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Enhancing Gingerol Production and Antibacterial Efficacy of *Zingiber officinale* Using Plant Growth Regulators

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Abstract

The paper aims to examine how plant growth regulators [indole-3-acetic acid (IAA), gibberellic acid (GA 3), and brassinosteroid (BR)] can be used to improve the gingerol levels in *Zingiber officinale* and its antimicrobial properties against multidrug-resistant (MDR) bacteria. The growth of *Zingiber officinale* was conducted in greenhouse conditions, with growth regulator concentrations of 100, 200, and 300 mg L⁻¹ applied through a randomized complete block design (RCBD) and three technical replications. High-performance liquid chromatography (HPLC) was used to determine gingerol in rhizomes. Also, the concentration of rhizomes ethanolic extract (25, 50, 100mg.L⁻¹) was determined in relation to four MDR bacterial strains, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, using the agar well diffusion method. The findings indicated that GA3 had a significant effect on raising the levels of gingerol, whereas BR boosted the rhizome biomass. The extract concentration had a direct proportional relationship with the antibacterial activity, with the best inhibition being observed at the 100% extract with BR. *P. aeruginosa* was the most resistant of the tested strains, and *S. pneumoniae* was the most susceptible one. These findings suggest the potential of plant growth regulators to stimulate the production of bioactive compounds in ginger, offering a promising natural solution for combating antibiotic-resistant pathogens.

Keywords: plant growth regulators, gingerol, *Zingiber officinale*, antibacterial activity, multidrug-resistant bacteria, MIC.

تحسين إنتاج الجينجول والنشاط المضاد للبكتيريا في الزنجبيل (*Zingiber officinale*) باستخدام منظمات النمو النباتية

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الخلاصة:

تبحث هذه الدراسة في تأثير منظمات النمو النباتية [حمض الأندول-3-الأسيتيك (IAA) ، حمض الجبريليك (GA₃) ، والبراسينوستيرويد—(BR)] في تعزيز تراكم الجينجول في نبات الزنجبيل *Zingiber*

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officinale ونشاطه المضاد للبكتيريا مقاومة لعدة مضادات حيوية (MDR). زُرعت النباتات في ظروف بيت زجاجي، وتمت معاملتها بتركيز 100 و 200 و 300 ملغم. لتر⁻¹ من منظمات النمو باستخدام تصميم القطاعات العشوائية الكاملة (RCBD) بثلاث مكررات. تم قياس محتوى الجينجرول في الريزومات باستخدام كروماتوغرافيا السائل عالية الأداء (HPLC)، كما و خضعت المستخلصات الإيثانولية للريزومات لاختبارات ضد أربع سلالات بكتيرية مقاومة *Streptococcus pneumoniae*، *Staphylococcus aureus*، *Pseudomonas aeruginosa*، *Klebsiella pneumoniae* باستخدام طريقة الحفر في الوسط وبتراكيز 25 و 50 و 100 ملغم. لتر⁻¹. أظهرت النتائج أن GA₃ عزز بشكل كبير من تراكم الجينجرول، بينما ساهم BR في زيادة الكتلة الحيوية للريزومات، في حين ارتفع النشاط المضاد للبكتيريا مع زيادة تركيز المستخلص، وكانت *P. aeruginosa* الأكثر مقاومة، بينما كانت *S. pneumoniae* الأعلى حساسية. تشير نتائج الدراسة الحالية إلى إمكانية استخدام منظمات النمو لتحفيز المركبات الفعالة في الزنجبيل كخيار طبيعي واعد ضد البكتيريا المقاومة للمضادات الحيوية.

Introduction

The increasing resistance of bacteria to traditional antibiotics is one of the most urgent threats to global public health. The World Health Organization (WHO) indicates that the occurrence and dissemination of resistant bacterial strains undermine the effectiveness of existing medicines, which in turn affect morbidity rates, mortality, and healthcare expenses [1]. As such, infections that previously could be treated very easily, e.g., urinary tract infections, pneumoniae, and sepsis, have become harder to deal with [2].

Also, a set of multidrug-resistant (MDR) bacteria has developed as a result of both inherent and acquired gene mechanisms. These pathogens develop in healthcare facilities, which depend on a wide spectrum of resistance mechanisms, such as the generation of 2-lactamases, efflux pumps, modified membrane permeability, and resistance genes to tetracycline, fluoroquinolones, and aminoglycosides [3]. The natural resistance mechanisms are encoded in the bacterial genome and are inherited vertically, whereas acquired resistance results from transformation, conjugation, or transduction, also known as horizontal gene transfer [4].

