

# Evaluation of the Antioxidant and Antifungal Efficacy of Azo Group-Related Synthetic Flavonoid Derivatives

Hadi Aqel Khdera <sup>1,\*</sup>, Sawsan Youseff Saad <sup>1</sup>, Ola Moustapha <sup>2</sup>, Farouk Kandil <sup>3</sup>

<sup>1</sup> Chemistry Department, Faculty of Sciences, Tishreen University, Lattakia, Syria

<sup>2</sup> Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Baath University, Homs, Syria

<sup>3</sup> Chemistry Department, Faculty of Sciences, Damascus University, Damascus, Syria

\* Correspondence: [hadi.aqel.khdera@tishreen.edu.sy](mailto:hadi.aqel.khdera@tishreen.edu.sy); [hadiaqelkhdera@gmail.com](mailto:hadiaqelkhdera@gmail.com);

Received: 19.04.2024; Accepted: 7.01.2025; Published: 20.12.2025

**Abstract:** The antioxidant activity of flavonoid derivatives (A<sub>1</sub>, C<sub>1</sub>-C<sub>9</sub>) was investigated using two methods: The diphenylpicrylhydrazyl radical inhibition method (DPPH) and the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) inhibition method. These methods were then compared with the activity of two well-known antioxidants, namely, ascorbic acid and quercetin. The inhibitory activity of flavonoids was assessed at four different concentrations (250, 500, 750, and 1000 µg/mL) at a wavelength of 516 nm using the DPPH method and at a wavelength of 230 nm using the H<sub>2</sub>O<sub>2</sub> method. The IC<sub>50</sub> value was calculated from the curves showing the change in percentage inhibition with solution concentration. The results indicated that compounds containing thiol and -OH groups exhibited greater efficacy than standard antioxidants. Additionally, the antifungal efficacy of all synthesized flavonoids was evaluated at two concentrations (0.5 mg/mL and 0.25 mg/mL) against three types of fungi: *Aspergillus flavus*, *Acremonium strictum*, and *Penicillium expansum*. Some of the compounds demonstrated superior activity against the tested fungi compared with quercetin and clotrimazole.

**Keywords:** azochalcone; azoflavone; antifungal; antioxidant; DPPH; hydrogen peroxide.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

## 1. Introduction

Flavonoids are low-molecular-weight polyhydroxy group phenolic compounds that are widely found in plants [1,2]. They have biological activity as antiviral [3], antibacterial [4], anti-inflammatory [5], and anticancer [6] agents. Flavonoids play an important role as antioxidants by eliminating reactive oxygen species, thereby protecting the heart and blood vessels from oxidative stress [7]. In the food industry, flavonoids are used as coloring agents, sweeteners [8], and flavor enhancers [8]. They work to inhibit free radicals by removing transition metals and binding to metal ions in the human body to prevent oxidation. Some flavonoids have the potential to chelate metal ions, such as Fe<sup>2+</sup> and Cu<sup>+</sup>, which play vital roles in oxygen metabolism and the formation of free radicals [9,10]. Flavonoids can also inhibit enzymes involved in the generation of free radicals, such as xanthine oxidase, lipoxygenase, and protein kinase [9].

Azo compounds are organic compounds that contain at least one azo group (N=N) bonded to one or more aromatic or heterocyclic systems [11-13]. They exhibit high stability to light and heat, making them useful in industrial applications such as coloring natural and

synthetic materials, ink manufacturing, leather dyeing, and the cosmetics industry. It is also used in the food industry as a coloring material [14-18].

Azo dyes are also used in solar energy cells [19], lasers, photovoltaic systems [20], and chemical sensors for detecting metal impurities in water [21]. Heterocyclic azo compounds and their derivatives, particularly those containing thiazoles and oxadiazoles, have attracted attention for their biological activities, including anti-inflammatory, antibacterial, antifungal, anticancer, and anti-tuberculosis properties [22-24].

Researchers have focused on the synthesis of azo compounds linked by heterocyclic rings [25]. However, there are no studies in the literature on the synthesis of flavonoid compounds containing azo groups and their biological activity. Therefore, flavonoid derivatives containing azo groups in their structure were synthesized in a previous study published in the *Arkivoc* journal [26].

The present paper aims to study the antioxidant and antifungal activities of flavonoid derivatives containing azo groups and heterocyclic rings, and to compare them with standard compounds.

## 2. Materials and Methods

### 2.1. General remarks.

The chemicals used include dry methanol, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), clotrimazole, quercetin, ascorbic acid (Sigma-Aldrich), hydrogen peroxide, phosphate buffer solution, and agar PDA. The instrumentation used is the Shimadzu UV-1700 spectrophotometer with 1 cm quartz cells.

### 2.2. Synthesis.

The tested compounds (C<sub>1</sub>-C<sub>9</sub>) have been synthesized in a previous study and can be found elsewhere [26]. The chemical structures of the new compounds were characterized by spectroscopic methods (<sup>13</sup>C-NMR, <sup>1</sup>H-NMR, FT-IR) in a previous study published in the *Arkivoc* journal [26]. Figure 1 shows the chemical formulas for the synthesized flavonoids. Table 1 shows the names of flavonoid derivatives (A<sub>1</sub>, C<sub>1</sub>-C<sub>9</sub>) according to the IUPAC system [26].

### 2.3. In vitro assays.

#### 2.3.1. DPPH radical scavenging assay.

A series of concentrations (250, 500, 750, 1000 µg/mL) for each of the flavonoids and the same concentrations of ascorbic acid and quercetin as a positive control were prepared. A solution of DPPH in methanol was prepared at a concentration of 0.2 mM/l by dissolving 78.86 mg of free radical DPPH in 100 mL of dry methanol in a 1000 mL volumetric flask, then diluting to the capacity mark with methanol.

The ability of flavonoids to inhibit free radicals was determined by the DPPH test using Blois' method [27].

