25 µg/ml	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**		
Quercetin	45.207 ± 1.89^{b}	61.877 ± 0.29^{a}	62.312 ± 0.14^{a}	63.085 ± 0.59^{a}	6.296	0.017
E1	48.88 ± 2.18^{c}	57.432 ± 0.8^{b}	61.829 ± 3.94^{ab}	67.289 ± 1.27^{a}	32.313	<0.001
E2	8.027 ± 0.36^{d}	22.885 ± 2.32^{c}	52.745 ± 1.45^{b}	61.152 ± 1.67^{a}	717.813	<0.001
Hesperidin	11.796 ± 1.09^{a}	15.782 ± 3.23^{a}	25.639 ± 3.62^{a}	31.437 ± 4.41^{a}	13.789	0.017
E3	11.94 ± 1.23^{c}	18.83 ± 2.32^{b}	24.407 ± 3.99^{b}	34.92 ± 1.16^{a}	46.909	<0.001
E4	8.433 ± 1.89^{c}	10.49 ± 0.36^{bc}	14.817 ± 1.48^{b}	23.573 ± 4.03^{a}	24.478	<0.001
E5	9.041 ± 1.38^{c}	15.782 ± 0.72^{b}	16.362 ± 4.49^{b}	28.683 ± 0.58^{a}	35.005	<0.001
E6	18.391 ± 1.38^{d}	30.76 ± 0.73^{c}	46.077 ± 0.38^{b}	58.93 ± 2.81^{a}	357.776	<0.001
E7	7.519 ± 2.75^{c}	11.433 ± 0.29^{b}	11.796 ± 0.07^{b}	18.681 ± 0.14^{a}	33.558	<0.001

$^{a-d:}$ There is no difference between groups with the same letter for each measurement value. There was analyzed by ANOVA Tukey HSD. **E1:** *Aspergillus flavus*; **E2:** *Penicillium claviforme;* **E3:** *Rhizopus stolonifer*; **E4:** *Sporobolomyces pararoseus*; **E5:** *Penicillium claviforme***; E6:** *Saccharomyces cerevisiae*; **E7:** *Fusarium solani*

Table 2. $ABTS^{\cdot+}$ Antioxidant activity (%) of the substrates and transformation extracts

	Final Concentration				F	*p*
	250 µg/ml	**500 µg/ml**	**1 mg/ml**	**2 mg/ml**		
Quercetin	71.006 ± 2.25^{a}	71.227 ± 2.86^{a}	74.492 ± 1.92^{a}	76.169 ± 0.79^{a}	5.242	0.074
E1	59.146 ± 5.14^{a}	59.611 ± 4.44^{a}	61.612 ± 0.28^{a}	63.985 ± 1.35^{a}	1.217	0.365
E2	21.941 ± 1.55^{c}	55.267 ± 2.95^{b}	63.111 ± 3.58^{b}	72.389 ± 4.05^{a}	143.611	<0.001
Hesperidin	11.796 ± 1.09^{a}	15.782 ± 3.23^{a}	25.639 ± 3.62^{a}	31.437 ± 4.41^{a}	13.789	0.017
E3	20.193 ± 3.56^{b}	22.68 ± 4.41^{b}	44.33 ± 1.35^{a}	45.002 ± 1.08^{a}	38.307	<0.001
E4	17.167 ± 1.08^{d}	24.608 ± 2.98^{c}	41.372 ± 2.97^{b}	58.92 ± 0.47^{a}	217.879	<0.001
E5	26.177 ± 1.08^{d}	21.112 ± 1.62^{c}	41.909 ± 1.61^{b}	58.247 ± 1.01^{a}	457.548	<0.001
E6	29.269 ± 0.40^{c}	42.649 ± 2.62^{b}	65.105 ± 1.55^{a}	66.428 ± 2.95^{a}	215.928	<0.001
E7	12.267 ± 3.00^{b}	15.268 ± 2.37^{b}	18.758 ± 3.35^{ab}	26.11 ± 2.72^{a}	12.818	0.002

$^{a-d:}$ There is no difference between groups with the same letter for each measurement value.
There was analyzed by ANOVA Tukey HSD. **E1:** *Aspergillus flavus*; **E2:** *Penicillium claviforme;* **E3:** *Rhizopus stolonifer*; **E4:** *Sporobolomyces pararoseus*; **E5:** *Penicillium claviforme***; E6:** *Saccharomyces cerevisiae*; **E7:** *Fusarium solani*

