



## Evaluation of the bioactivities of turmeric spices of different origins

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## Farklı kökenli zerdeçal baharatlarının biyoaktivitelerinin değerlendirilmesi

**Abstract:** Our study aimed to compare the in vitro bioactivities of turmeric spice samples obtained from three different sources (India, Pakistan, and Indonesia). Our study involved the determination of total phenolic and flavonoid content, in vitro antioxidant activities, tyrosinase enzyme activity, and antimicrobial activity (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans*) analyses in three different spice samples. Each spice sample was dissolved in 250 mL of ethanol and stirred on a magnetic stirrer for 36 hours. After filtering out the solid parts, the residues were dissolved again in 250 mL of ethanol separately. This process was repeated three times. After the filtration steps, all filtrates were combined. The remaining solvents in the filtrate were evaporated using an evaporator. The residue of the extracts was placed in Eppendorf tubes and stored in a freezer until use. It has been determined that turmeric of Indian origin, with its high phenolic and flavonoid content, exhibits more potent antioxidant and antityrosinase effects than those from Indonesia and Pakistan. However, antimicrobial activity could not be detected within the studied concentration range of 10 µg/mL to 400 mg/mL.

**Key words:** Antimicrobial, antioxidant, antityrosinase, turmeric

**Özet:** Çalışmamız, üç farklı kaynaktan elde edilen zerdeçal baharat örneklerinin in vitro biyoaktivitelerini (Hindistan, Pakistan ve Endonezya) karşılaştırmayı amaçlamıştır. Çalışmamızda, toplam fenolik ve flavonoid içeriğin belirlenmesini, in vitro antioksidan aktivitelerini, tirozinaz enzim aktivitesini ve antimikrobiyal aktiviteyi (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* ve *Candida albicans*) üç farklı baharat örneğinde analiz edildi. Her bir baharat örneği 250 mL etanol içinde çözüldü ve 36 saat boyunca manyetik bir karıştırıcıda karıştırıldı. Katı parçalar süzildükten sonra artık kalıntılar ayrı ayrı 250 mL etanol içinde tekrar çözüldü. Bu işlem üç kez tekrarlandı. Süzme adımlarının ardından, tüm süzme sıvıları birleştirildi. Süzme sıvısındaki artan çözücüler buharlaştırıcı kullanılarak uzaklaştırıldı. Özütle kalıntısı Eppendorf tüplerine konuldu ve kullanıma kadar dondurucuda saklandı. Hindistan kökenli zerdeçalın yüksek fenolik ve flavonoid içeriği ile Endonezya ve Pakistan'dan gelenlere göre daha güçlü antioksidan ve antitirozinaz etkileri sergilediği belirlendi. Bununla birlikte, 10 µg/mL ile 400 mg/mL arasındaki incelenen konsantrasyon aralığında antimikrobiyal aktivite tespit edilemedi.

**Anahtar Kelimeler:** Antimikrobiyal, antioksidan, antitirozinaz, zerdeçal

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### 1. Introduction

Medicinal herbs have been used as a source of medicine from ancient times to the present day. Among these, turmeric is a spice derived from the rhizomes of *Curcuma longa* (*C. longa*), a flowering plant in the ginger family. *C. longa* is commonly cultivated in China, India, and other Asian countries, and it is a perennial herbaceous plant (Trigo-Gutierrez et al., 2021; Artar and Öztürk, 2022). Turmeric is a polyphenolic plant, and like many other plant materials, variations in the polyphenolic content of *C. longa* from different geographical regions have been observed (Zorofchian Moghadamtousi et al., 2014; Karaman and Köseleler, 2017). More than 100 components have been isolated from turmeric. Turmeric comprises approximately 60-70% carbohydrates, 8.6% protein, 5-10% fat, 2-7% dietary fiber, 3-5% curcuminoids (with 50-70% curcumin content), and 5% volatile oil and resin, as indicated by Trujillo et al. (2013). It also contains noteworthy quantities of vitamins and minerals, including vitamin C (ascorbic acid), vitamin E ( $\alpha$ -tocopherol), vitamin B3 (niacin), potassium (K), magnesium (Mg), phosphorus (P), and calcium (Ca), as outlined by Karaman and Köseleler (2017).

