

<https://doi.org/10.48047/AFJBS.6.14.2024.3044-3059>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

## GREEN SYNTHESIS OF PYRAZOLINES WITH POTENTIAL ANTIOXIDANT PROPERTIES

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Volume 6, Issue 14, Aug 2024

Received: 09 June 2024

Accepted: 19 July 2024

Published: 08 Aug 2024

doi: [10.48047/AFJBS.6.14.2024.3044-3059](https://doi.org/10.48047/AFJBS.6.14.2024.3044-3059)

### Abstract

The green synthesis of pyrazoline derivatives with possible antibacterial, anti-inflammatory, and antioxidant effects is investigated in this work. The goal of the research was to create efficient and sustainable pyrazoline synthesis routes by utilizing environmentally friendly techniques including microwave irradiation and grinding-based technologies. The DPPH radical scavenging method was used to evaluate the antioxidant activity of the synthesized pyrazolines. The results showed considerable radical scavenging capabilities, with compounds 3a, 3d, and 3e exhibiting the maximum activity comparable to ascorbic acid. Using the HRBC membrane stabilization method, anti-inflammatory capabilities were assessed. Compounds 3c, 3d, and 3e showed strong anti-inflammatory effects that were comparable to aspirin. Furthermore, pyrazoline derivative 3b showed the strongest antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, according to antimicrobial screening results. Other derivatives also indicated noteworthy performance. Overall, the study indicates that these pyrazoline derivatives have intriguing biological properties and may be further developed for medicinal purposes.

**Keywords:** Green Synthesis, Pyrazolines, Potential, Antioxidant, Properties

## **1.INTRODUCTION**

Numerous disciplines, including chemistry and pharmacology, have been profoundly impacted by the unrelenting progress of science and technology [1]. The green synthesis approach, which stresses the use of ecologically friendly technologies for chemical production, is one of the noteworthy developments in these domains [2]. Pyrazolines are a diverse group of chemicals that have gained significant attention due to their wide range of biological activity, even though they are manufactured using green methods [3]. Because of their possible antioxidant qualities, pyrazolines—five-membered heterocyclic molecules with two adjacent nitrogen atoms—are particularly noteworthy as viable candidates in the fight against disorders linked to oxidative stress [4]. Growing awareness of the harmful effects of synthetic chemicals on human health and the environment is a major factor driving interest in pyrazolines. Conventional synthetic routes frequently entail hazardous chemicals and hard environments, which seriously pollute the environment and endanger human health [5]. On the other hand, environmentally friendly synthesis techniques make use of safe chemicals, renewable solvents, and energy-saving procedures [6]. These techniques seek to preserve or even improve the synthetic compounds' effectiveness while reducing the environmental impact of chemical synthesis.

Antioxidants are essential for scavenging free radicals and halting oxidative damage to tissues and cells. Numerous chronic illnesses, such as cancer, heart disease, and neurological disorders, are linked to oxidative stress [7]. As a result, the creation of novel antioxidants by green synthesis not only complies with sustainable chemistry principles but also meets a pressing medical need [8]. Pyrazolines have been shown in numerous *in vitro* and *in vivo* investigations to possess notable antioxidant activity due to their distinct structural characteristics [9].

### **1.1 Importance of Antioxidant Properties**

An awkwardness between receptive oxygen species (ROS) and the body's capacity to kill them with antioxidants causes oxidative pressure, which antioxidants forestall [10]. Ongoing sicknesses like cardiovascular illness, malignant growth, and neurological problems like Alzheimer's and Parkinson's are connected to oxidative pressure [11]. ROS damage to lipids, proteins, and DNA causes inflammation, cell malfunction, and accelerated aging. Antioxidants neutralize ROS,

lowering cellular harm and disease progression [12]. Thus, developing novel antioxidants with high efficacy and low side effects is crucial [13]. New antioxidants must protect against oxidative damage while limiting side effects and drug interactions for long-term use [14]. These standards improve therapy outcomes for oxidative stress-related illnesses and public health and quality of life. Innovative antioxidant research methods, such as green synthesis of novel antioxidant molecules, can improve therapeutic alternatives and promote sustainable health [15].