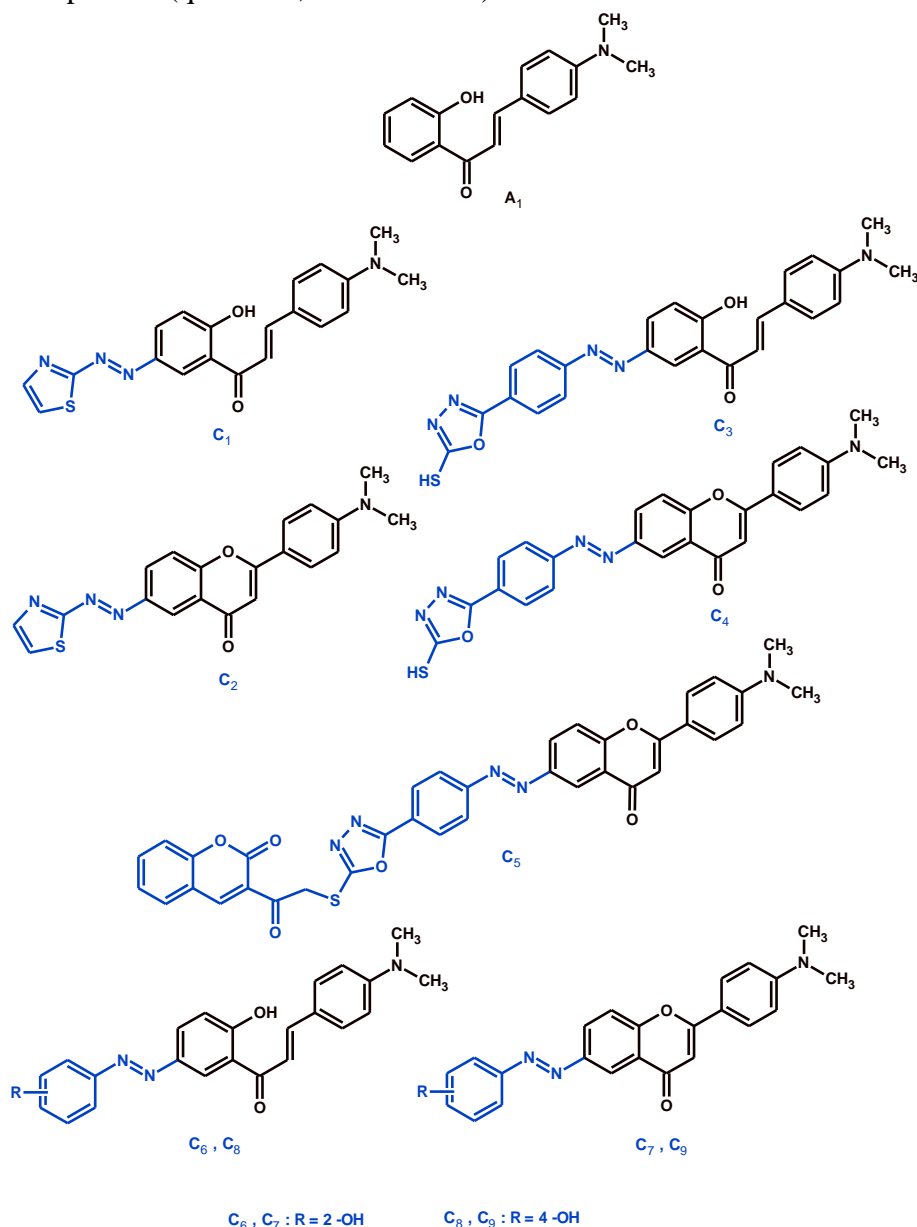
Each test tube contained 1 mL of the solution to be tested (separately for each flavonoid and standard compound), and 5 mL of DPPH solution was added with stirring. The tubes were placed in a dark place at room temperature for 20 minutes. The absorbance was measured using a UV-Vis device at 516 nm (the control solution consisted of 1 mL of dry methanol + 5 mL of

DPPH methanol solution). The percentage of free radical inhibition was calculated by the following arithmetic method:

$$\text{DPPH \%} = [(A_0 - A_1) / A_0] \times 100$$

Where  $A_1$  is the absorbance of the sample, and  $A_0$ : is the absorbance of the control sample.

A diagram was sketched showing the percentage of DPPH inhibition as a function of concentration, which follows a first-order equation. The IC<sub>50</sub> value was then calculated, defined as the concentration of the solution ( $\mu\text{g/mL}$ ) required to clear 50% of the DPPH radicals. The ability of flavonoids ( $C_1$ - $C_9$ ) to inhibit free radicals was compared with the ability of standard compounds (quercetin, ascorbic acid).



**Figure 1.** Synthetic structures of synthesized flavonoids ( $A_1, C_1$ - $C_9$ ) [26].

**Table 1.** The following are the names of flavonoid derivatives ( $A_1, C_1$ - $C_9$ ) according to the IUPAC system [26].

code	Name of the compound according to the IUPAC system
$A_1$	( <i>E</i> )-3-(4-(dimethylamino)phenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one
$C_1$	( <i>E</i> )-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-5-(( <i>E</i> )-thiazol-2-ylidiazenyl)phenyl) prop-2-en-1-one
$C_2$	( <i>E</i> )-2-(4-(dimethylamino)phenyl)-6-(thiazol-2-ylidiazenyl)-4 <i>H</i> -chromen-4-one
$C_3$	( <i>E</i> )-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-5-(( <i>E</i> )-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl) diazenyl)phenyl)prop-2-en-1-one

code	Name of the compound according to the IUPAC system
C <sub>4</sub>	(E)-2-(4-(dimethylamino)phenyl)-6-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazanyl)-4H-chromen-4-one
C <sub>5</sub>	(E)-3-(2-((5-(4-((2-(4-(dimethylamino)phenyl)-4-oxo-4H-chromen-6-yl)diazanyl)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetyl)-2H-chromen-2-one
C <sub>6</sub>	(E)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-5-((E)-(2-hydroxyphenyl)diazanyl)phenyl) prop-2-en-1-one
C <sub>7</sub>	(E)-2-(4-(dimethylamino)phenyl)-6-((2-hydroxyphenyl)diazanyl)-4H-chromen-4-one
C <sub>8</sub>	(E)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxy-5-((E)-(4-hydroxyphenyl)diazanyl)phenyl) prop-2-en-1-one
C <sub>9</sub>	(E)-2-(4-(dimethylamino)phenyl)-6-((4-hydroxyphenyl)diazanyl)-4H-chromen-4-one

### 2.3.2. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging assay.

The ability of flavonoids to inhibit hydrogen peroxide was determined using the following method [28]: 1 mL of the flavonoid compound was added to each test tube (prepared at different concentrations). Afterwards, it was dissolved in methanol and 1 mL of hydrogen peroxide solution (40 mM) in a phosphate buffer solution (50 mM, pH=7.4). The tubes were placed in a dark place at room temperature for 10 minutes. The absorbance for hydrogen peroxide was measured in a UV-VIS (Shimadzu) at a wavelength of 230 nanometers. The control solution consisted of a mixture of methanol, a phosphate buffer, and hydrogen peroxide.