The main components of the plant are volatile oil called turmerone and coloring agents called curcuminoids (Noorafshan and Ashkani-Esfahani, 2013; Artar and Öztürk, 2022). Curcumin ( $C_{21}H_{20}O_6$ ), one of the three curcuminoids found in turmeric, is considered as the most important polyphenol associated with health. It has been reported that curcumin positively reduces oxidative stress, and its activities extend to various chronic diseases. It reduces oxidative stress and increases the activities of antioxidant enzymes, thus reducing the risk of various chronic diseases (Artar and Öztürk, 2022). While curcumin is soluble in acetone and ethanol, it is insoluble in water. Curcumin is a hydrophobic polyphenolic flavonoid (Akbay and Pekcan, 2016). The methoxy, hydroxyl, and carbonyl groups in curcumin, which have a polyphenolic structure, confer antioxidant and radical scavenging properties on turmeric (Wright, 2002; Boroumand et al., 2018).

Additionally, some curcumin analogs have recently exhibited inhibitory properties against the enzyme tyrosinase (Du et al., 2011). Tyrosinase is a multifunctional enzyme containing copper. Tyrosinase catalyzes the oxidation of phenolic compounds to quinone derivatives. Tyrosinase is an enzyme involved in melanin formation.

Melanin is the black pigment in hair and skin, and it is necessary to protect the skin against ultraviolet rays. This pigment is produced by melanocyte cells. However, abnormal melanin production leads to dermatological disorders (Akter et al., 2021). The increase in tyrosinase activity in some cancer cells has drawn attention to the importance of tyrosinase in treating these cancer types. Lee et al. (2009) reported that some curcumin analogs exhibited inhibitory activity against tyrosinase (Lee et al., 2009). This situation has attracted our interest in evaluating turmeric's antityrosinase and antioxidant activities.

Furthermore, the antimicrobial properties of turmeric have been included in the literature. Empirical evidence substantiates that the antimicrobial effect is linked to the methoxy and hydroxyl groups present in the chemical configuration of curcumin within turmeric. Molecular docking studies have suggested that functional groups in curcumin form hydrogen bonds and nonspecific hydrophobic interactions with the catalytic region of the FtsZ protein, which is involved in bacterial cell division, thereby inhibiting bacterial cell division (da Silva et al., 2018).

Curcuminoids, with their polyphenolic structure, are present in turmeric at a rate of 3-5%. An average teaspoon of turmeric contains approximately 30-90 mg of curcumin. Studies in humans have shown that curcumin consumption of up to 12000 mg/day is well-tolerated. For the most effective therapeutic effect, it is recommended to consume 4000-8000 mg/day of curcumin based on safety and toxicity profiles (Çöteli and Karataş, 2017). However, the phenolic composition within turmeric, specifically the curcuminoid content, may vary, ranging from 2% to 9%, depending on geographical conditions (Trujillo et al., 2013).

Therefore, comparing the bioactivities of turmeric with varying phenolic contents based on geographical conditions can provide valuable insights into its consumption, which has a positive impact on public health and is a part of our daily diet. Our study aimed to compare the *in vitro* bioactivities of turmeric spice samples obtained from three different sources (India, Pakistan, and Indonesia). Our study involved the determination of phenolic and flavonoid content, antioxidant activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ABTS cation radical scavenging activity, and cupric ion-reducing (CUPRAC) activity. Additionally, we analyzed tyrosinase enzyme activity and antimicrobial activity in three different spice samples (India, Pakistan, and Indonesia).

## 2. Materials and Method

### 2.1. Reagent and standards

The chemicals utilized for investigating bioactivity were procured from Sigma-Aldrich (Schnelldorf, Germany). The microorganisms used for assessing antimicrobial properties were obtained from the American Type Culture Collection (ATCC). The spice samples from India, Pakistan, and Indonesia were obtained from international online sales platforms.