MDR pathogens also possess several virulence factors, including adhesion proteins, outer membrane proteins, biofilm formation, gelatinase, lipase, protease enzymes, and siderophores [5]. The bacteria cause more than 73 percent of infection-related deaths, and they are a major health problem in the world. The ESKAPE group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) is considered to have a critical priority, as they are multidrug-resistant and highly virulent, which is especially true in intensive care units [6].

The Gram-negative bacteria use all four strategies above as major resistance strategies with the assistance of their double outer membrane structure containing hydrophobic lipid components, lipopolysaccharides, phospholipids, and porin proteins. Gram-positive bacteria, in turn, are more dependent on restricted permeability and efflux pumps due to the lack of a membrane in the outer part [7].

Since the level of drug resistance is alarming, more and more interest is being taken in medicinal plants as alternative antimicrobial agents. They tend to be safe, readily available, and affordable, requiring no special preparation or administration skills [8]. Medicinal plants and their extracts are characterized by a wide spectrum of biological activities, including antioxidant, antiviral, antimicrobial, immunomodulatory, and anti-inflammatory properties [9].

Zingiber officinale (ginger) is a member of the family Zingiberaceae comprising approximately 80 species with medicinal and aromatic properties [10]. The pharmacology of

ginger, along with the availability of bioactive constituents, particularly gingerol, has a long history of use in both traditional and modern medicine [11]. Gingerol has been researched for its use as an antiemetic, anti-inflammatory, anti-obesity, antioxidant, and anticancer agent, with a focus on its action on colon cancer and its role in metabolic regulation [12-14].

In addition to its therapeutic potential, ginger is an economically significant crop grown in tropical and subtropical regions. Major producing countries include Nigeria, India, China, Indonesia, and Bangladesh, with India leading global exports [15].

Plant growth regulators play critical roles in regulating plant development, including flowering, fruiting, seed formation, and response to environmental stress [16]. Key classes include indole-3-acetic acid (IAA), gibberellic acid (GA₃), cytokinin, ethylene, abscisic acid, and brassinosteroid [17]. IAA promotes root initiation and cell elongation, while gibberellins stimulate stem elongation and seed germination, whereas BRs enhance vascular development and stress tolerance [18-20].

Despite the increasing global interest, ginger cultivation and production remain limited in Iraq. Therefore, this study aims to evaluate the effect of selected plant growth regulators, IAA, GA₃, and BR, on the growth of *Zingiber officinale* rhizomes and their gingerol content, as well as to investigate the antibacterial activity of rhizome extracts against key multidrug-resistant bacterial pathogens.

2. Materials and methods

2.1 Plant cultivation and growth conditions

Fresh rhizomes of *Zingiber officinale* were obtained from the Agricultural Supplies Office in Diyala, Iraq. Each rhizome was cut into segments of approximately 100 g containing 2–3 viable buds. The segments were planted in 10 kg capacity plastic pots containing a 1:1 mixture of sterilized river soil and peat moss. Cultivation was conducted inside a controlled plastic greenhouse (25 m × 8 m × 3 m) located in Baqubah, Diyala Governorate, from the 15th of February 2024, to the 15th of February 2025 [15].

Irrigation was carried out consistently in accordance with the prevailing climatic conditions. Fertilizers application was performed in two stages: initially, triple superphosphate (46% P₂O₅ at 120 kg.ha⁻¹) and potassium sulfate (41.5% K at 100 kg.ha⁻¹) were applied before planting. At the 15 cm plant height stage, urea (46% N at 60 kg.ha⁻¹) was administered to support vegetative growth [15].

2.2 Application of plant growth regulators (PGRs)

Three plant growth regulators were tested for their impact on rhizome cultivation: IAA, GA₃, and BR, each applied at concentrations of 100, 200, and 300 mg.L⁻¹. Foliar applications were made at three growth stages (60, 120, and 180 days after planting) with 1 percent Tween-20 surfactant to enhance absorption on the leaves. The experiment was conducted using a completely randomized design (RCBD) with three technical replications per treatment [17].

2.3 HPLC analysis and extraction.

When physiological maturity was achieved, the rhizomes were gathered, washed, dried in air at room temperature, and milled into a fine powder. To extract, 20g of powdered sample was combined with 100mL of 1:1 ethanol-acetone solution. The mixture was placed in a shaking incubator and stirred at 160 rpm. After 24 hours, it was centrifuged at 10,000 rpm at 20 °C. The supernatant was filtered and stored at 4°C until analysis [11].