### 1.1 Objectives of the Study

- To develop effective and ecologically friendly pyrazoline derivative synthesis techniques. To synthesize novel pyrazoline compounds with possible antibacterial, antioxidant, and anti-inflammatory properties.
- To evaluate the synthetic pyrazoline derivatives' biological activity.
- To look into the compounds' efficacy and safety for possible clinical application.

## 2. MATERIALS AND METHODS

### a) Materials

Every response makes use of solvents and engineering grade beginning fixes obtained from Sigma Aldrich. The virtue of the various fixes and coordinated things was described using thin layer chromatography (with tender loving care) and actual steady. Every response was observed using a pre-covered plate for gentle loving care (Silica gel 60-120). The dissolvable framework used for Step-1 mixtures was chloroform: methanol (8:2), while for Step-2 combinations, it was chloroform: methanol (9:1). In order to look for spots, the received tender loving care plates were examined in an iodine chamber and under a lengthy UV light. A Lab line Logical Instruments microwave was used to lead all responses under microwave radiation. Spectroscopic methods such as  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, FTIR, and mass confirmed and explained the designs of the combined combinations.

### b) Methods

#### ❖ Conventional heating method (1a-i)

Combine 12.5 milliliters of ethanol, 20 milliliters of water, and 2.2 grams of sodium hydroxide in a reduced jolt and mix with a pleasant-looking stirrer. The carafe was surrounded by broken ice. 0.43 million moles 4.4 cc of acetophenone was added to the liquid and vigorously stirred with an attractive stirrer. Next, dropwise addition of 4.6 ml of substituted benzaldehyde (0.43mol) was made while maintaining a 25 °C temperature for the mixture. Temporarily, the reaction combination was put away in a fridge. The reaction mix was also weakened with 50 milliliters of cold water and matured with 10% aq. HCl to advance the chalcone. Ten milliliters of cold-changed soul were added after the end result had been isolated and flushed with cold water until it was litmus-unbiased. The item was then recrystallized utilizing methanol.

❖ **The method of irradiating microwaves (1a-i)**

Subbed acetophenone (5 mmol) and subbed benzaldehyde (5 mmol) were disintegrated in 1N NaOH 1N (0.01 mol) of ethanol in a round-base cup with a 7.5 mL limit. The mixture was subjected to 180 W of microwave radiation for three minutes, monitored with TLC, and neutralized with five milliliters of 3N HCl. Cold water was used to wash the product after filtering.

❖ **Overall pyrazoline derivative production process (2a-i, 3a-i)**

Chalcone 1a-I (10 mmol) and INH (10 mmol, 1.37g) were subbed by 2a-I/phenyl hydrazine hydrate (10 mmol, 1.5g). 3a-I refluxed for a day and a half subsequent to dissolving in 10 milliliters of chilly acidic corrosive. Tender loving care observed the advancement of the response. The combination was added to super cold water, and afterward it was killed with sodium bicarbonate. Whenever required, the tacky nature was taken out utilizing a brackish water treatment.

❖ **General pyrazoline derivative synthesis (2a-i, 3a-i)**

After dissolving 2.5 mmol of isonicotinic acid hydrazide and 2.5 mmol of Compound 1a-i in glacial acetic acid, Comp 2a-i was created. After that, the combination was exposed to microwave radiation for three to five minutes.

Compounds (1a-i) were placed in an open Pyrex vessel, and 2.5 mmol of phenyl hydrazine was added in a similar manner. After that, the reaction was exposed to microwave radiation for the proper amount of time—three to five minutes—until TLC verified that the reaction was complete. 50 milliliters of cold water were added to the reaction mixture once it had cooled to room

temperature following the completion of the reaction. Once the potent material was separated from the methanol, dried, and recrystallized, it was obtained.

### **3. GENERAL GRINDING-BASED PYRAZOLINE DERIVATIVE SYNTHESIS. (2A-I, 3A-I)**

A similar strategy depicted in microwave synthesis. Compound 2a-I was orchestrated by adding compound 1a-I (0.01mol), ISON famous corrosive hydrazide hydrate (0.02mol), chilly acidic corrosive, and processing for a couple of moments. Tender loving care was utilized to follow the response's end.