### 2.2. Preparation of extracts

Samples of turmeric spice from three different origins, namely India, Pakistan, and Indonesia, were individually weighed (20 grams each). Each weighed spice sample was

dissolved in 250 mL of ethanol and stirred on a magnetic stirrer for 36 hours. After filtering out the solid particles, the residues were dissolved again in 250 mL of ethanol separately. This process was repeated three times. Following the filtration steps, all the filtrates were combined. The remaining solvents in the filtrate were evaporated using an evaporator at 40°C. The residue from the extracts was placed in Eppendorf tubes and stored in a freezer until use. Sample preparations were conducted across a wide concentration range (10 µg/mL - 400 mg/mL), and subsequent analyses were carried out.

### 2.3. The total phenolic and total flavonoid contents

The quantification of total phenolic content (TPC) was conducted using the Folin-Ciocalteu method with slight modifications, following the approach described in earlier studies (Sarikurkcu et al., 2018; Yırtıcı et al., 2022). A 96-well microplate was employed, where 12.5 µL of Folin-Ciocalteu reagent (diluted 1:9) was added to 25 µL of the extract and 187.5 µL of ultrapure water in each well. Subsequently, a mixture of 25 µL of 20% (w/v) sodium carbonate was introduced. The absorbance was gauged at 760 nm through a microplate reader. The outcomes of TPC were presented in milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract).

For evaluating total flavonoid content (TFC), a modified aluminum chloride colorimetric technique was used (Sarikurkcu et al., 2018; Yırtıcı et al., 2022). In this procedure, the extract was combined with methanol (25 µL), 5% sodium nitrite (10 µL), and ultrapure water (100 µL) within each well of a 96-well microplate. Then, 10% aluminum chloride (15 µL) was added to the mixture. After an incubation period of five minutes, 100 µL of 1 M sodium hydroxide and 50 µL of ultrapure water were incorporated, and the absorbance was assessed at 510 nm. A standard curve was devised using rutin, and the findings were expressed as milligrams of rutin equivalent (RE) per gram of extract (mg RE/g extract).

### 2.4. Bioactivities

#### 2.4.1. Antioxidant tests

The assessment of DPPH radical scavenging activity was conducted with specific adaptations based on prior research (Sarikurkcu et al., 2018; Yırtıcı et al., 2022). Different concentrations (10 µg/mL - 400 mg/mL) of the extracts (10 µL) were blended with 190 µL of a DPPH solution (0.004%). Following a 30-minute incubation period at room temperature, the absorbance was gauged at 517 nm. The proportion of DPPH radical scavenging manifested by the spice extracts was calculated using Equation 1. The same procedures were replicated utilizing Trolox as a standard, and the DPPH radical scavenging activities of the spice extracts were conveyed in terms of Trolox equivalent (mgTE/g extract).

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (1)$$

$A_{\text{control}}$ ; control absorbance and  $A_{\text{sample}}$ ; sample absorbance

ABTS cation radical scavenging activity was assessed with adjustments derived from the methodology presented by Sarikurkcu et al. (2018). The ABTS<sup>+</sup> was generated by reacting 7 mmol/L of 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt with 2.45 mmol/L of potassium persulfate over 16 hours at room temperature.

Varied concentrations (10 µg/mL - 400 mg/mL) of the extracts (10 µL) were combined with 190 µL of the prepared ABTS solution and incubated for six minutes at room temperature. Subsequently, the absorbance was measured at 734 nm. The proportion of ABTS cation radical scavenging demonstrated by the spice extracts was determined using the supplied equation. The activities of the spice extracts were conveyed in terms of Trolox equivalent (mgTE/g extract).