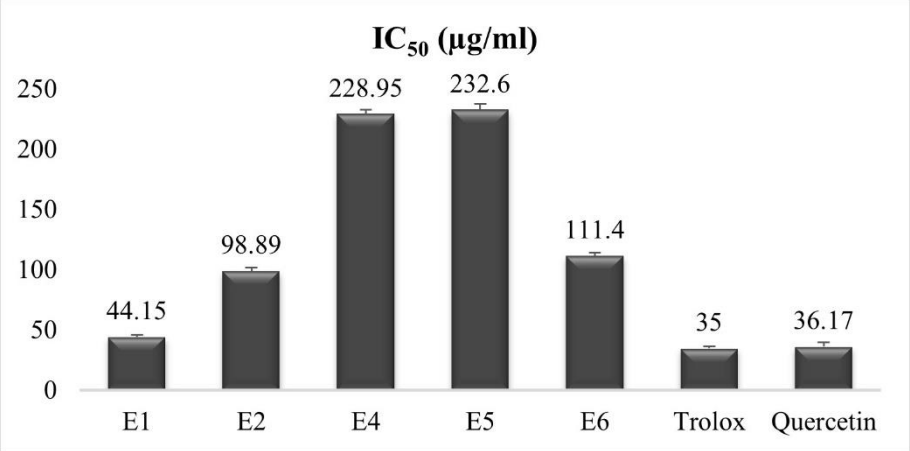

Figure 2. $ABTS^{\cdot+}$ IC_{50} values of the extracts, trolox and quercetin
E1: *Aspergillus flavus*; **E2:** *Penicillium claviforme;* **E4:** *Sporobolomyces pararoseus*; **E5:** *Penicillium claviforme*; **E6:** *Saccharomyces cerevisiae*

In vitro Anti-inflammatory Activity

As for the enzyme inhibitory activity in this work, the inhibitory effects of hesperidin, quercetin and the biotransformation extracts on 5-Lipoxygenase (5-LOX). The results of 5-LOX enzyme inhibition were given in Table 3. In this method, the extracts, hesperidin and quercetin were tested three different doses. % inhibitions, and IC_{50} values were calculated.

In our study, while the extracts of *Aspergillus flavus* (E1) and *Penicillium claviforme* (E2) had strong inhibitory activity against 5-LOX, the extract of *Rhizopus stolonifer* (E3) had no activity. The IC_{50} value of E3 from the hesperidin transformation extracts is 25.93±0.36 µg/ml. NDGA was used as a positive control and the IC_{50} value was found to be 3.63±0.29 µg/ml.

Table 3. Anti-inflammatory effect (%) of the substrates and transformation extracts

	Concentration			F	*p*
	10 µg/ml	**20 µg/ml**	**40 µg/ml**		
Quercetin	41.603 ± 3.7^{c}	63.194 ± 1.09^{b}	70.139 ± 1.29^{a}	120.676	<0.001
E1	26.092 ± 0.95^{c}	41.921 ± 4.27^{b}	64.52 ± 1.24^{a}	162.125	<0.001
E2	32.205 ± 2.56^{b}	34.989 ± 5.08^{b}	47.38 ± 2.86^{a}	14.502	0.005
Hesperidin	43.584 ± 4.53^{b}	51.587 ± 3.34^{ab}	61.508 ± 4.01^{a}	15.202	0.004
E3	3.363 ± 1.74^{b}	5.714 ± 0.71^{b}	33.75 ± 5.04^{a}	37.765	<0.001
E4	12.143 ± 1.24^{c}	22.857 ± 1.43^{b}	32.262 ± 1.8^{a}	134.069	<0.001
E5	21.786 ± 1.79^{b}	25.893 ± 1.61^{b}	35.00 ± 2.14^{a}	39.720	<0.001
E6	22.857 ± 2.5^{b}	25.476 ± 2.32^{b}	35.238 ± 2.86^{a}	19.298	0.002
E7	22.262 ± 0.74^{c}	32.679 ± 0.18^{b}	41.25 ± 1.61^{a}	256.916	<0.001

[a-c:] There is no difference between groups with the same letter for each measurement value. There was analyzed by ANOVA Tukey HSD. **E1:** *Aspergillus flavus*; **E2:** *Penicillium claviforme;* **E3:** *Rhizopus stolonifer*; **E4:** *Sporobolomyces pararoseus*; **E5:** *Penicillium claviforme***; E6:** *Saccharomyces cerevisiae*; **E7:** *Fusarium solani*

In a previous study, biotransformation was performed in an industrial citrus residue extract by *Paecilomyces variotii*. The anti-inflammatory activity in the cell line was investigated without the structure determination of the extract. It was shown further to increase the efficiency of the biotransformation extract through fermentation. It has been shown to further increase the efficiency of the biotransformation extract through fermentation [17].