The previous method was followed in the preparation of titer solutions (quercetin, ascorbic acid).

The hydrogen peroxide suppression percentage was calculated by the following arithmetic method:

$$\text{Percent inhibition \%} = [(A_0 - A_1) / A_0] \times 100$$

Where A<sub>1</sub> is the absorbance of the sample, and A<sub>0</sub> is the absorbance of the control sample.

A diagram was sketched for the percentage of inhibition H<sub>2</sub>O<sub>2</sub> % in terms of concentrations, which is a first-order straight equation, then the value of IC<sub>50</sub> for H<sub>2</sub>O<sub>2</sub> was calculated.

The ability of flavonoids to inhibit hydrogen peroxide was compared with the ability of standard compounds (quercetin, ascorbic acid).

### 2.3.3. Study of antifungal efficacy.

The antifungal efficacy of synthetic flavonoid derivatives was studied using the Petri dish method against three types of fungi: *Aspergillus flavus* (A.f), *Acremonium strictum* (A.s), and *Penicillium expansum* (P.ex). This study followed the reference method of Suarez-Jimenez et al. [29] with some modifications to suit our research in the microbiology laboratory. Solutions of flavonoid compounds were prepared at two different concentrations (0.25 mg/mL and 0.5 mg/mL) for each sample, as well as the same concentrations for quercetin and clotrimazole as positive controls. The nutrient medium, composed of prepared agar and flavonoids, was poured into 9 cm diameter Petri dishes and allowed to cool and solidify.

A 5 mm disk was placed at the edges of the active 7-day-old fungal colony for each fungus, at the two concentrations studied. The plates were then incubated at 1 ± 25°C for 7 days.

Growing fungi on flavonoid-free PDA prepared control plates.

Three replicates were conducted for each flavonoid compound at the two concentrations studied, as well as for the control dishes.

Readings of colony growth diameters were taken for both treatments and controls, and their averages were calculated. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition\%} = (dc - dt) / dc \times 100$$

Where *dc* represents the average diameter of the control colonies, and *dt* represents the average diameter of the treated colonies. The same steps were followed for the comparison of both quercetin and clotrimazole.

### 3. Results and Discussion

#### 3.1. DPPH, H<sub>2</sub>O<sub>2</sub> assays.

The inhibitory effect of flavonoids was measured using the DPPH method and the hydrogen peroxide method at four different concentrations, i.e., at (250, 500, 750, and 1000 µg/mL) at a wavelength of 516 nm using the DPPH method and 230 nm using the hydrogen peroxide method. The percentages of the ability of the flavonoid compounds (A<sub>1</sub>, C<sub>1</sub>-C<sub>9</sub>) to inhibit DPPH and H<sub>2</sub>O<sub>2</sub> were calculated and summarised in Table 2. Figures (2) and (3) show the graphical curves of inhibition of synthetic flavonoids, quercetin, and ascorbic acid as a function of concentration. The value IC<sub>50</sub> for each compound was calculated from the graph of percentage inhibition versus concentration, as shown in Table 3.

The results of the inhibitory effects of the synthesized flavonoids (A<sub>1</sub>, C<sub>1</sub>-C<sub>9</sub>) showed that the percentage of free radical suppression was variable, and all had higher IC<sub>50</sub> values than the two standard compounds, ascorbic acid and quercetin, indicating they are weaker antioxidants. It was found that the percentage of inhibition of chalcone A<sub>1</sub> was low, reaching 41.74% in the DPPH method and 27.08% in the H<sub>2</sub>O<sub>2</sub> method, and the IC<sub>50</sub> values were 1053.045 and 1909.311 µg/mL, respectively.

The percentages of inhibition increased when the azo group (C<sub>1</sub>-C<sub>9</sub>) was introduced. Table 2 shows the percentage of inhibition when azothiazoles were introduced for the two compounds (C<sub>1</sub>, C<sub>2</sub>) by the DPPH method (42.32%, 34.21%), and their IC<sub>50</sub> values were 1045.197, 1357.448 µg/mL, and by the hydrogen peroxide method (1821.190, 2146.476) µg/mL, respectively.

The percentage of free radical inhibition in the two compounds C<sub>4</sub> and C<sub>3</sub> containing the oxadiazole ring by DPPH method was (60.30%, 53.66%). The values of IC<sub>50</sub> = 774.568 and 706.677 µg/mL respectively and by hydrogen peroxide method the percentage inhibition percentages were (31.34%, 39.04%), and the values of IC<sub>50</sub> were (1538.055, 1149.399) µg/mL. While the percentage inhibition of free radicals in compound C<sub>5</sub> decreased when the coumarin ring was introduced, with the DPPH method, it reached 51.23 %. The value of IC<sub>50</sub> was equal to 862.081 µg/mL, and with the hydrogen peroxide method, the percentage inhibition reached (24.06 %), and the value of IC<sub>50</sub> = 2023.788 µg/mL.

It was also found that compounds C<sub>6</sub> and C<sub>8</sub>, each containing two groups, ortho-hydroxyphenyl and para-hydroxyphenyl, exhibited higher free radical inhibition rates. The inhibition percentages by the DPPH method were 53.66% and 54.22%, and the IC<sub>50</sub> values were 706.130 and 700.663 µg/mL, respectively. For the hydrogen peroxide method, the percentages were 43.45 % and 39.04 %, and the IC<sub>50</sub> values were 974.658 and 1149.247 µg/mL, respectively.

While the values of the percentage inhibition of free radicals for compounds C<sub>7</sub> and C<sub>9</sub> decreased when the cyclization reactions of compounds C<sub>6</sub> and C<sub>8</sub> with iodine in dimethyl sulfoxide were performed, the percentage of inhibition by the DPPH method was (44.32 %, 44.32 %), and the IC<sub>50</sub> values were 1045.197 and 1357.448 µg/mL, respectively.

and 49.54 %). The IC<sub>50</sub> values for them were 949.723 and 810.273 µg/mL, respectively, while the percentage inhibition by the peroxide method was 33.29 %, 33.67 % and their IC<sub>50</sub> values were 1386.447 and 1427.375 µg/mL, respectively.