CUPRAC reagent was freshly prepared by combining 10 mM copper(II) chloride, 7.5 mM neocuproine, and 1 M ammonium acetate (pH 7) at a ratio of 10:1:1 (v/v/v). Various extracts (10 µg/mL - 400 mg/mL) concentrations (25 µL) were mixed with 175 µL of the CUPRAC reagent and incubated for 30 minutes at room temperature. Subsequently, the absorbance was measured at 450 nm. The antioxidant potential of the extracts was quantified in terms of milligrams of standard equivalent per gram of extract and compared against the positive controls represented by Trolox.

#### 2.4.2. Enzyme inhibition test

The tyrosinase inhibitory potential of the extracts was evaluated utilizing the dopachrome method outlined by Sarikurkcu et al. (2018). In alignment with this procedure, 25 µL of each extract (10 µg/mL - 400 mg/mL), 40 µL of tyrosinase solution, and 100 µL of sodium phosphate buffer (pH 6.8) were combined and thoroughly mixed. The resultant mixture was subjected to an incubation period at 25°C for 15 minutes. After adding 40 µL of L-3,4 dihydroxyphenylalanine (L-DOPA), the mixture was again incubated at 25°C for 10 minutes. Subsequently, the absorbance was gauged at 492 nm. To determine the inhibitory effect of the scutellarin compound, employed as the positive control, against the tyrosinase enzyme, a well-defined procedure was followed. Initially, 1 mg of scutellarin compound was dissolved in dimethyl sulfoxide (DMSO) and then subjected to a tenfold dilution with distilled water. The enzyme activity was assessed across five distinct concentrations of the scutellarin compound.

#### 2.4.3. Antimicrobial test

The antimicrobial activity of ethanol extracts of the spices was determined using a combination of the microdilution method, the NCCLS M07-A9 method, and the method of Özcan Ateş and Kanbur (2023) (NCCLS, 2012; Özcan Ateş and Kanbur, 2023). Initially, cultures of *Escherichia coli* (*E. coli*) ATCC 25922, *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 700603, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, and *Candida albicans* (*C. albicans*) ATCC 10231 were revived on Tryptic Soy Agar (Merck, Germany) Petri dishes at 37°C for 24 hours. Then, ethanol extracts of the samples were prepared in the range of 10 µg/mL - 400 mg/mL using Muller Hinton Broth (MHB) (BD, USA) medium. From the prepared concentrations, 100 µL was added to the wells of U-bottomed microplates. In row, A of the plates, only MHB (4 mg/mL) medium was added as a sterility control. Samples were distributed in rows B and G. In row H, MHB + inoculum suspension (100 µL) was added to control microbial growth. After incubating at 37°C for 24 hours, 20 µL of sterile 1% 2,3,5 triphenyl tetrazolium chloride (Merck, Germany) solution was added to the wells and further incubated for 30 minutes. The lowest concentration without a visible red-pink

coloration was identified as the minimum inhibitory concentration (MIC). The study was conducted in triplicate.

### 2.5 Statistical evaluation

Statistical analysis of the substance content and biological activities of spices from different sources was conducted using SPSS version 23. The results are expressed as the mean ± standard error of the mean (SEM) based on three separate experiments performed in triplicate. A one-way analysis of variance (ANOVA) was employed, followed by the Tukey post hoc statistical test, as the groups showed a normal distribution according to Normality Analysis. Differences were considered statistically significant when the p-value (P) was less than 0.05 at a 95% confidence interval.

### 3. Results

The quantities of extracts obtained from 20 grams of turmeric spice samples originating from India, Pakistan, and Indonesia were determined to be 1.30 g, 1.15 g, and 1.86 g, respectively. The spice content and in vitro bioactivity analysis results obtained from the extracts are presented in Table 1, based on three repeated analyses.