The previous research was on the biotransformation of the lime peel by *Aspergillus saitoi* in solid medium. The $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical scavenging activity of the biotransformation extract was increased compared with unfermented lime peel [18].

On the other hand, the microbial transformation of hesperidin was shown by Dodda et al. There was used for *Cunninghamella elegans*, *C. echinulata*, *C. blakesleeana*, *Aspergillus terreus*, *A. ochraceus*, *A. flavus*, *Gliocladium roseum* and *Rhizopus stolonifer*. *Cunninghamella elegans* was observed to convert hesperidin to hesperitin through hydrolysis, demonstrating a similarity to human metabolism of hesperidin [19]. Also, the microbial transformation was converted from hesperidin to hesperetin (aglycone) by *Streptomyces griseus* and *Pichia kluyveri* [20,21]. Previously the other study, hesperidin was transformed cartamidin and isocartamidin by *Rhodotorula* species [22].

Microbial transformation studies have been carried out with quercetin by *Gliocladium deliquescens* (NRRL 1086) and *Cunninghamella elegant* (ATCC 9245) in the literature [23,24]. 2-Protocatechuic-phloroglucinol carboxylic acid, quercetin 3-*O*-*β*-*D*-glucoside, 2,4,6-trihydroxy benzoic acid, and protocatechuic acid were transformed from quercetin by *G. deliquescens*.

Microbial transformation of quercetin with *C. elegans* converted to isorhamnetin 3-*O*-*β*-*D*-glucopyranoside, kaempferol 3-*O*-*β*-*D*-glucopyranoside, and quercetin 3-*O*-*β*-*D*-glucopyranoside. Quercetin was first glycosylated, then modified by dehydroxylation and methylation to its metabolites.

The results of this study show us that the extracts obtained with the *Aspergillus* and *Penicillium* strains are more effective in terms of antioxidant and anti-inflammatory activities. The microbial transformation of hesperidin by *Penicillium* strain has not been researched previously.

The high activity of the extracts in this study suggests a synergistic effect between the compounds. In this context, future studies can explain the chemical compositions of the extracts. In addition, our research suggests that the microbial transformation extracts of hesperidin and quercetin can be used as natural antioxidants and anti-inflammatory in the food and pharmaceutical industries.

ACKNOWLEDGEMENTS

This study is part of the PhD thesis of Damla KIRCI was financially supported as a Scientific Research Project (1901S001) by Anadolu University.

AUTHOR CONTRIBUTIONS

Concept: D.K., B.D.; Design: D.K., B.D.; Control: B.D.; Sources: D.K., B.D.; Materials: D.K., B.D.; Data Collection and/or Processing: D.K.; Analysis and/or Interpretation: D.K., B.D.; Literature Review: D.K.; Manuscript Writing: D.K., B.D.; Critical Review: D.K., B.D.; Other: D.K., B.D.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Hosoda, R., Horio, Y., Shimoda, K., Hamada, M., Hamada, H., Hamada, H. (2013). Regioselective hydroxylation and glucosylation of flavanones with cultured plant cells of Eucalyptus perriniana. Natural Product Communications, 8(7), 905-906. [CrossRef]
2. Cao, H., Chen, X., Jassbi, A.R., Xiao, J. (2015). Microbial biotransformation of bioactive flavonoids. Biotechnology Advances, 33(1), 214-223. [CrossRef]