We conclude that the highest activity of the compound as an antioxidant among the flavonoid derivatives (C<sub>1</sub>-C<sub>9</sub>) is the compounds (C<sub>3</sub>, C<sub>4</sub>, C<sub>6</sub>, C<sub>8</sub>). The reason for this is due to the presence of a thiol group (-SH) in the compounds C<sub>3</sub> and C<sub>4</sub> and a phenolic -OH group in the compounds (C<sub>6</sub>, C<sub>8</sub>), which increases the acidic properties of the compound and is thus capable of binding hydrogen to the free radical DPPH, Hydrogen to the free radical DPPH (and its conversion to the stable form DPPH-H) or to hydrogen peroxide (and its conversion to water molecules) [30, 31], and the molecules of the hydrogen donor compounds are resonantly converted to stable free radicals, as shown by the mechanism of the compounds shown in Figure 5. The least effective compound as an antioxidant is C<sub>2</sub>, as it has no hydroxyl group.

The results showed that flavonoids had a greater inhibitory effect on DPPH free radicals than hydrogen peroxide. The reason for this could be that DPPH is an intermediate compound (unstable) compared to hydrogen peroxide (stable compound). Therefore, DPPH is characterized by high activity as it reacts faster with the flavonoid compound than hydrogen peroxide. Figure 4 shows the IC<sub>50</sub> values for the two methods mentioned.

The compound is an antioxidant if the phenoxy radicals it produces are stabilized. If it fails to stabilize unstable radicals, it triggers a chain reaction of free radicals that can lead to harmful effects in the human body. However, the stabilization of unstable free radicals in phenolic compounds (especially flavonoids) can be easily achieved by electron transfer, as they have a conjugated structural system either in the aromatic ring or the pyran ring (as in the proposed mechanism in Figure 5). Thus, the radicals formed undergo delocalization of their electrons within the flavonoid structure and are converted into a stable form [30].

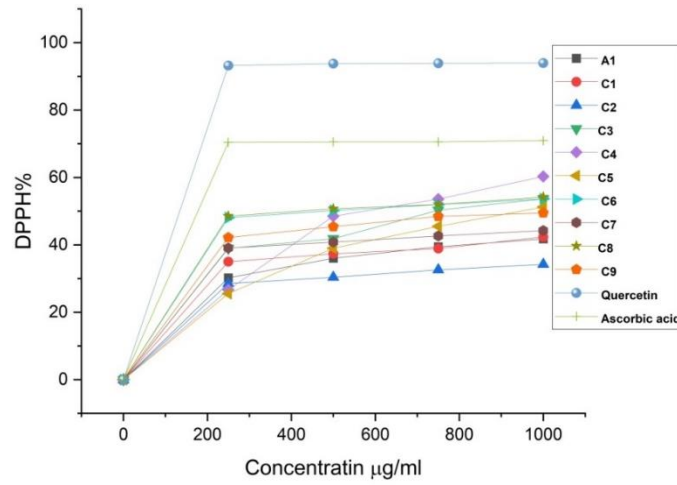
**Table 2.** Results of percentages of inhibition of DPPH, H<sub>2</sub>O<sub>2</sub> for synthetic flavonoids (A<sub>1</sub>, C<sub>1</sub> - C<sub>9</sub>).

Concs µg/mL	0	250		500		750		1000	
Comp.	0	H <sub>2</sub> O <sub>2</sub> %	DPPH %	H <sub>2</sub> O <sub>2</sub> %	DPPH %	H <sub>2</sub> O <sub>2</sub> %	DPPH %	H <sub>2</sub> O <sub>2</sub> %	DPPH %
A <sub>1</sub>	0	12.68	30.20	15.92	36.00	20.31	39.43	27.08	41.74
C <sub>1</sub>	0	14.26	35.00	19.09	37.32	23.12	38.90	27.08	42.32
C <sub>2</sub>	0	15.12	28.51	18.29	30.36	19.74	32.57	23.91	34.21
C <sub>3</sub>	0	18.37	38.95	23.12	41.80	27.66	50.28	31.34	53.66
C <sub>4</sub>	0	18.94	26.67	31.05	48.49	36.67	53.55	39.04	60.30
C <sub>5</sub>	0	13.61	25.51	17.14	39.00	22.26	45.49	24.06	51.23
C <sub>6</sub>	0	33.88	47.98	37.29	50.18	40.69	51.92	43.45	53.66
C <sub>7</sub>	0	19.14	39.06	23.77	40.80	31.91	42.64	33.29	44.32
C <sub>8</sub>	0	31.17	48.55	34.29	50.71	36.74	51.85	39.04	54.22
C <sub>9</sub>	0	17.99	42.12	23.02	45.42	29.69	48.39	33.67	49.54
Quercetin	0	91.49	93.19	91.78	93.77	91.93	93.88	92.57	93.93
Ascorbic acid	0	75.86	70.42	76.80	70.53	77.80	70.53	78.53	70.90

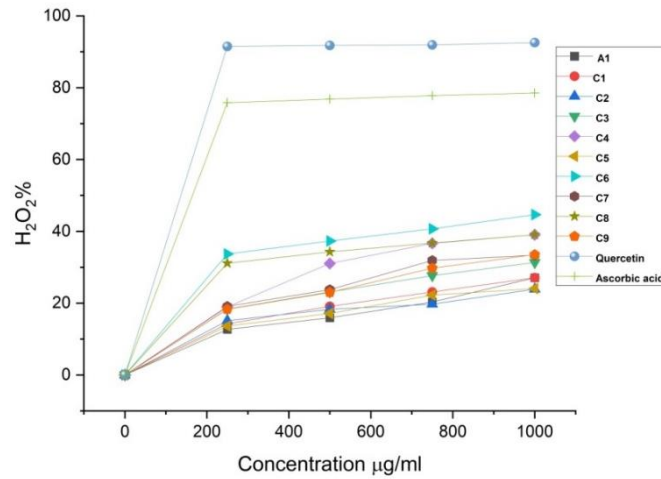
**Table 3.** IC<sub>50</sub> values for synthetic flavonoids (A<sub>1</sub>, C<sub>1</sub> - C<sub>9</sub>).