**Table 1.** Substance content and bioactivity analysis results of three different spice samples

Analyze	Sample	Mean ± std.dev	One way ANOVA (P)
Fenolic (mgGAE/g extract)	India	75.46 ± 1.82	P < 0.05
	Pakistan	24.74 ± 2.57	
	Indonesia	68.03 ± 1.30	
Flavanoid (mgRE/g extract)	India	42.11 ± 1.77	P < 0.05
	Pakistan	13.54 ± 2.14	
	Indonesia	60.56 ± 1.80	
ABTS (mgTE/g extract)	India	132.23 ± 4.86	P < 0.05
	Pakistan	61.58 ± 3.60	
	Indonesia	129.32 ± 4.96	
DPPH (mgTE/g extract)	India	44.58 ± 3.86	P < 0.05
	Pakistan	34.09 ± 3.86	
	Indonesia	48.39 ± 3.83	
CUPRAC (mgTE/g extract)	India	86.42 ± 5.63	P < 0.05
	Pakistan	46.15 ± 1.74	
	Indonesia	71.08 ± 2.79	
Tyrosinase Enzyme inhibition (IC <sub>50</sub> µg/mL)	India	38.50 ± 2.28	P < 0.05
<i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , <i>C. albicans</i> (MIC; mg/mL)	Pakistan	301.36 ± 4.21	
	Indonesia	46.29 ± 1.80	
	India	>400	
	Pakistan	>400	
	Indonesia	>400	

The outcomes are presented as the mean ± standard deviation (std.dev). Values denoted by distinct superscripts within the same row differ as determined by Tukey's honestly significant difference post hoc test at a 5% significance level. IC<sub>50</sub> (mg/mL) signifies the concentration at which 50% inhibition is achieved for all samples. MIC (mg/mL) stands for Minimum Inhibitory Concentration.

When Table 1 is examined, TPC and TFC in each spice sample were statistically different (P < 0.05). Regarding phenolic content, the Indian extract exhibited the highest level, whereas the Pakistani extract showed the lowest (Figure 1). On the other hand, the Indonesian extract displayed the greatest quantity of flavonoid content, whereas the Pakistani extract demonstrated the lowest (Fig. 1).

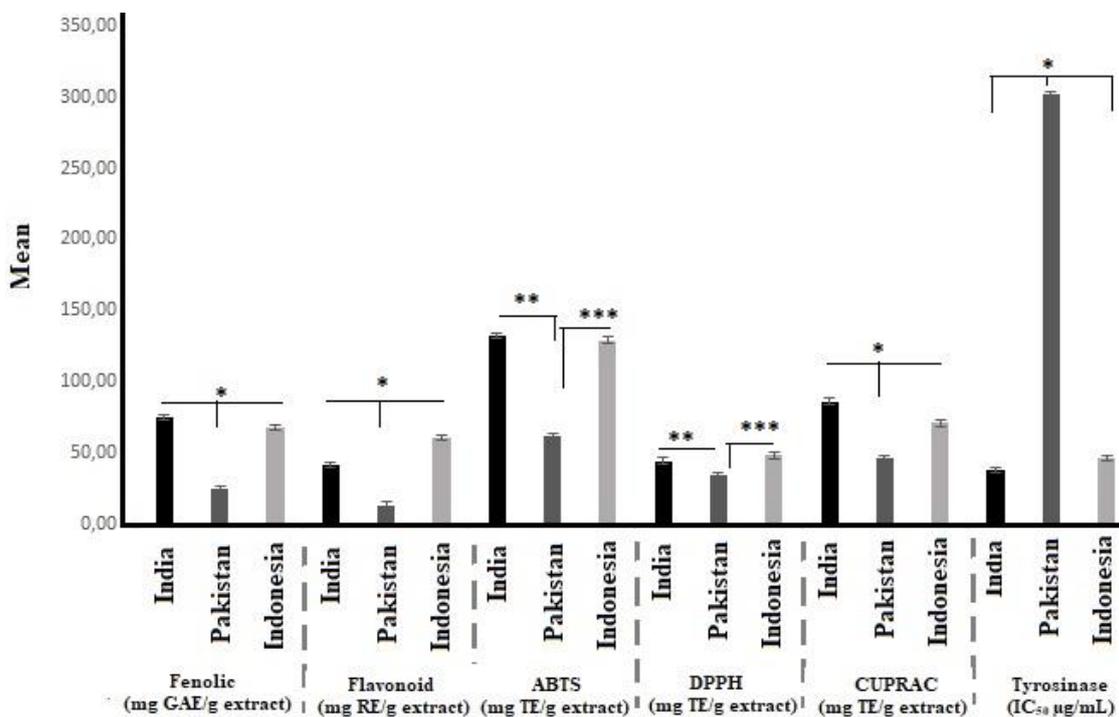
The antioxidant activity assessments of the spices were reported as mgTEs/g using ABTS, DPPH, and CUPRAC