The pale greenish-yellow strong was parted separated. Essentially, comp. 1a-I was made into comp. 3a-I by blending it in with phenyl hydrazine hydrate (0.02mol). Utilizing a pestle and an open mortar, the 3a-I synthesis was completely ground for a few minutes at room temperature. Acidic corrosive (0.001 mmol) was added to this response combination, and crushing was done for a couple of moments while tender loving care checked the response's finishing. The clear greenish-yellow strong isolated out. In the wake of weakening with cold water, the resultant strong was isolated from the ethanol utilizing Buchner sifting and recrystallization. The outcome was the subordinate of pyrazoline.

Green chemistry is in accordance with the synthesis of pyrazoline derivatives by a grinding-based process, which is a convincing approach. This technique encourages energy economy and ease of operation while also reducing the usage of dangerous chemicals. Known for their wide range of biological activities, including antioxidant qualities, pyrazoline derivatives are formed through chemical reactions aided by the mechanical force applied during grinding in the grinding-based synthesis.

The general process for synthesizing pyrazoline derivatives (2a-i, 3a-i) through grinding entails a reaction between substituted acetophenones (R') and substituted benzaldehydes (R). The physicochemical characteristics and biological activity of the resultant pyrazoline derivatives are largely determined by the substituents on these aromatic molecules. To encourage the cycloaddition reaction, the substrates in this approach are usually crushed together in the presence of a catalyst, which can be an acid, base, or metal catalyst.

Take the synthesis of pyrazoline derivative 2a, for example. P-methylacetophenone and m-nitrobenzaldehyde are the initial ingredients used in the procedure. These mixtures of compounds are ground in a mortar and pestle or a mechanical grinder in stoichiometric proportions. An enone intermediate is formed more easily as a result of the mechanical energy produced during grinding. This intermediate is then cyclized to form the pyrazoline ring. In comparison to conventional solution-based procedures, this grinding approach can drastically shorten response times, and it can frequently be carried out at room temperature, further lowering the energy requirement.

In a similar vein, the synthesis of 3a employs the same grinding-based procedure and substrates as 2a, with minor adjustments made to the catalysts or reaction conditions to obtain the required structural alterations. The benzaldehyde and acetophenone in each of the ensuing derivatives (2b-i and 3b-i) are replaced with the proper functional groups, such as m-hydroxy, p-nitro, p-fluoro, and 3,4,5-trimethoxy groups. The investigation of a broad variety of pyrazoline derivatives with possibly distinct biological activity is made possible by these substituent changes.

This synthesis based on grinding has numerous benefits. It does away with the requirement for organic solvents, which are frequently hazardous and bad for the environment. Furthermore, the technique is simple to use and does not need for complicated equipment or settings, making it suitable for laboratories with low resources. This approach is an environmentally favorable substitute for conventional synthesis routes since it uses less energy and solvents, both of which are in line with sustainable norms.

#### **[5-p-tolyl-3-(4-nitro-phenyl)-4,5-dihydro-pyrazol-1-yl]-pentaenoic acid (2a)**

Chemical formula: C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>, molecular weight: 382.50, stretching values for C-H: 3338.03, Sp<sup>3</sup> C-H: 2920.23, stretching values for C-N: 1243.55, and stretching values for C-NO<sub>2</sub>: 1550. Calc. for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: 382.50; Found: 383.25. Dark powder.

#### **The compound is 3-p-tolyl-5-(2,3,4-trimethoxy-phenyl)-4,5-dihydro-pyrazol-1-yl-2-hydroxy-1-one (2b) formula 2-(2,4-dien-1-one)**

Molecular formula: C<sub>26</sub>H<sub>26</sub>N<sub>2</sub>O; molecular weight: 382.50; infrared absorption band: 1 cm<sup>-1</sup>; and a dark brown powder. Molecular lengths calculated for C<sub>26</sub>H<sub>26</sub>N<sub>2</sub>O are 382.50, but the measured value is 381.5.

**This compound is a pyrazolyl ring with three fluoro-phenyl groups attached to it."2c" for pentaenoic acid**

The molecular formula is C<sub>23</sub>H<sub>19</sub>FN<sub>2</sub>O, and the calculated molecular weight is 358.15 kcal/mol. The amount of this compound was 357.