Compound	IC <sub>50</sub> (µg/mL)	
	DPPH	H <sub>2</sub> O <sub>2</sub>
A1	1053.045	1909.311
C1	1045.197	1821.190
C2	1357.448	2146.476
C3	774.568	1538.055
C4	706.677	1149.399
C5	862.081	2023.788
C6	706.130	974.658
C7	949.723	1386.447
C8	700.663	1149.247
C9	810.243	1427.375

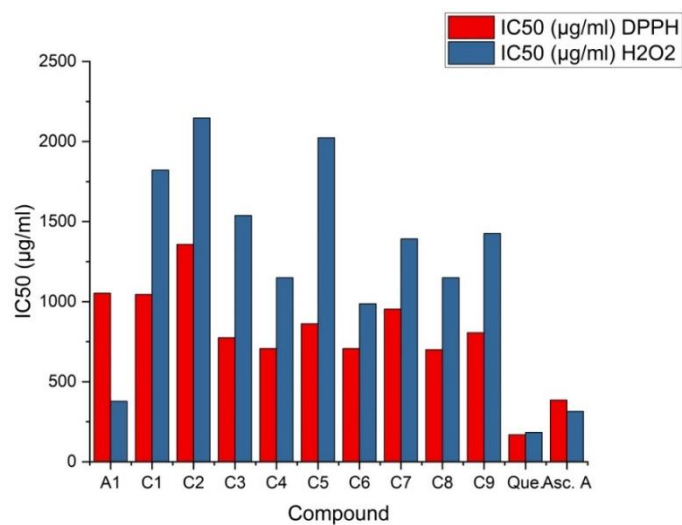
Compound	IC <sub>50</sub> (µg/mL)	
	DPPH	H <sub>2</sub> O <sub>2</sub>
Quercetin	169.177	182.776
Ascorbic acid	385.669	314.496



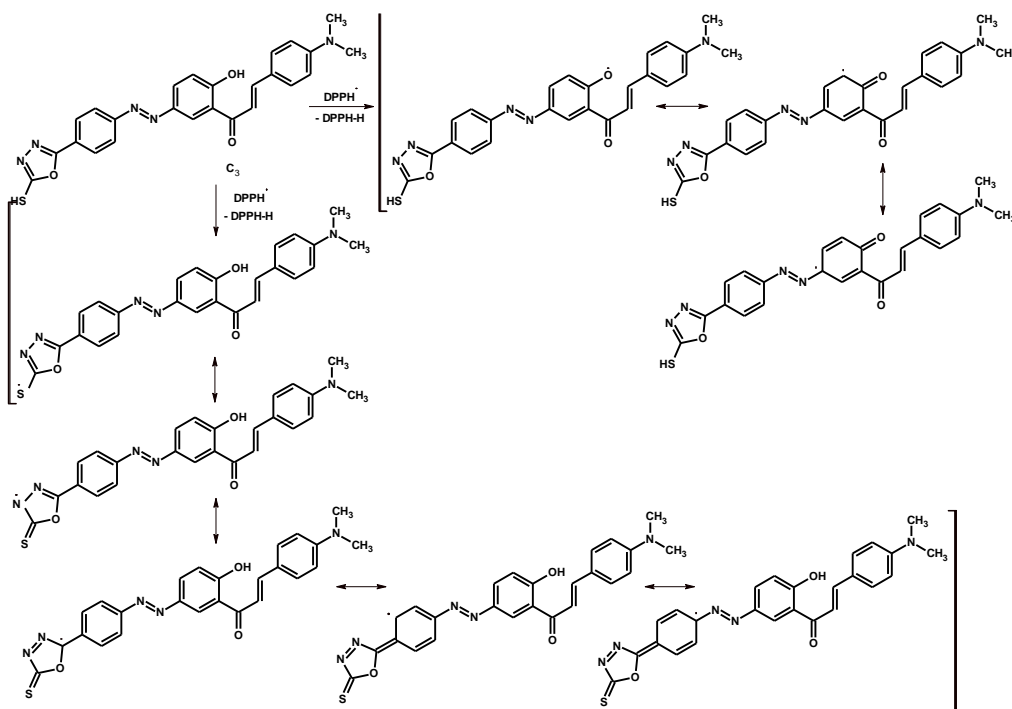
**Figure 2.** Results of the DPPH % test for flavonoids (A<sub>1</sub>, C<sub>1</sub> – C<sub>9</sub>).



**Figure 3.** Results of the H<sub>2</sub>O<sub>2</sub> % test for flavonoids (A<sub>1</sub>, C<sub>1</sub> – C<sub>9</sub>).



**Figure 4.** IC<sub>50</sub> values for flavonoids (A<sub>1</sub>, C<sub>1</sub> - C<sub>9</sub>) by DPPH and H<sub>2</sub>O<sub>2</sub> methods.



**Figure 5.** Proposed mechanism of compound C<sub>3</sub> as an antioxidant.

### 3.2. Antifungal efficacy.

The inhibitory effect of the synthesized flavonoids (A<sub>1</sub>, C<sub>1</sub>-C<sub>9</sub>) was investigated on three fungal species (*Acremonium strictum*, *Aspergillus flavus*, *Penicillium expansum*) at two different concentrations (0.5 mg/mL, 0.25 mg/mL). Table 4 shows that the inhibitory effect of the flavonoids compares favorably with that of quercetin (a natural flavonoid) and the standard drug, clotrimazole. Quercetin was chosen because its chemical structure is very similar to that of the flavonoids analyzed. Clotrimazole was selected as an antimycotic with broad-spectrum activity. Clotrimazole contains a heterocyclic ring (imidazole) and a chloro group.

Table 4 shows that the highest inhibitory effect of compound A<sub>1</sub> was observed against the fungus *Penicillium expansum*, with percentage inhibition values of 60.78% and 44.11% at the two concentrations tested, and the inhibitory effect was comparable to that of quercetin and clotrimazole. In contrast, the effect on the two species, *Acremonium strictum* and *Aspergillus flavus*, was lower.

The inhibitory effect of the two flavonoid compounds (C<sub>1</sub>, C<sub>2</sub>) containing the thiazole ring was slightly higher than the inhibitory effect of the main compound A<sub>1</sub> on *Aspergillus flavus* and *Penicillium expansum*. In contrast, their inhibitory effect on *Acremonium strictum* was high, as the percentage of inhibition was (86.66 %, 100 %).

The inhibitory effect of compounds C<sub>3</sub> and C<sub>4</sub>, which contain an oxadiazole ring and a thiol group, on *Aspergillus flavus* and *Penicillium expansum* was lower than that of the previous compounds A<sub>1</sub>, C<sub>1</sub>, and C<sub>2</sub> at two concentrations tested due to the presence of a thiol group with uncharged polar properties.

While the inhibitory effect of these compounds on *Acremonium strictum* was higher than that of compound A<sub>1</sub>, this is due to the structure of the cell wall of *Acremonium strictum*. The inhibitory effect of the flavonoid complex C<sub>5</sub>, which contains an oxadiazole ring and a coumarin, was lower in all fungal species.