3. Kim, J., Wie, M.B., Ahn, M., Tanaka, A., Matsuda, H., Shin, T. (2019). Benefits of hesperidin in central nervous system disorders: a review. Anatomy & Cell Biology, 52(4), 369-377. [CrossRef]
4. Umeno, A., Horie, M., Murotomi, K., Nakajima, Y., Yoshida, Y. (2016). Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. Molecules, 21(6), 708. [CrossRef]
5. Ali, A.M., Gabbar, M.A., Abdel-Twab, S.M., Fahmy, E.M., Ebaid, H., Alhazza, I.M., Ahmed, O.M. (2020). Antidiabetic potency, antioxidant effects, and mode of actions of Citrus reticulata fruit peel hydroethanolic extract, hesperidin, and quercetin in nicotinamide/streptozotocin-induced Wistar diabetic rats. Oxidative Medicine and Cellular Longevity, 2020. [CrossRef]
6. Chikara, S., Nagaprashantha, L.D., Singhal, J., Horne, D., Awasthi, S., Singhal, S.S. (2018). Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. Cancer Letters, 413, 122-134. [CrossRef]
7. Pandey, P., Khan, F. (2021). A mechanistic review of the anticancer potential of hesperidin, a natural flavonoid from Citrus fruits. Nutrition Research, 92, 21-31. [CrossRef]
8. Miles, E.A., Calder, P.C. (2021). Effects of Citrus fruit juices and their bioactive components on inflammation and immunity: a narrative review. Frontiers in Immunology, 12, 2558. [CrossRef]
9. Wang, W., Sun, C., Mao, L., Ma, P., Liu, F., Yang, J., Gao, Y. (2016). The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. Trends in Food Science & Technology, 56, 21-38. [CrossRef]
10. David, A.V.A., Arulmoli, R., Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy Reviews, 10(20), 84-89. [CrossRef]
11. Meneguzzo, F., Ciriminna, R., Zabini, F., Pagliaro, M. (2020). Review of evidence available on hesperidin-rich products as potential tools against COVID-19 and hydrodynamic cavitation-based extraction as a method of increasing their production. Processes, 8(5), 549. [CrossRef]
12. Garg, A., Garg, S., Zaneveld, L.J.D., Singla, A.K. (2001). Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytotherapy Research, 15(8), 655-669. [CrossRef]
13. Batur, Ö.Ö., Kıran, İ., Berger, R.G., Demirci, B. (2019). Microbial transformation of β-caryophyllene and longifolene by Wolfiporia extensa. Natural Volatiles and Essential Oils, 6(3), 8-15.
14. Clarke, G., Ting, K.N., Wiart, C., Fry, J. (2013). High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. Antioxidants, 2(1), 1-10. [CrossRef]
15. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231-1237. [CrossRef]
16. Baylac, S., Racine, P. (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. International Journal of Aromatherapy, 13(2-3), 138-142. [CrossRef]
17. Nakajima, V.M., Moala, T., Moura, C.S., Amaya-Farfan, J., Gambero, A., Macedo, G.A., Macedo, J.A. (2017). Biotransformed citrus extract as a source of anti-inflammatory polyphenols: Effects in macrophages and adipocytes. Food Research International, 97, 37-44. [CrossRef]
18. Pérez-Nájera, V.C., Lugo-Cervantes, E., Amaya-Delgado, L., Madrigal-Pulido, J.A., Rueda-Puente, E.O., Borboa-Flores, J., Del-Toro-Sánchez, C.L. (2018). Biotransformation of hesperidin from lime peel (Citrus limetta Risso) in solid fermentation by Aspergillus saitoi. CyTA-Journal of Food, 16(1), 537-543. [CrossRef]
19. Dodda, S., Bandlapalli, S., Vidyavathi, M. (2012). Biotransformation of hesperidine to hesperitine by Cunninghamella elegans. Asian Journal of Pharmaceutical and Clinical Research, 5(2), 174-178.
20. Wang, Y.Y., Liu, J.H., Yu, B.Y. (2005). Biotransformation of flavonoids by *Streptomyces griseus* ATCC 13273. Pharmaceutical Biotechnology-Beijing-, 12(5), 308.
21. Escudero-López, B., Cerrillo, I., Gil-Izquierdo, Á., Hornero-Méndez, D., Herrero-Martín, G., Berná, G., Medina, S., Ferreres, F., Martin, F., Fernández-Pachón, M.S. (2016). Effect of thermal processing on the profile of bioactive compounds and antioxidant capacity of fermented orange juice. International Journal of Food Sciences and Nutrition, 67(7), 779-788. [CrossRef]
22. Sordon, S., Madej, A., Popłoński, J., Bartmańska, A., Tronina, T., Brzezowska, E., Juszczyk, P., Huszcza, E. (2016). Regioselective ortho-hydroxylations of flavonoids by yeast. Journal of Agricultural and Food Chemistry, 64(27), 5525-5530. [CrossRef]
23. Jia-Qi, X., Ni, F., Bo-Yang, Y., Qian-Qian, W., Zhang, J. (2017). Biotransformation of quercetin by Gliocladium deliquescens NRRL 1086. Chinese Journal of Natural Medicines, 15(8), 615-624. [CrossRef]
24. Zi, J., Valiente, J., Zeng, J., Zhan, J. (2011). Metabolism of quercetin by Cunninghamella elegans ATCC 9245. Journal of Bioscience and Bioengineering, 112(4), 360-362. [CrossRef]