



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Article

To Take a Look at Impact of 2,4-D and Kinetin on Callus Induction in *Asparagus racemosus* and its Antimicrobial Assay

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ARTICLE INFO

Published: 14 Oct 2026

Keywords:

Asparagus racemosus,
Callus induction, 2,4-D,
Kinetin, Antibacterial
activity, Well-diffusion
assay

DOI:

10.5281/zenodo.23376785

ABSTRACT

Asparagus racemosus (Shatavari) is an endangered and economically important medicinal plant known for its diverse bioactive secondary metabolites. In the present study, an efficient protocol was established for in vitro callus induction from internodal and root explants obtained from germinated seedlings. Explants were cultured on Murashige and Skoog (MS) media supplemented with B₅ vitamins, 3% sucrose, 0.8% agar, and varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5–2.0 mg/L) in combination with Kinetin (Kin; 0.25 and 0.50 mg/L). The highest callus induction frequency was observed on MS medium containing 2.0 mg/L 2,4-D and 0.5 mg/L Kin, yielding 94% callusing for internodal explants and 90% for root explants. Well-proliferated calli were extracted using aqueous and ethanolic solvents and subjected to an antibacterial assay using the agar well-diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The 20% ethanolic callus extract exhibited superior antibacterial activity, producing maximum zones of inhibition of 22 /{ mm} against *E. coli* and 20 /{ mm} against *S. aureus*. These results demonstrate that in vitro-derived callus of *A. racemosus* can serve as a potent source of antimicrobial metabolites.

INTRODUCTION

Plant tissue culture is an essential branch of biotechnology enabling the aseptic growth of plant cells, tissues, and organs on nutrient media under controlled environmental conditions. It plays a critical role in micropropagation, germplasm conservation, genetic transformation, and the

production of bioactive secondary metabolites without relying on wild populations. Auxins (such as 2,4-D) and cytokinins (such as Kinetin) act as master regulators of cell division, elongation, and organogenesis in vitro.

Asparagus racemosus Willd. (family Asparagaceae), commonly known as Shatavari, is

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



a woody climber widely distributed across tropical and subtropical regions. It is extensively utilized in Ayurveda, Unani, and Siddha systems of medicine for its nutritive, immunomodulatory, antioxidant, and antimicrobial properties, largely attributed to steroidal saponins (shatavarins), alkaloids, and flavonoids. Due to destructive overexploitation and habitat loss, *A. racemosus* faces severe conservation threats in its natural habitat. Consequently, establishing standardized plant tissue culture protocols provides a sustainable approach for germplasm preservation and mass production of pharmaceutical compounds.

The present study aims to evaluate the synergistic impact of 2,4-D and Kinetin on callus induction using internodal and root explants of *A. racemosus*, and to investigate the antibacterial efficacy of the resulting callus extracts against pathogenic bacterial strains.

2. MATERIALS AND METHODS

2.1 Plant Material and Surface Sterilization

Mature seeds of *A. racemosus* were collected from plants maintained under hydroponic conditions. Seeds were surface sterilized using 70% alcohol for 5 minutes, followed by immersion in 0.1% HgCl_2 for 7 minutes. Sterilized seeds were thoroughly rinsed 2–4 times with sterile distilled water, blotted dry on sterile tissue paper, and inoculated onto half-strength MS medium for germination. Seeds were kept in the dark at 27 °C for 2 days before transfer to a 16-hour photoperiod relative humidity 60%).

2.2 Explant Inoculation and Callus Induction

Internodal and root explants obtained from 15–20-day-old germinated seedlings were inoculated onto full-strength MS media containing B₅ vitamins, 3% (w/v) sucrose, and 0.8% (w/v) agar

at pH 5.8. Ten media formulations (CIM₁ to CIM₁₀) were prepared using different combinations of 2,4-D (0.5, 0.75, 1.0, 1.5, and 2.0 mg/L) and Kinetin (0.25 and 0.50 mg/L):

1. CIM₁: MS + B₅ vitamins + 3% sucrose + 2,4-D (0.5 mg/L) + Kin (0.25 mg/L)
2. CIM₂: MS + B₅ vitamins + 3% sucrose + 2,4-D (0.75 mg/L) + Kin (0.25 mg/L)
3. CIM₃: MS + B₅ vitamins + 3% sucrose + 2,4-D (1.0 mg/L) + Kin (0.25 mg/L)
4. CIM₄: MS + B₅ vitamins + 3% sucrose + 2,4-D (1.5 mg/L) + Kin (0.25 mg/L)
5. CIM₅: MS + B₅ vitamins + 3% sucrose + 2,4-D (2.0 mg/L) + Kin (0.25 mg/L)
6. CIM₆: MS + B₅ vitamins + 3% sucrose + 2,4-D (0.5 mg/L) + Kin (0.50 mg/L)
7. CIM₇: MS + B₅ vitamins + 3% sucrose + 2,4-D (0.75 mg/L) + Kin (0.50 mg/L)
8. CIM₈: MS + B₅ vitamins + 3% sucrose + 2,4-D (1.0 mg/L) + Kin (0.50 mg/L)
9. CIM₉: MS + B₅ vitamins + 3% sucrose + 2,4-D (1.5 mg/L) + Kin (0.50 mg/L)
10. CIM₁₀: MS + B₅ vitamins + 3% sucrose + 2,4-D (2.0 mg/L) + Kin (0.50 mg/L)

Cultures were maintained at 25 with a 16-hour photoperiod under cool white fluorescent light (3000 lux) and 50–70% relative humidity. Subculturing was carried out every 20 days.