Table 4 also shows that the inhibitory effect of compounds (C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, and C<sub>9</sub>) on *Penicillium expansum* is greater than the inhibitory effect of compound A<sub>1</sub>, with the highest

value obtained for compound C<sub>7</sub> (77.45 %), followed by compounds C<sub>8</sub> and C<sub>9</sub> (72.54 %). The inhibitory effect of the two titer compounds was slightly less at a concentration of 0.5 mg/mL and slightly less at a concentration of 0.25 mg/mL. The inhibitory effect of the compounds (C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, and C<sub>9</sub>) on *Aspergillus flavus* was stronger than the inhibitory effect of chalcone A<sub>1</sub> but weaker than the effect of the two standard compounds.

When comparing the inhibitory effect of flavonoids (A<sub>1</sub>, C<sub>1</sub>-C<sub>9</sub>) on fungal species (*Acremonium strictum*, *Aspergillus flavus*, *Penicillium expansum*) with the inhibitory effect of flavonoids (A<sub>2</sub>, A<sub>5</sub>, A<sub>7</sub>, A<sub>8</sub>, A<sub>9</sub>, A<sub>12</sub>) on the same fungal species [32], it was found that the inhibitory effect of the compounds (C<sub>2</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, C<sub>9</sub>) was close to the Inhibitory compound (A<sub>2</sub>) 2-[4-(dimethylamino)phenyl]-3-hydroxy-4*H*-1-benzopyran-4-one which contains an enolic -OH group, and the compound (A<sub>5</sub>) 2-({2-[4-(dimethylamino)phenyl]-4-oxo-4*H*-1-benzo pyran -3-yl} oxy) acetamide which contains an aliphatic amide group, and the compound (A<sub>9</sub>) 2-[4-(dimethylamino)phenyl]-4-oxo-4*H*-1-benzopyran -3-yl chloroacetate which contains a chloroacetyl group, and compound (A<sub>12</sub>) 2-[4-(dimethylamino) phenyl]-4-oxo-4*H*-1-benzopyran -3-yl 2-carbamoyl benzoate which contains an aromatic amide group, on the fungus species *Acremonium strictum* at the concentration 500 ppm.

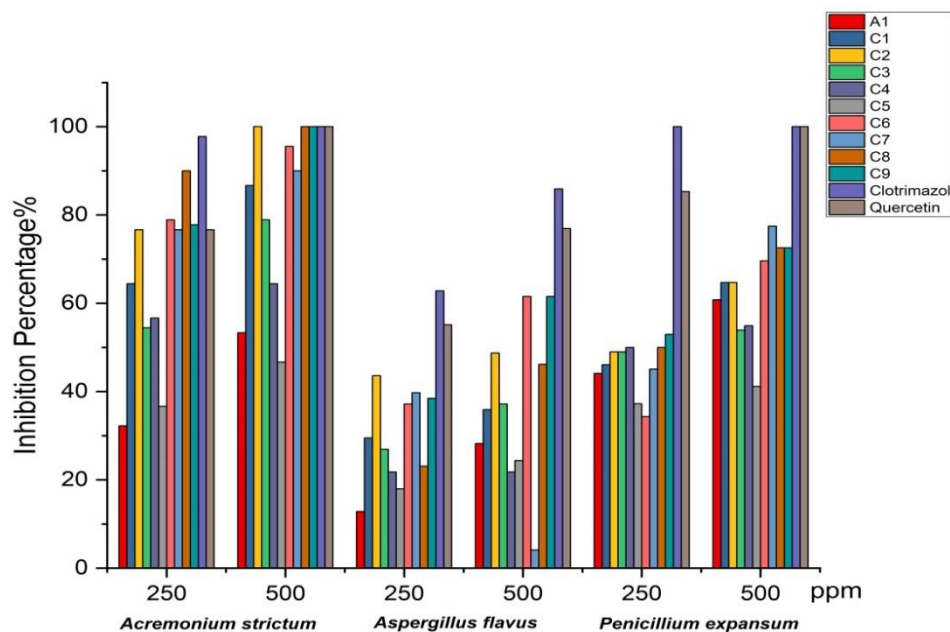
All compounds (C<sub>1</sub>-C<sub>9</sub>) showed a lower inhibitory effect on the fungal species *Penicillium expansum* compared to the compounds in the reference study (A<sub>2</sub>, A<sub>5</sub>, A<sub>8</sub>) [32], while convergence was observed between the inhibitory effect of compounds (C<sub>1</sub>, C<sub>2</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, C<sub>9</sub>) and the inhibitory effect of compound A<sub>12</sub> at a concentration of 500 ppm. In addition, a convergence was observed between the inhibitory effect of the two compounds (C<sub>3</sub>, C<sub>4</sub>) on the fungus *Penicillium expansum* and the inhibitory effect of compound A<sub>7</sub>, which contains a coumarin group in its structure. When comparing the inhibitory effect of compounds A<sub>7</sub> and C<sub>5</sub>, both of which contain a coumarin structure, on the fungus *Penicillium expansum*, compound A<sub>7</sub> was found to be more inhibitory than compound C<sub>5</sub>. The inhibitory effect of the compounds (C<sub>6</sub>, C<sub>7</sub>, C<sub>9</sub>) on the fungus *Aspergillus flavus* at a concentration of 500 ppm was close to the inhibitory effect of the two compounds (A<sub>2</sub>, A<sub>9</sub>).

**Table 4.** Results of the biological activity of flavonoid derivatives against fungal strains (Inhibition percentage %).

Comp	<i>Acremonium strictum</i>		<i>Aspergillus flavus</i>		<i>Penicillium expansum</i>	
	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm
Cons.						
A <sub>1</sub>	32.22	53.33	12.82	28.20	44.11	60.78
C <sub>1</sub>	64.44	86.66	29.48	35.89	46.07	64.70
C <sub>2</sub>	76.66	100	43.58	48.71	49.01	64.7
C <sub>3</sub>	54.44	78.88	26.92	37.17	49.01	53.92
C <sub>4</sub>	56.66	64.44	21.79	21.79	50.00	54.90
C <sub>5</sub>	36.66	56.66	17.94	24.35	37.25	41.17
C <sub>6</sub>	78.88	95.55	37.17	61.53	34.31	69.60
C <sub>7</sub>	76.66	90.00	39.74	64.10	45.09	77.45
C <sub>8</sub>	90.00	100	23.07	46.15	50.00	72.54
C <sub>9</sub>	77.77	100	38.46	61.53	52.94	72.54
Clotrimazol	97.77	100	62.82	85.89	100	100
Quercetin	76.66	100	55.12	76.92	85.29	100

The inhibitory effect of flavonoids is attributed to their ability to bind cellular proteins and form a water-soluble extracellular protein complex after penetrating the cell wall and plasma membranes of the microbe [33, 34]. The study also showed that some flavonoid derivatives contain a hydroxyl group linked to an aromatic ring, which is associated with bacterial intoxication via enzymatic oxidation of phenols and interference with sulfhydryl groups during protein synthesis [34].