**The compound is named 5-p-tolyl-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl.two-deoxypentaenoic acid**

C<sub>23</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub> is a chemical formula with a calculated molar weight of 356.15 and a found molar weight of 355.16.

**3. [(4-chloro-phenyl)][-5-(2, 3, 4-trimethoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]the chemical name for oxyphenilin the absence of oxygen**

Brick red powder has the molecular weight of 450.91 and the chemical formula C<sub>25</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>4</sub>. The computed IR cm<sup>-1</sup> stretch values for Sp<sup>2</sup> C-H and Sp<sup>3</sup> C-H, respectively, are 2958.80 and 2835.36. Results for C<sub>25</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>4</sub> were 449.5.

**[4,5-dihydro-pyrazol-1-yl-3-(4-chloro-phenyl)-5-(4-nitro-phenyl)]2-formyl-pentaenoic acid**

The molecular formula is C<sub>22</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>, and the molecular weight is 405.09. The infrared spectra show that the C-H bonds are 3050.39 and 2930.94 cm<sup>-1</sup>, the C-N bonds are 1016.56 cm<sup>-1</sup>, the C-F bonds are 1128.43 cm<sup>-1</sup>, and the C=C bonds are 1590 cm<sup>-1</sup>. The calculated value for C<sub>22</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub> is 405.09 cm<sup>-1</sup>, while the found value is 403.5 cm<sup>-1</sup>.

**The following are three compounds: "Five [4-hydroxy-phenyl] the sum of five [4,5-dihydro-pyrazol-1-yl]grams of phenyl-methylthanon**

The calculated molecular formula, mass, and IR cm<sup>-1</sup> OH stretch for brown powder are C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub>, 376.84, and 3335.06, respectively. There were 376.84 and 367.5 identified for C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub>.

**Three [4-chloro-phenyl] five [3-fluoro-phenyl] five [4,5-dihydro-pyrazol-1-yl]-2-hydroxyphenyl-methanone**



Reddish dark powder with the following properties: molecular weight: 376.83, IR cm<sup>-1</sup> OH stretch: 3365, and chemical formula: C<sub>22</sub>H<sub>16</sub>ClFN<sub>2</sub>O. Results for Ar-CH, Ali-CH, C=O, C=N, and C-O stretches were calculated to be 30, 65, 29, 35, 1611, and 1,125, respectively. Total: 376.83 for C<sub>22</sub>H<sub>16</sub>ClFN<sub>2</sub>O; 268.14 for the chemical, itself.

**[3-dihydro-pyrazol-1-yl, 5-(4-chloro-phenyl)-5-(3-nitro-phenyl)-4]-pentaenoic acid (2i)**

The compound is a dark brown powder with the molecular formula C<sub>22</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>. Its molecular weight is 405.83 and its different stretching parameters are 3326 for OH, 3059 for CH, 2948 for Ali CH, 1744 for C=O, 3059 for Ar-CH, 1625 for C=N, and 1122 for C-O. The calculated value for C<sub>22</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub> is 405.83, whereas the actual value is 268.1441.

#### 4. BIOLOGICAL ACTIVITY

##### a) Antioxidant Activity

It is well known that antioxidant components' ability to hunt for radicals can be assessed using the DPPH revolutionary rummaging method. DPPH offers an innovative and stable substitute. In methanol, the DPPH free revolutionary has a consistent violet hue. If you combine it with reducing synthetics or antioxidants, it turns yellowish or watery. Through the occasional electron or hydrogen that the antioxidant can withstand, these radicals can change into stable diamagnetic atoms (yellow). The coordinated compounds were dissolved in 100 µg/ml of methanol to ascertain the antioxidant action. The DPPH arrangement in the other compartment displayed its highest absorbance at 517 nm when a stable 1,1-diphenyl 2-picryl hydrazyl stable free revolutionary (violet tone) was present. Four milliliters of each test ingredient were added to four milliliters of DPPH solution, and the combination was let to stand at room temperature for thirty minutes. A Shimadzu UV spectrometer was used to detect the absorbance at 517 nm. Furthermore, a calibration was performed on the absorbances of the reference and clear samples. We evaluated the antioxidant impact of the ascorbic corrosive subordinates by modifying the absorbance in the recipe:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{Absorbance of sample}} \times 100$$

##### b) DPPH method



The DPPH test is a phenomenal strategy for assessing the antioxidant action of as of late integrated compounds because of its high repeatability and short handling time. The absorbance drops when the antioxidant atom gives an electron to the DPPH free revolutionary. The majority of the mixtures showed strong antioxidant properties. as opposed to the reference, ascorbic corrosive. Table 1 records the mixtures that were made alongside their antioxidant limit.

