

Triggering Enhanced Production of Secondary Metabolites in *Jatropha Curcas* Callus through Salicylic Acid: an Investigation Combining in Vitro Experiments with In Silico Molecular Docking

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Abstract

Under salicylic acid (SA) elicitation, the build-up of secondary metabolites in *Jatropha curcas* callus tissue was the focus of this work. Across 30 days of in vitro culture maintained at controlled settings, leaf-derived calli received SA doses of 0, 0.5, 1.0, 2.0 and 4.0 mg/l. Distinct shifts in fresh as well as dry biomass of the *Jatropha* callus followed the elicitor exposure. A notable lift in fresh weight relative to unexposed cultures emerged at the lower dose end, which can be read as evidence of intensified cell division alongside improved physiological vigor in the callus cells. Through quantitative profiling, SA was found to substantially raise yields of steroids, glycosides, saponins, terpenoids, alkaloids, anthocyanins, and phorbol esters; the optimum response sat at the 2.0 mg/l dose. Set against untreated controls, the rises measured 42% for steroids, 38% for saponins, 33% for alkaloids, 29% for terpenoids, 26% for anthocyanins, 24% for glycosides, and 21% for phorbol esters. Since the elicitation outcome scaled in proportion to dose, a plausible reading is that SA re-tunes secondary metabolism by triggering biosynthetic enzymes together with defense-linked routes. Read together, the outcomes mark SA as a practical biotechnological elicitor for raising bioactive yields from *Jatropha curcas* callus, with applications stretching across pharmaceutical, agricultural, and industrial bioproduct domains. Via molecular docking, the in silico arm of the work was designed to unpack the molecular basis of SA-driven antioxidant gains and metabolite enrichment in *Jatropha* callus, with attention given to how SA engages central antioxidant enzymes alongside enzymes of the phenylpropanoid and flavonoid routes.

Keywords: *Jatropha curcas*, Salicylic acid, Secondary metabolites, Callus culture, Phorbol esters, Molecular Docking.

1. Introduction

A small tree or fleshy shrub of the broadly distributed Euphorbiaceae family is what *Jatropha curcas* L. represents (Carels, 2013). Throughout history this species has stood among the more notable medicinal plants, and its presence in contemporary Materia Medica has not waned. Its medicinal worth and tolerance to harsh environments aside, the plant is prized for its versatility, an oilseed crop role included. Phytochemicals of many kinds populate the different organs of *J. curcas* organic acids, saponins, tannins,

quercetin, amerin, phytase, and sitosterol among them (Heller, 1996). Mexico and Central America are the tropical home of the species, which today is grown widely throughout Central and South America, Southeast Asia, India, and Africa (Maes et al., 2009).

Considerable interest has been drawn to *Jatropha* across tropical and subtropical zones, an interest grounded in the plant's resilience, the breadth of agro-climatic settings under which it can thrive, its modest demand for irrigation and external inputs, its pest resistance, the short interval before reproductive maturity, and the ease with which its fruits can be harvested. From these very traits flows clear medicinal worth in the treatment of various disorders (Edrisi et al., 2015). Within the plant tissues sits a varied set of phytochemicals tannins, terpenoids, alkaloids, and flavonoids among them and several have registered antimicrobial activity in vitro (Talib and Mahasneh, 2010). Leaves, alongside other organs, find use in remedies for diverse illnesses. According to Ogodo et al. (2021), reported compounds from *Jatropha curcas* leaf extracts include the flavonoid apigenin together with its glycosides vitexin and isovitexin, as well as the sterols stigmasterol, β -D-sitosterol, and the β -D-glucoside derivative of the latter.

Conventionally the species is multiplied through seeds or vegetative cuttings. Plantlet production from seed remains the dominant route, yet poor seed viability, depressed germination percentages, weak rooting in small containers, and slow seedling root development frequently constrain it. Stem-cutting propagation, although workable, produces individuals with shallow root architectures that are easily uprooted on poorly developed top soils (Openshaw, 2000). Wider cultivation therefore depends on moving past these inefficient classical methods and on developing reliable techniques for the bulk propagation of *Jatropha*.

Bulk plant production gains a viable route through in vitro multiplication. Unhindered by seasonal cycles, the approach yields uniform, pathogen-free planting material at scale within a comparatively brief window (George, 2008). Three pillars of any propagation programme fine-tuning callus initiation, sustaining the culture, and securing regeneration in vitro were stressed by Morinuzzaman (2016). Reliable callus induction backed by an in vitro regeneration platform is, as a consequence, indispensable for moving forward with modern genetic improvement strategies (Jose et al., 2012). Long-term tissue conservation under slow-growth settings is another use to which callus material can be put (Ali et al., 2007).