This is also due to morphological and cellular changes in microorganisms, including alterations in colony color and shape, as well as changes in cell shape and size. In addition, phenolic compounds interfere with the formation of the cell wall and cell membrane, leading to their destruction and killing the fungi [35]. Figure 6 shows the results of the biological activity of flavonoid derivatives against fungal species (*Acremonium strictum*, *Aspergillus flavus*, *Penicillium expansum*).



**Figure 6.** Results of the biological activity of flavonoid derivatives against fungal strains (*Acremonium strictum*, *Aspergillus flavus*, *Penicillium expansum*).

#### 4. Conclusions

The percentage inhibition of free radicals was good, with the compounds (C4, C6, C8) showing the highest values using the DPPH and hydrogen peroxide methods. The IC<sub>50</sub> values were (706.677, 707.146, 699.686 µg/mL) using the DPPH method and (1149.399, 986.649, 1149.313 µg/mL) using the hydrogen peroxide method, compared with ascorbic acid and quercetin, known antioxidants. The results of inhibition of some flavonoid compounds on the growth of three fungal species (*Acremonium strictum*, *Aspergillus flavus*, *Penicillium expansum*) were different, and the best compounds (C1, C2, C6, C7, C8, C9) were against *Acremonium strictum* compared to quercetin and clotrimazole.

#### Author Contributions

Conceptualization, H.K.; methodology, H.K.; software, H.K. and M.O.; validation, H.K., S.S., F.K., and M.O.; formal analysis, H.K.; investigation, H.K., S.S., F.K., and M.O.; resources, F.K.; data curation, H.K. and S.S.; writing—original draft preparation, H.K.; writing—review and editing, H.K. and S.S.; visualization, H.K.; supervision, S.S.; project administration, S.S.; funding acquisition, not applicable. All authors have read and agreed to the published version of the manuscript.

#### Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

## Funding

This research received no external funding.

## Acknowledgments

None.

## Conflicts of Interest

The authors declare no conflict of interest.

## References

1. Dias, M.C.; Pinto, D.; Silva, A.M.S. Plant flavonoids: Chemical characteristics and biological activity. *Molecules* **2021**, *26*, 5377, <https://doi.org/10.3390/molecules26175377>.
2. Rodríguez De Luna, S.L.; Ramírez-Garza, R.E.; Serna Saldívar, S.O. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *Sci. World J.* **2020**, *2020*, 6792069, <https://doi.org/10.1155/2020/6792069>.
3. Xing, N.; Meng, X.; Wang, S. Isobavachalcone: A comprehensive review of its plant sources, pharmacokinetics, toxicity, pharmacological activities and related molecular mechanisms. *Phytother. Res.* **2022**, *36*, 3120–3142, <https://doi.org/10.1002/ptr.7520>.
4. Huang, W.; Wang, Y.; Tian, W.; Cui, X.; Tu, P.; Li, J.; Shi, S.; Liu, X. Biosynthesis investigations of terpenoid, alkaloid, and flavonoid antimicrobial agents derived from medicinal plants. *Antibiotics* **2022**, *11*, 1380, <https://doi.org/10.3390/antibiotics11101380>.
5. Rakha, A.; Umar, N.; Rabail, R.; Butt, M.S.; Kieliszek, M.; Hassoun, A.; Aadil, R.M. Anti-inflammatory and anti-allergic potential of dietary flavonoids: A review. *Biomed. Pharmacother* **2022**, *156*, 113945, <https://doi.org/10.1016/j.biopha.2022.113945>.
6. Luo, Y.; Jian, Y.; Liu, Y.; Jiang, S.; Muhammad, D.; Wang, W. Flavanols from nature: A phytochemistry and biological activity review. *Molecules* **2022**, *27*, 719, <https://doi.org/10.3390/molecules27030719>.
7. Khan, J.; Deb, P.K.; Priya, S.; Medina, K.D.; Devi, R.; Walode, S.G.; Rudrapal, M. Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. *Molecules* **2021**, *26*, 4021, <https://doi.org/10.3390/molecules26134021>.
8. Addi, M.; Elbouzidi, A.; Abid, M.; Tungmunnithum, D.; Elamrani, A.; Hano, C. An overview of bioactive flavonoids from citrus fruits. *Appl. Sci.* **2022**, *12*, 29, <https://doi.org/10.3390/app12010029>.
9. Procházková, D.; Bousová, I.; Wilhelmová, N. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* **2011**, *82*, 513–523, <https://doi.org/10.1016/j.fitote.2011.01.018>.
10. Malešev, D.; Kunti, V.; Investigation of metal-flavonoid chelates and the determination of flavonoids via metal-flavonoid complexing reactions. *J. Serb. Chem. Soc.* **2007**, *72*, 921–939, <https://doi.org/10.2298/JSC0710921M>.
11. Hu, D.; Wang, Y.; Liu, J.; Mao, Y.; Chang, X.; Zhu, Y. Light-Driven Sequential Shape Transformation of Block Copolymer Particles through Three-Dimensional Confined Self-Assembly. *Nanoscale* **2022**, *14*, 6291–6298, <https://doi.org/10.1039/D2NR01172G>.
12. Shimizu, T.; Tanifuji, N.; Yoshikawa, H. Azo Compounds as Active Materials of Energy Storage Systems. *Angew. Chem. Int. Ed.* **2022**, *61*, e202206093, <https://doi.org/10.1002/anie.202206093>.