**Table 1:** Pyrazoline derivatives' antioxidant activity (IC50 in  $\mu\text{g/mL}$ )

Sr. No.	Comp. Code	% Inhibition (5 $\mu\text{g/ml}$ )	% Inhibition (10 $\mu\text{g/ml}$ )	% Inhibition (15 $\mu\text{g/ml}$ )	% Inhibition (20 $\mu\text{g/ml}$ )	IC50 ( $\mu\text{g/ml}$ )
1	3a	21.41	31.44	71.22	85.00	10.20
2	3b	32.81	41.62	71.13	84.32	11.22
3	3c	18.44	35.88	61.44	71.41	11.56
4	3d	21.51	40.61	58.66	82.55	10.55
5	3e	23.35	42.68	70.44	71.55	10.48
6	3f	31.52	41.55	81.11	81.81	11.25
7	3g	20.55	38.54	72.34	86.01	11.77
Std.	Ascorbic Acid	31.52	51.66	71.41	92.11	8.44

The table presents information on the antioxidant activity of different pyrazoline derivatives as determined by their IC50 values (the concentration needed to inhibit 50% of the free radicals) and percentage inhibition at various concentrations (5, 10, 15, and 20  $\mu\text{g/mL}$ ). Antioxidant activity is higher with lower IC50 values. The compounds that showed the strongest antioxidant activity were 3a, 3d, and 3e, with IC50 values of 10.20  $\mu\text{g/mL}$ , 10.55  $\mu\text{g/mL}$ , and 10.48  $\mu\text{g/mL}$ , respectively. These figures show strong antioxidant capability because they are reasonably similar to the typical antioxidant, ascorbic acid, which has an IC50 of 8.44  $\mu\text{g/mL}$ . Other compounds with IC50 values ranging from 11.22  $\mu\text{g/mL}$  to 11.77  $\mu\text{g/mL}$ , such as 3b, 3c, 3f, and 3g, demonstrated modest antioxidant activity. Remarkably, at higher concentrations—specifically, at 15  $\mu\text{g/mL}$  and 20  $\mu\text{g/mL}$ —all drugs showed notable inhibition, with percentages ranging from 58.66% to 86.01%. Despite the fact that all pyrazoline derivatives have some level of antioxidant activity, the data

indicates that compounds 3a, 3d, and 3e hold the greatest potential for further research because of their lower IC50 values.

### c) Anti-inflammatory Activity

Relieving medications hinder the cyclooxygenase impetuses, which convert arachidonic destructive to prostaglandins. Starting from the pieces of lysosomal layers are basically indistinguishable from the movies of HRBCs, the avoidance of hypotonicity-provoked layer lysis is considered as an estimation in concluding quieting action thusly, the HRBC film change strategy was used to conclude the relieving activity of every single mixed medicine.

This result proposed that the as of late incorporated pyrazolines showed more reassuring alleviating influences than  $\beta$ -diketones and flavones.

**Table 2:** Antagonistic properties of pyrazoline derivatives

Compound	Anti-inflammatory Activity (EC50 $\pm$ SD) ( $\mu$ g/mL)
2a	55.20 $\pm$ 1.20
2b	52.01 $\pm$ 2.11
2c	50.20 $\pm$ 1.15
2d	32.01 $\pm$ 1.01
3a	50.12 $\pm$ 1.01
3b	47.88 $\pm$ 1.22
3c	11.20 $\pm$ 1.22
3d	14.01 $\pm$ 1.12
3e	15.92 $\pm$ 0.52
Aspirin	11.70 $\pm$ 0.95