The amount of gingerol was determined by HPLC with an FLC NUCLOSIL C18 column (125 × 4.0 mm, 7 mm). The mobile phase was a linear gradient of phosphate buffer and Tris-acetate. It was kept at 1.5mL. min, and it was detected at a wavelength of 280nm [11].

2.4 Antibacterial assay

The ginger extract was examined concerning the antibacterial action against four MDR bacteria, namely *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, and *P. aeruginosa*. The disc diffusion method was used to test the extract at concentrations of 25, 50, and 100 ug.mL⁻¹. Each concentration of the extract was impregnated with sterile paper discs (6mm) and incubated on Mueller-Hinton agar plates pre-inoculated with bacterial suspensions (1x10⁶ CFU.mL⁻¹). Plates were incubated at 37 °C for 24 hours, and the areas of inhibition were then measured in millimeters [6].

2.5 Determination of MIC

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the minimum inhibitory concentration (MIC) was assessed using the broth microdilution technique in 96-well microtiter plates.

Serial two-fold dilutions of each extract (ranging from 1000 to 15.625 µg.mL⁻¹) were prepared in Mueller-Hinton broth. Each well was inoculated with 100 µL of bacterial suspension. Plates were incubated at 37 °C for 24 hours, and MIC was defined as the lowest concentration with no visible bacterial growth [4].

2.6 Statistical analysis

All data were statistically analyzed using one-way analysis of variance (ANOVA) through SPSS software version 23. Differences between treatment means were determined using Duncan's multiple range test (DMRT) at a significance level of $p \leq 0.05$. Where applicable, least significant difference (LSD) values and independent t-tests were used to validate results.

3. Results and Discussion

3.1 Effect of Plant Growth Regulators on Fresh Weight of Ginger Rhizomes

All three tested types of PGRs, IAA, GA₃, and BR, induced an increase in fresh rhizome weight compared to the untreated control (Table 1). The greatest impact was observed when 300 mg.L⁻¹ of BR was used, yielding 171.0 g of fresh rhizome weight, which was significantly better compared to the other treatments ($p < 0.05$). These findings indicate that BRs could increase cell elongation and nutrient absorption, which agrees with the results of Cheng *et al.*, and Jiang *et al.*, [18,19].

Table 1: Effect of plant growth regulators on fresh weight (g) of ginger rhizomes

Treatment	Concentration (mg.L ⁻¹)	Fresh weight (g)
IAA	100	82.0
	200	107.0
	300	104.7
GA ₃	100	120.3
	200	127.0
	300	135.7
BR	100	159.0
	200	153.0
	300	171.0
Control	0	84.3

LSD 5%: treatments = 12.9, concentrations = 14.8, interaction = 25.7

3.2 Effect of PGRs on gingerol content (HPLC analysis)

The impact of PGRs on the gingerol content (HPLC analysis) was investigated. The concentration of gingerol was determined by using the HPLC. The highest concentration of gingerol was 8.325mg.L⁻¹ at a concentration of 300mg.L⁻¹ of GA₃, then BR with a concentration of 7.543mg.L⁻¹ of gingerol. It means that GA₃ can somehow inhibit gingerol production by increasing secondary metabolite biosynthesis [20-22]. Our results are consistent with those of Jinhui *et al.*, and Xu *et al.*, [23,24], who also found a hormonal effect on metabolic flux to phenolic compounds.

Table 2: Effect of treatments on gingerol content (mg.L⁻¹) by HPLC

Treatment	Retention time (min)	Peak area	Gingerol concentration (mg L ⁻¹)
Standard	3.803	180.613	10.000
Control	3.840	53.512	2.962
IAA 100	3.248	71.668	3.968
IAA 200	3.953	94.803	5.248
IAA 300	3.624	113.132	6.263
GA ₃ 100	3.673	79.513	4.402
GA ₃ 200	3.877	98.643	5.461
GA ₃ 300	3.776	150.363	8.325
BR 100	3.836	75.143	3.163
BR 200	3.523	95.874	5.308
BR 300	3.604	136.244	7.543

3.3 Antibacterial activity of ginger extracts treated with IAA

The effects of ginger extracts on bacteria under the influence of IAA are presented in Table 3. The results demonstrate a strong synergistic effect between IAA and ginger extract in enhancing antibacterial activity, as indicated by the diameter of the inhibition zone. IAA alone and ginger extract alone, with a 0% concentration, did not exhibit any antibacterial effect. IAA, combined with the growing concentration of ginger extract, had a significant impact in inhibiting the growth of all bacterial strains tested, including *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, and *P. aeruginosa*.