analyses (Table 1). Upon statistical evaluation of the ABTS and DPPH analysis results, it was found that the antioxidant activity level of the Pakistan extract was significantly lower compared to the Indian and Indonesian extracts ( $P < 0.05$ ) (Fig. 1). When comparing the CUPRAC analysis activities of the spices, it was observed that the activity levels of each spice were significantly different ( $P < 0.05$ ). The extract with the highest activity was the Indian extract (Fig. 1).

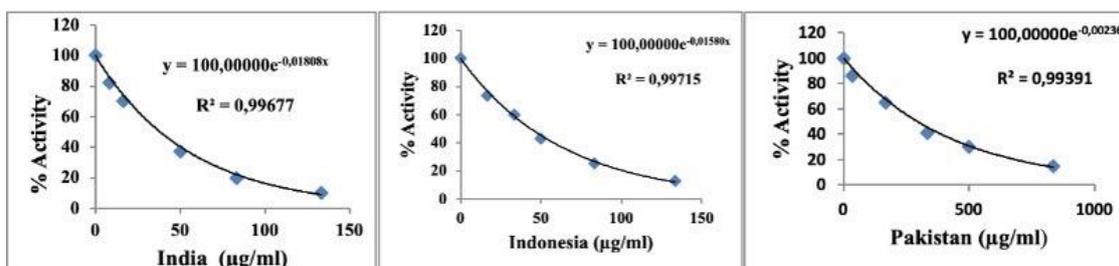
When the Tyrosinase Enzyme inhibition ( $IC_{50}$   $\mu\text{g/mL}$ , 50% inhibition concentration) of the spice extracts is examined

in Table 1, the  $IC_{50}$  level of each spice sample was found to be significantly different from each other ( $P < 0.05$ ) (Figure 1). The most effective spice extract in enzyme inhibition was the Indian extract (Figure 1). Tyrosinase Enzyme activity levels of each spice are given in Figure 2.

The MIC (mg/mL) values obtained from the antimicrobial analysis of the spice samples extracted in ethanol are provided in Table 1. At the end of the analysis, it was determined that the MIC values of the spice samples were  $>400$  mg/mL.



**Figure 1.** The substance content and bioactivity analysis outcomes for three spice samples were subjected to a one-way analysis of variance (ANOVA) along with the Tukey post hoc statistical test. Differences were deemed significant when the p-value (P) was less than 0.05 within a 95% confidence interval. \*, \*\*, \*\*\*; Signs of statistical significance.



**Figure 2.** Tyrosinase Enzyme Activity results of spices

#### 4. Discussions

This study has highlighted significant variations in bioactivity, TPC, and TFC among different types of turmeric. Among the three analyzed turmeric types, the extracts from Indian and Indonesian origins exhibited considerably stronger antioxidant activity. Additionally, these extracts contained higher levels of TPC and TFC than the extract from Pakistan. An appropriate solvent is crucial for extracting active compounds from plants and spice species containing bioactive substances. In the study by Akter et al. (2019), methanol was used as the extraction solvent to determine turmeric's antioxidant and flavonoid content (Akter et al., 2019). In the research conducted by