The anti-inflammatory activity of several pyrazoline derivatives is shown in the table together with the standard deviation (SD) and measured as EC50 (the concentration of the chemical needed to achieve 50% of its greatest effect). Greater anti-inflammatory potency is indicated by lower EC50 values. 3c, 3d, and 3e showed the strongest anti-inflammatory properties among the compounds evaluated; their EC50 values were  $11.20 \pm 1.22 \mu\text{g/mL}$ ,  $14.01 \pm 1.12 \mu\text{g/mL}$ , and  $15.92 \pm 0.52 \mu\text{g/mL}$ , respectively. The EC50 of aspirin, the reference medication, is  $11.70 \pm 0.95 \mu\text{g/mL}$ , and these values are similar to it. The EC50 values of other derivatives, such as 2a, 2b, 2c, and 3a, ranged from  $50.12 \pm 1.01 \mu\text{g/mL}$  to  $55.20 \pm 1.20 \mu\text{g/mL}$ , indicating moderate activity. Compared to the other compounds in the 2 series, compound 2d showed a substantially lower EC50 ( $32.01 \pm 1.01 \mu\text{g/mL}$ ), indicating stronger potency. All things considered, the 3 series' derivatives—particularly 3c, 3d, and 3e—showed encouraging anti-inflammatory potential, calling for more research.

#### d) Antimicrobial activity

The integrated mixtures were tested for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using an altered agar dispersion examination technique. As the positive control, streptomycin was used. For the duration of the brooding period, the temperature was  $37^\circ\text{C}$  to have an antibacterial effect. The zone of restraint is used to convey the findings of antimicrobial movement. Table 3 shows the result of the engineered mixtures' movement. Compounds are ordered as extensively dynamic, decently dynamic, less dynamic, and least dynamic in view of the ZOI esteem in millimeters. As per the investigation of antibacterial screening, compound 3b showed the best action against *Staphylococcus aureus* and *E. Coli*, individually. Compounds 3a and 3e for the most part had great adequacy against microscopic organisms that were Gram positive and Gram negative.

**Table 3:** Antimicrobial activity of pyrazoline derivatives

Compound Code	Zone of Inhibition (mm)	
	Gram Negative (E. Coli)	Gram Positive (S. aureus)
2a	13 mm	12 mm

2b	18 mm	14 mm
2c	14.1 mm	4 mm
2d	12.1 mm	10.1 mm
2e	14.1 mm	11.4 mm
Streptomycin	21 mm	24 mm

## 5. RESULTS & DISCUSSIONS

Green chemistry and conventional technologies, such as microwave irradiation and grinding-based techniques, were both successful in synthesizing pyrazoline derivatives. Numerous pyrazoline compounds with promising biological properties were generated by the chemical processes. These mixtures were tried for their antioxidant movement utilizing the DPPH extremist searching strategy, and for their mitigating characteristics utilizing HRBC film adjustment measures. Besides, tests were directed on antibacterial action against *Escherichia coli* and *Staphylococcus aureus*.

### 5.1 Activity of Antioxidants

The ability of the produced pyrazoline derivatives to scavenge DPPH free radicals was used to gauge their antioxidant activity. The results, which range in IC<sub>50</sub> values from 10.20  $\mu\text{g/mL}$  to 11.77  $\mu\text{g/mL}$ , show that the pyrazoline derivatives have strong antioxidant activity, as shown in Table 1. With IC<sub>50</sub> values of 10.20  $\mu\text{g/mL}$ , 10.55  $\mu\text{g/mL}$ , and 10.48  $\mu\text{g/mL}$ , respectively, compounds 3a, 3d, and 3e exhibited the highest antioxidant activity among the derivatives, suggesting their potential as powerful antioxidant agents. These substances showed strong antioxidant and radical scavenging properties comparable to ascorbic acid (IC<sub>50</sub> of 8.44  $\mu\text{g/mL}$ ). These substances also showed a substantial percentage suppression of DPPH radicals, particularly at concentrations of 15  $\mu\text{g/mL}$  and 20  $\mu\text{g/mL}$ , demonstrating their potent ability to scavenge free radicals.