The strongest antibacterial activity was noticed when 300 mg.L⁻¹ of the used plant auxin reacted with 100 percent ginger extract, and the zones of inhibition against *S. aureus* were as high as 30.00 mm. The findings indicated that *P. aeruginosa* was the most reactive species (average = 14.31 mm), the next reactor was *S. aureus* (13.71 mm), *K. pneumoniae* (12.83 mm), and *S. pneumoniae* (12.31 mm). This finding aligns with previous studies that reported gingerols and shogaols, the major bioactive compounds of ginger, as having potent antibacterial effects through the disturbance of bacterial cell membranes and metabolic pathways [25].

Table 3: Antibacterial properties of IAA and ginger extract mixtures against selected bacteria (inhibition zone diameter, mm).

IAA (mg·L ⁻¹)	Ginger extract (%)	<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
0	0	00.00	00.00	00.00	00.00
	25	10.67	10.00	11.33	10.33
	50	15.00	15.00	19.67	16.33
	100	22.00	20.00	18.67	24.00
100	0	00.00	00.00	00.00	00.00
	25	7.33	8.33	9.33	9.33
	50	11.00	11.67	11.33	14.33
	100	19.33	19.67	20.00	23.67
200	0	00.00	00.00	00.00	00.00
	25	14.33	11.67	7.67	17.67
	50	23.67	19.33	23.33	24.67
	100	28.33	23.67	28.00	29.00
300	0	00.00	00.00	00.00	00.00
	25	15.33	13.00	12.67	9.67
	50	24.00	17.67	18.67	24.33
	100	28.33	27.00	24.67	25.67

Mean effect on bacteria: *S. aureus*: 13.71 mm, *S. pneumoniae*: 12.31 mm, *K. pneumoniae*: 12.83 mm, *P. aeruginosa*: 14.31 mm. LSD (0.05) for bacterial species = 0.792.

The antibacterial effect of the ginger extract, as observed, could be attributed to the facilitating effect of auxin, which regulates the secondary metabolism of plants and increases phenolic compounds known to have antimicrobial effects [26]. This might be the reason that it was more effective with higher levels of auxins.

The GA3 and ginger extract on pathogenic bacteria.

The synergistic antibacterial effect of GA3 and ginger extract is demonstrated in Table 4. As with IAA, GA3 alone did not have any observable effect on antibacterial. Nevertheless, when mixed with ginger extract, it produced large areas of inhibition. The best response was recorded with 300 mg 3 -GA and 100% ginger extract with an inhibition zone of 28.00 mm to *K. pneumoniae*. *K. pneumoniae* presented the highest mean sensitivity (14.12 mm), *S. aureus* (13.96 mm), *P. aeruginosa* (13.95 mm), and *S. pneumoniae* (12.33 mm) had a close result. The increased effect of GA 3 on bacterial growth inhibition can be explained by the fact that it can activate the synthesis of gingerols and other antimicrobial proteins by activating the phenylpropanoid metabolic pathway [26]. Additionally, GA3 is reported to enhance the biomass and oil content of medicinal plants, which can enhance the antibacterial activity of the extract.

Table 4: The antimicrobial effect of gibberellin and ginger extract on bacterial species of pathogen (inhibition zone diameter in mm)

GA ₃ concentration (mg.L ⁻¹)	Ginger extract concentration (%)	<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
0	0	00.00	00.00	00.00	00.00
	25	10.67	10.00	11.33	10.33
	50	15.00	15.00	19.67	16.33
	100	22.00	20.00	18.67	24.00
100	0	00.00	00.00	00.00	00.00
	25	09.67	08.33	11.67	09.33
	50	13.00	11.67	15.00	15.00
	100	20.33	21.67	23.00	25.67
200	0	00.00	00.00	00.00	00.00
	25	14.33	09.67	15.00	18.67
	50	23.67	19.33	25.00	23.00
	100	28.33	26.00	27.33	25.33
300	0	00.00	00.00	00.00	00.00
	25	15.33	13.00	12.67	11.00
	50	24.00	17.67	18.67	21.00
	100	27.00	25.00	28.00	23.67

Mean inhibition by bacterial species (mm): *S. aureus*: 13.96, *S. pneumoniae*: 12.33, *K. pneumoniae*: 14.12, *P. aeruginosa*: 13.95, LSD (0.05) for bacterial species = 0.792

Effect of BR and ginger extract on antibacterial activity

The results in Table 5 reveal that BRs, especially when used at higher concentrations, markedly enhanced the antibacterial potential of ginger extract. The best treatment (300 mg.L⁻¹ + 100% extract) gave a growth inhibition zone of 30.00 mm with *S. aureus* and 29.33 mm with *S. pneumoniae*. The BR-enhanced combinations were consistently better than the lower concentrations and were significant concerning *P. aeruginosa* (mean = 14.35 mm) and *S. aureus* (mean = 13.62 mm).