2.3 Preparation of Callus Extracts

Proliferated calli from internodal and root cultures were collected, sun-dried, and ground into fine powder using a mortar and pestle. For extraction,



2.0 g of dried callus powder was soaked separately in 10 mL of ethanol and distilled water for 24–48 hours at room temperature. The mixture was filtered through Whatman No. 1 filter paper, and filtrates were concentrated to 2 mL at 40°C in a porcelain dish and stored at 4°C

2.4 Antibacterial Well-Diffusion Assay

Antibacterial activity was evaluated against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the agar well-diffusion method. Bacterial suspensions (10^5 CFU/mL) were spread onto nutrient agar plates. Wells were punched using a sterile metal borer, and callus extracts at concentrations of 25 µL, 50 µL, and 100 µL were loaded into respective wells. Plates were incubated at 37 °C for 24 hours in the dark, and zones of inhibition were recorded in millimeters (mm).

3. RESULTS AND DISCUSSION

3.1 Effect of 2,4-D and Kinetin on Callus Induction

Explants began to swell within 10–12 days of culture, and callus initiation occurred at the cut margins after 15 days. The percentage and quality of callus induction varied significantly depending on plant growth regulator concentrations. Lower concentrations of 2,4-D (0.5 mg/L) and Kin (0.25 mg/L) resulted in low callus induction rates and compact, hard calli.

Increasing concentrations of 2,4-D up to 2.0 mg/L in combination with 0.50 mg/L Kinetin

significantly enhanced callus induction frequency. Light yellow, friable, highly proliferative calli were achieved on medium containing 2.0 mg/L 2,4-D and 0.50 mg/L Kinetin, achieving maximum induction rates of 94% for internodal explants and 90% for root explants.

Table 1: Effect of 2,4-D and Kinetin concentrations on callus induction from internodal and root explants of *Asparagus racemosus*.

Growth Hormones (mg/L)		Callus Induction (%)	
2,4-D	Kinetin	Internodal Explant	Root Explant
0.50	0.25	37%	26%
0.75	0.25	44%	30%
1.00	0.25	49%	47%
1.50	0.25	59%	51%
2.00	0.25	66%	59%
0.50	0.50	59%	56%
0.75	0.50	70%	68%
1.00	0.50	89%	82%
1.50	0.50	92%	88%
2.00	0.50	94%	90%

3.2 Antibacterial Efficacy of Callus Extracts

Both aqueous and ethanolic callus extracts demonstrated dose-dependent antibacterial activity against *E. coli* and *S. aureus*. The ethanolic extract exhibited superior inhibitory potential compared to the aqueous extract, particularly against Gram-negative *E. coli*.

At a maximum dose of 100 µL, the ethanolic extract yielded an inhibition zone of 22 mm against *E. coli* and 20 mm against *S. aureus*. The aqueous extract displayed maximum inhibition zones of 18 mm against *E. coli* and 15 mm against *S. aureus* at 100 µL.

Table 2: Antibacterial activity of aqueous and ethanolic callus extracts of *Asparagus racemosus*.

Bacterial Strain	Extract Type	Zone of Inhibition (mm) at 25 µL	Zone of Inhibition (mm) at 50 µL	Zone of Inhibition (mm) at 100 µL
<i>E. coli</i>	Aqueous	12 mm	14 mm	18 mm
	Ethanolic	20 mm	20 mm	22 mm
<i>S. aureus</i>	Aqueous	15 mm	18 mm	15 mm
	Ethanolic	16 mm	18 mm	20 mm



4. CONCLUSION

This study establishes a reproducible and efficient protocol for in vitro callus induction in *Asparagus racemosus* using combinations of 2,4-D and Kinetin. The combination of 2.0 mg/L 2,4-D and 0.50 mg/L Kinetin proved optimal for high-frequency callus proliferation. Furthermore, antibacterial screening demonstrated that ethanolic callus extracts possess significant inhibitory activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. In vitro tissue culture of *A. racemosus* presents a viable mechanism for germplasm conservation and industrial production of bioactive antimicrobial compounds.

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HOW TO CITE: Rahul Varma, Geetika Agrawal, Manjit Rajput, To Take a Look at Impact of 2,4-D and Kinetin on Callus Induction in *Asparagus racemosus* and Its Antimicrobial Assay, *Int. J. of Pharm. Sci.*, 2026, Vol 4, Issue 10, 2236-2239. <https://doi.org/10.5281/zenodo.23376785>