Ivanović et al. (2021), turmeric samples were extracted using different solvents, including 80% methanol, 80% ethanol, and ultrapure water. The study indicated the highest phenolic content in the ethanol-extracted sample (Ivanović et al., 2021). Considering the findings from the literature, ethanol solvent was chosen in our study to effectively extract phenolic and flavonoid compounds from turmeric, to achieve high extraction yields. The analysis results revealed the following TPC (mg GAE/g extract) and TFC (mg RE/g extract) values: for the Hindustani extract,  $75.46 \pm 1.82$  and  $42.11 \pm 1.77$ ; for the Pakistani extract,  $24.74 \pm 2.57$  and  $13.54 \pm 2.14$ , and for the Indonesian extract,  $68.03 \pm 1.30$  and  $60.56 \pm 1.80$  (Table 1, Figure 1). In general, our extraction results align with the findings of

Sahin (2018), who investigated the effects of turmeric extraction (Sahin, 2018). In the study conducted by Sahin in 2018, using various extraction methods, TPC in turmeric was found to be approximately 46.50 (mg GAE/g extract) (Sahin, 2018). The ethanol extraction method employed in our study has facilitated the extraction of high levels of phenolic and flavonoid compounds from turmeric samples. Existing literature studies have identified that the antioxidant property of turmeric originates from its contained phenolic and flavonoid compounds (Çöteli and Karatas, 2017). In the study conducted by Eleazu et al. in 2015, the polyphenolic content and in vitro antioxidant capacity of six new turmeric accessions were investigated. At the end of the study, the antioxidant capacity of two specific accessions was found to be different from the others. It was reported that the ratio of phytochemical compounds in turmeric directly influenced its antioxidant capacity (Eleazu et al., 2015). Our study observed that the antioxidant activity analysis of the Pakistan extract, which contains lower levels of phenolic and flavonoid compounds compared to the extracts from India and Indonesia, was significantly lower when compared to the other spices (Table 1, Figure 1). Our study is consistent with existing literature data. Upon assessing the DPPH<sup>•</sup> and ABTS<sup>•+</sup> scavenging activity of distinct turmeric varieties in our study, as detailed in Table 1 and Figure 1, it becomes evident that the extracts originating from India and Indonesia, characterized by elevated phenolic content, demonstrate correspondingly heightened DPPH<sup>•</sup> and ABTS<sup>•+</sup> scavenging activity. In the study conducted by Du et al. in 2011, the antioxidant activities of polyphenolic curcumin analogs were investigated using DPPH analysis.

Based on the analysis results, the study concluded that the samples exhibited higher antioxidant activity than vitamin C (Du et al., 2011). In the study conducted by Ak and Gulcin in 2008, the antioxidant and radical scavenging properties of curcumin were investigated. The study concluded that curcumin could be an antioxidant in both pharmacology and the food industry (Ak and Gulcin, 2008). In the study conducted by Tanvir et al. (2017), the antioxidant capacity of various turmeric species grown in Bangladesh was investigated. Turmeric samples from the Khulna and Chittagong regions in Bangladesh were collected for the study. The antioxidant capacities of the extracts obtained using different solvents were also examined. At the end of the study, the ethanolic extract of turmeric grown in the Khulna region was found to have the highest antioxidant capacity. Similarly, in our study, the antioxidant capacity of turmeric plants varied depending on the region and extraction solvent. Our data and literature indicate that turmeric's levels of bioactive compounds and antioxidant capacity can vary according to climatic and geographical conditions (Tanvir et al., 2017).

Antioxidants are recognized as reducers. Their capacity to donate electrons enables them to counteract the effects of free radicals and reactive oxygen species, reducing these elements from higher-valence states to lower-valence states. The redox potential, or the reducing ability of antioxidants, holds substantial importance as an indicator of their effectiveness in combating oxidative stress. In our study, when evaluating the reducing power of turmeric extracts on copper ions, it was found that the one with the highest effect was turmeric from India (Table 1, Figure 1). Although all the turmeric species used in the study exhibited high antioxidant activity, the turmeric variety

originating from India was identified to have the highest activity among the three spice types. Our findings are consistent with the literature regarding the antioxidant activity levels of turmeric, as supported by previous studies (Noori et al., 2022).