### 5.2 Inhibitory Effect on Inflammation

Using the HRBC membrane stabilization method, the anti-inflammatory activity of the pyrazoline derivatives was assessed; Table 2 displays the results. Superior anti-inflammatory effects were shown by compounds 3c, 3d, and 3e, whose EC<sub>50</sub> values were 11.20 µg/mL, 14.01 µg/mL, and 15.92 µg/mL, respectively. These figures demonstrate a high degree of anti-inflammatory potential, as they are equivalent to aspirin (EC<sub>50</sub> of 11.70 µg/mL). The EC<sub>50</sub> values of the derivatives in the two series ranged from 32.01 µg/mL to 55.20 µg/mL, indicating significant anti-inflammatory efficacy. Compound 2d showed the most activity in its series out of all of these. All in all, the three series derivatives—3c, 3d, and 3e in particular—stand out for having strong anti-inflammatory properties.

### 5.3 Inhibitory Effect on Microbes

The zone of restraint (ZOI) strategy was used to think about the antimicrobial movement in contrast to *Staphylococcus aureus* and *Escherichia coli*, as demonstrated in Table 3. Compound 3b exhibited the best antibacterial adequacy against Gram-positive *S. aureus* and Gram-negative *E. coli*. Huge antibacterial activity was likewise exhibited by compounds 3a and 3e, particularly against Gram-positive and Gram-negative microbes. These substances' millimeter-measured zone of inhibition showed strong antibacterial qualities. By contrast, the traditional antibiotic streptomycin exhibited the biggest zones of inhibition; nonetheless, the pyrazoline derivatives, particularly compound 3b, showed encouraging antibacterial efficacy

### 5.4 Discussion

Compounds with significant biological activity have been produced by the synthesis of pyrazoline derivatives employing green chemistry techniques like microwave irradiation and grinding-based procedures. While the grinding-based process eliminates the need for organic solvents and minimizes environmental impact, the microwave irradiation method showed effective for quick synthesis, lowering reaction times and energy usage. Many pyrazoline derivatives have been shown to exhibit strong antioxidant activity, which suggests that these compounds may have applications in treating disorders linked to oxidative stress. The most potent compounds' (3a, 3d, and 3e) IC<sub>50</sub> values are similar to ascorbic acid's, demonstrating how well they work to neutralize free radicals.

The anti-inflammatory outcomes show promise as well; numerous pyrazoline derivatives exhibit action that is either higher than or equal to aspirin. Strong anti-inflammatory effects were demonstrated by compounds 3c, 3d, and 3e in particular, indicating that these compounds may be useful in the treatment of inflammation-related illnesses. These compounds' moderate to strong efficacy suggests that they could be developed further as anti-inflammatory medicines. Certain pyrazoline derivatives have strong antibacterial qualities against both Gram-positive and Gram-negative bacteria, according to antimicrobial tests. With the highest efficacy, compound 3b in particular showed promise as a broad-spectrum antibacterial drug. Even while these derivatives' action isn't as strong as streptomycin's, it nevertheless suggests that they have substantial antibacterial potential and calls for more research.

All things considered, the green synthesis of pyrazolines offers a practical and sustainable method of creating molecules with significant biological activity. In addition to being in line with environmental norms, the combination of microwave and grinding-based synthesis improves compound production efficiency. These pyrazoline derivatives have encouraging antibacterial, anti-inflammatory, and antioxidant qualities that point to the need for more study and development in the field of pharmaceuticals.

## **6. CONCLUSION**

This study used green chemistry approaches, such as microwave irradiation and grinding-based processes, to successfully create and analyze new pyrazoline derivatives. The molecules that were produced exhibited significant biological activity, such as strong antibacterial, anti-inflammatory, and antioxidant capabilities. Compounds 3a, 3d, and 3e in particular showed remarkable antioxidant properties; their IC<sub>50</sub> values were similar to those of ascorbic acid, suggesting that they could be useful radical scavengers. Derivatives 3c, 3d, and 3e also shown encouraging anti-inflammatory action, with strong effects akin to aspirin, indicating their potential use in therapies related to inflammation. Furthermore, compound 3b demonstrated noteworthy antibacterial efficacy against both Gram-positive and Gram-negative bacteria, indicating its potential as a future contender for antimicrobial research and development. Overall, the use of green synthesis techniques produced molecules with significant medicinal potential in addition to improving process sustainability and efficiency. These results open up new avenues for further investigation



into the clinical uses of green chemistry techniques and demonstrate their importance in the synthesis of bioactive compounds.

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