Table 5: Antibacterial activity of BR and ginger extract

BR concentration (mg.L ⁻¹)	Ginger extract concentration (%)	<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
0	0	0.00	0.00	0.00	0.00
	25	10.67	10.00	11.33	10.33
	50	15.00	15.00	19.67	16.33
	100	22.00	20.00	18.67	24.00
100	0	0.00	0.00	0.00	0.00
	25	7.33	8.33	9.33	9.33
	50	11.00	11.67	11.33	14.67
	100	19.33	19.67	20.00	26.00
200	0	0.00	0.00	0.00	0.00
	25	14.33	9.67	15.00	18.67
	50	23.67	19.33	23.00	24.00
	100	28.33	23.67	26.00	29.33
300	0	0.00	0.00	0.00	0.00
	25	12.33	10.67	12.67	9.67
	50	24.00	16.33	18.67	22.00
	100	30.00	26.33	24.67	25.33

Mean bacterial inhibition zone diameter (mm): *S. aureus*: 13.62 mm, *S. pneumoniae*: 11.91 mm, *K. pneumoniae*: 13.14 mm, *P. aeruginosa*: 14.35 mm, LSD at 0.05 for ginger extract = 0.721

This suggests that BRs can play a vital role in the preparation of the ginger defense system, possibly by stimulating the formation of reactive oxygen species (ROS) and activating secondary metabolic pathways that enhance the synthesis of gingerol [26]. Past research has attributed stress effects caused by BR to the enhancement of antimicrobial phenolic compound synthesis in several medicinal plants [27,28]. The differences imply the ability to vary depending on the cell wall structure and the metabolic pathways of bacteria. Regarding *S. pneumoniae*, being a Gram-positive bacterium with a thick peptidoglycan layer, it is possible that some phytochemicals may penetrate it in a less effective manner. The LSD ($p = 0.05$, $p = 0.721$) proves the statistical significance of the differences in bacterial inhibition, which adds to the conviction in the validity of the observed trends. The communication between BR and ginger extract exhibits considerable antibacterial effects, with *P. aeruginosa* and *S. aureus* being the most sensitive. The findings highlight the significance of BR-phytochemical formulations as effective, naturally found antimicrobial agents [28]. Future research can focus on their use in the medical, agricultural, or food preservation settings. Plant growth regulators have a profound impact on the yield and phytochemical content of ginger. The BR application generated the best rhizome fresh weight, and the gibberellin treatment resulted in the best gingerol accumulation [28]. The ginger extracts were also found to have strong antibacterial activity against MDR strains, which underscores the potential application of ginger in the antibacterial field as a natural agent. The increased bioactivity may be explained by the increased concentrations of gingerol resulting from PGR treatments [29]. Such results are consistent with the earlier research, which indicated that the growth condition manipulation and PGRs application may increase medicinal values of *Z. officinale* [27-29].

General comparison and statistical relevance.

The LSD 0.792 among all bacterial species supports the statistical significance of the differences between the treatment groups. The overall bacterial growth inhibition of ginger extract combined with BRs was the highest, followed by BRs, then gibberellin, and finally auxin, indicating that the BR-mediated alteration of phytochemical profiles may be the most efficient approach to improving the antibacterial activity of ginger.

Conclusion

This paper has demonstrated that plant growth regulators, particularly GA3 and BR, play a significant role in ginger growth, gingerol production, and antibacterial activity. BR enhanced the weight of the rhizome, and GA 3 (300mg L⁻¹) produced the highest amount of gingerol. Ginger extracts exhibited a strong inhibitory effect against multidrug-resistant bacteria. In this order, *S. pneumoniae* and *P. aeruginosa* were the most susceptible, while the latter was the most resistant. The conclusions demonstrate the advantages of using ginger as a natural antimicrobial and the importance of maximizing PGRs production to enhance the effectiveness of medicines. The efficacy and safety need to be confirmed through future studies involving advanced antimicrobial tests and animal models. There should be a conflict of interest.

Conflict of interest

The authors declared that there is no conflict of interest.

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