All of the turmeric extracts studied for tyrosinase inhibition exhibit effective inhibitory capabilities. Regarding tyrosinase inhibition, the most effective extract is from India, followed by extracts from Indonesia and Pakistan, respectively (Table 1, Figure 1, Figure 2). Previous studies have reported that phenolic compounds possess inhibitory abilities through competitive inhibition or by exhibiting chelating properties with the copper atom at the center of the tyrosinase enzyme (Kim and Uyama, 2005). In this context, the high tyrosinase inhibition ability of the Indian extract among the investigated turmeric extracts can be attributed to its elevated phenolic content. Various studies have also observed similar approaches (Oskoueian et al., 2012). The study by Du et al. (2011) investigated the antioxidant and antityrosinase activities of curcumin analogs. The research revealed that curcumin analogs exhibited potent activity (Du et al., 2011). Akter et al. (2021) reported that active molecules extracted from turmeric exhibited antityrosinase activity (Akter et al., 2021). The turmeric extracts used in our study also showed high antityrosinase activity (Table 1, Figure 1, Figure 2). Our study results are in accordance with the existing literature.

Our study determined that the MIC values of spice samples from India, Pakistan, and Indonesia, extracted in ethanol, were >400 mg/mL (Table 1). Niamsa and Sittiwet (2009) determined the MIC values of *C. longa* rhizome water extract as follows: 400 mg/mL for *E. coli* ATCC 25922, 600 mg/mL for *S. aureus* ATCC 25923, and 1600 mg/mL for *K. pneumoniae* ATCC 10031 (Niamsa and Sittiwet, 2009). In their study, Gupta et al. (2015) determined that petroleum ether, benzene, chloroform, methanol, and aqueous solutions of turmeric extracts at concentrations of 50 and 100 mg/mL exhibited inhibition zones similar to the gentamicin antibiotic disk against *S. aureus* ATCC 6571 and two clinical *S. aureus* isolates (Gupta et al., 2015). In the study by Kasta et al. (2020), it was observed that the ethanol extract of *C. longa* exhibited inhibition zones similar to antibiotic disks at a concentration of 500 mg/mL against *E. coli*, *S. aureus*, and *C. albicans* cultures. In our study, despite conducting bioactivity analyses over a wide concentration range (10 µg/mL - 400 mg/mL), the MIC values could not be determined for concentrations lower than 400 mg/mL, as the microdilution method was employed for evaluation. In our study, the samples exhibited no antimicrobial activity within the 10 µg/mL concentration range to 400 mg/mL. Additionally, concentrations exceeding 400 mg/mL were not investigated in our study. As a result, no antimicrobial effects of the samples against the tested microorganisms were observed within the specified concentration range. Based on our study's data and existing literature, it was observed that antimicrobial activity exhibited a dose-dependent pattern. Our findings indicated that the samples displayed robust antioxidant and antityrosinase activity within the range of 10 µg/mL to 400 mg/mL, while no antimicrobial activity was evident within this concentration range. Consequently, the principal bioactive attributes of the samples were identified as antioxidant and antityrosinase activity.

## 5. Conclusion

Our findings highlight that the phenolic and flavonoid content variations of turmeric plants grown in different geographical regions also influence their bioactivities. Moreover, we have determined that turmeric originating from India, with its high phenolic and flavonoid content, exhibits more potent antioxidant and antityrosinase effects than those from Indonesia and Pakistan. However, antimicrobial activity could not be detected within the studied concentration range. These results suggest that turmeric spice samples extracted using ethanol as a solvent may possess antimicrobial activity at higher concentrations. These insights underscore the potential of turmeric, particularly in terms of antioxidant activity, in the context of health benefits.

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## Conflict of Interest

Authors have declared no conflict of interest.

## Authors' Contributions

F.C. is the first author of this study. F.C. contributed to the study's design, conceptualization, methodology, TPC, TFC, antioxidant and antityrosinase activity analysis, and manuscript writing. G.O.A. contributed to the methodology, antimicrobial analysis, and manuscript writing.

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