13. Udoikono, A.D.; Louis, H.; Eno, E.A.; Agwamba, E.C.; Unimuke, T.O.; Igbalagh, A.T.; Edet, H.O.; Odey, J.O.; Adeyinka, A.S. Reactive Azo Compounds as a Potential Chemotherapy Drugs in the Treatment of Malignant Glioblastoma (GBM): Experimental and Theoretical Studies. *J. Photochem. Photobiol* **2022**, *10*, 100116, <https://doi.org/10.1016/j.jpap.2022.100116>.
14. Jarad, A.; Obaid, S.; Abd-Almonuim, A. Synthesis, characterization, industrial and biological application of co(ii),ni(ii),cu(ii) and zn(ii) complexes with azo ligand derived from metoclopramide hydrochloride and 3,5-dimethylphenol. *Egyptian Journal of Chemistry* **2020**, *63*, 4719-4729, <https://doi.org/10.21608/ejchem.2020.21400.2283>.
15. Selvaraj, V.; Swarna Karthika, T.; Mansiya, C.; Alagar, M. An over review on recently developed techniques, mechanisms and intermediate involved in the advanced azo dye degradation for industrial applications. *Journal of Molecular Structure* **2021**, *1224*, 129195, <https://doi.org/10.1016/j.molstruc.2020.129195>.
16. Bafana, A.; Devi, S.S.; Chakrabarti, T. Azo dyes: Past, present and the future. *Environmental Reviews* **2011**, *19*, 350-371, <https://doi.org/10.1139/a11-018>.
17. Tasli, P.T.; Atay, Ç.K.; Demirturk, T.; Tilki, T. Experimental and computational studies of newly synthesized azo dyes based materials. *Journal of Molecular Structure* **2020**, *1201*, 127098, <https://doi.org/10.1016/j.molstruc.2019.127098>.
18. Mezgebe, K.; Mulugeta, E. Synthesis and pharmacological activities of azo dye derivatives incorporating heterocyclic scaffolds: A review. *RSC advances* **2022**, *12*, 25932-25946, <https://doi.org/10.1039/D2RA04934A>.
19. Neghabi, S.; Ghadari, R. Enhanced efficiency of the azo dye-sensitized solar cell via the cooperation of graphene oxide and graphene oxide/polypyrrole: Experimental and computational studies. *Electrochimica Acta* **2024**, *479*, 143865, <https://doi.org/10.1016/j.electacta.2024.143865>.
20. Popa, S. Aspects regarding colour fastness and adsorption studies of a new azo-stilbene dye for acrylic resins. *Sci. Rep.* **2021**, *11*, 5889, <https://doi.org/10.1038/s41598-021-85452-7>.
21. Patel, M.; Likhar, A.R.; Bhojani, A.K.; Vaishnani, A.; Patel, H.; Singh, D.K.; Asthana, D.; Gour, N. New azo dyes for detection of metallic impurities. *Microchemical Journal* **2024**, *200*, 110351, <https://doi.org/10.1016/j.microc.2024.110351>.
22. Mi, Y.; Zhang, J.; Han, X.; Tan, W.; Miao, Q.; Cui, J.; Li, Q.; Guo, Z. Modification of carboxymethyl inulin with heterocyclic compounds: synthesis, characterization, antioxidant and antifungal activities. *Int. J. Biol. Macromol.* **2021**, *181*, 572–581, <https://doi.org/10.1016/j.ijbiomac.2021.03.109>.
23. Singh, K.; Pal, R.; Khan, S.A.; Kumar, B.; Akhtar, M.J. Insights into the structure activity relationship of nitrogen-containing heterocyclics for the development of antidepressant compounds: an updated review. *J. Mol. Struct.* **2021**, *1237*, 130369, <https://doi.org/10.1016/j.molstruc.2021.130369>.
24. Sahilu, R.; Eswaramoorthy, R.; Mulugeta, E.; Dekebo, A. Synthesis, DFT Analysis, Dyeing Potential and Evaluation of Antibacterial Activities of Azo Dye Derivatives combined with in silico molecular docking and ADMET predictions. *J. Mol. Struct.* **2022**, *1265*, 133279, <https://doi.org/10.1016/j.molstruc.2022.133279>.
25. Muhammad, S.; Abdul, J.; Salma, F.; Idrees, B.; Muhammad, S. K. R. New heterocyclic azo-disperse dyes; their synthesis, characterization, application, photo physical properties and solvatochromic studies. *Journal of Molecular Structure* **2023**, *1287*, 135664, <https://doi.org/10.1016/j.molstruc.2023.135664>.
26. Khdera, H.A.; Saad, S.Y.; Moustapha, A.; Kandil, F. Design, synthesis and characterization of azo group-containing flavonoid derivatives. *Arkivoc* **2023**, *Vii*, 12010, <https://doi.org/10.24820/ark.5550190.p012.010>.
27. Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods in Enzymology* **1990**, *186*, 343-355, [https://doi.org/10.1016/0076-6879\(90\)86128-i](https://doi.org/10.1016/0076-6879(90)86128-i).
28. Ruch, R.J.; Cheng, S.J.; Klaunig, J.E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* **1989**, *10*, 1003-1008, <https://doi.org/10.1093/carcin/10.6.1003>.
29. Suárez-jiménez, G.; cortez-rocha, M.; rosas-burgos, C.; burgos-hernández, A.; plascencia-jatomea, M.; cinco-moroyoqui, F. Antifungal Activity of Plant Methanolic Extracts Against *Fusarium verticillioides* (Sacc.) Nirenb. *And Fumonisin B1 Production* **2007**, *25*, 134 – 142.
30. Assa, J. R.; Widjanarko, S. B.; Kusnadi, J. K.; Berhimpon, S. Antioxidant Potential of Flesh, Seed and Mace of Nutmeg (*Myristica fragrans* Houtt). *Int. J. ChemTech Res.* **2014**, *6*, 2460-2468.
31. Prahadeesh, N.; Sithambaresan, M.; Mathiventhan, U. A Study on Hydrogen Peroxide Scavenging Activity and Ferric Reducing Ability of Simple Coumarins. *Emerging Science Journal* **2018**, *2*, 417-427.

32. Khdera, H. A.; Saad, S. Y.; Moustapha, A.; Kandil, F. Synthesis of new flavonoid derivatives based on 3-hydroxy-4'-dimethylamino flavone and study the activity of some of them as antifungal. *Heliyon* **2022**, *8*, e12062, <https://doi.org/10.1016/j.heliyon.2022.e12062>.
33. Tim Batchelder, B. A. The chemical anthropology of antimicrobial plants (Medical anthropology). Townsend letter for Doctors and Patients. **2004**.
34. Tsuchiya, H.; Sato, M.; Miyazaki, T.; Fujiwara, S.; Linum, M. Comparative study on the antibacterial activity of the phytochemical flavonones against methicillin - resistant *Staphylococcus aureus*. *J. Ethopharmacol.* **1996**, *50*, 27-34, [https://doi.org/10.1016/0378-8741\(96\)85514-0](https://doi.org/10.1016/0378-8741(96)85514-0).
35. Arif, T.; Bhosale, J. D.; Kumar, N. Natural products-antifungal agents derived from plants. *J. Asian Nat. Prod. Res.* **2009**, *11*, 621-638, <https://doi.org/10.1080/10286020902942350>.

### **Publisher's Note & Disclaimer**

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.