Among the parameters that can be steered during in vitro callus induction, plant growth regulators (PGRs) rank as the most influential. The two PGR families that matter most for directing tissue and organ development are auxins together with cytokinins (Ismail et al., 2013 and Nabil, 2022). Callus cultures provide a sustainable option for the consistent synthesis of essential secondary metabolites, overcoming the limitations of inconsistent harvests from field-grown plants (Mohammed et al., 2025).

As a promising route for consistent, scalable production of secondary metabolites, plant tissue culture has gained ground in recent years. Of the strategies aimed at lifting in vitro metabolite output, elicitation has shown clear effectiveness at triggering plant defense responses and at amplifying metabolite build-up. Recent studies have confirmed their utility abiotic elicitors for increasing production of secondary metabolites, including Dimethyl sulphoxide LR (Taha et al., 2010), methyl jasmonate and silver nanoparticles (Shahin, 2018), Mannitol (Mousa et al., 2022), growth stimulation in *Astragalus* spp. (Alhaithloul et al., 2024) and date palm seeds (Elsherif et al., 2024). This thorough review of how elicitation drives secondary metabolite production in plant cell cultures gives weight to the regulatory and mechanistic functions of key elicitors, salicylic acid among them (Mashal et al., 2024 and Selwal et al., 2025).

Owing to its abundance of phenolic and flavonoid constituents, *Jatropha* offers a fitting platform for studying secondary metabolism. Bench experimentation paired with molecular docking simulations can shed light on how salicylic acid interacts with central

antioxidants and with phenylpropanoid/flavonoid enzymes, thereby clarifying the molecular logic behind salicylic acid-driven metabolic regulation. Under both biotic and abiotic stress, salicylic acid governs critical reprogramming of gene transcription that underlies defense, with sets of defense genes being switched on in a spatial and temporal pattern coordinated through salicylic acid-mediated signaling (Herrera-Vásquez et al., 2015).

Assessing how salicylic acid supplementation shapes the formation of secondary constituents in *Jatropha curcas* callus tissue culture was the chief aim of the present work. Defining the most favorable BA-NAA combinations for triggering callus formation from *Jatropha* leaves was a further aim. Quantification of the secondary constituents' terpenoids, sterols, phenols, flavonoids, alkaloids, tannins, anthocyanins, saponins, glycosides, and phorbol in the callus was also part of the analyses. So that a mechanistic basis could be furnished for the salicylic acid-driven shifts in antioxidant performance and secondary metabolite accumulation in *Jatropha curcas* callus, in silico screening of SA against central antioxidant and phenylpropanoid/flavonoid enzymes was performed, with the docked complexes interrogated through receptor-ligand interaction fingerprints alongside docking energetics.

2. Materials and Methods

2.1- Preparation of explants

Healthy young shoots were carefully detached from adult *J. curcas* specimens raised in the greenhouse of the Horticulture Research Institute at the Agriculture Research Center, Cairo, Egypt, during the period from January 2024 to April 2025. Without delay, the harvested young leaves were carried to the laboratory.

2.2- Sterilization of Explants

To begin, leaf-explant surfaces were freed of dust by a one-hour wash under tap water, followed by a single rinse with sterilized distilled water. Within a sterile Laminar flow hood, a one-minute treatment with 70% (v/v) ethanol was applied and two further rinses in sterile distilled water followed. For surface decontamination, a 20-minute exposure to commercial bleach (Clorox, 5.25% w/v NaOCl) was used at three NaOCl strengths 10, 15, and 20% each carrying two drops of Tween 20. Three additional rinses in sterilized double-distilled water then cleared away any residue from the sterilants.

Pinpointing the regime that would deliver the lowest contamination, the lowest mortality, and the highest survival of the explants was the goal of this experiment. At the experimental endpoint, six weeks in, survival, contamination, and mortality (%) were the data points captured.

2.3- Culture Media

As the initial phase in numerous tissue culture experiments, it is essential to induce callus formation from the primary explant.

Onto an initial medium MS basal nutrient medium (Murashige and Skoog, 1962) carrying myo-inositol at 100 mg/l, thiamine-HCl at 0.4 mg/l, and sucrose at 30 g/l, with 6.0 g/l agar serving as the gelling agent surface-sterilized leaf segments was placed.

Three leaf segments per jar was the loading.

Culture medium at 25 ml was dispensed into each jar, which was immediately capped with polypropylene closures. Autoclaving of the media at 121°C and 1.5 Kg/cm² then proceeded for 20 minutes. In a growth room kept at (25±°C), cultures were held for three months under 16-hour light / 8-hour dark. Five replicates served each treatment, each replicate containing nine culture jars, each jar holding three explants.

2.4- Callus induction

This experiment was designed to identify the growth-regulator concentration that produced the greatest callus induction.

With sterilization complete, the leaf explants were sectioned into 0.5 cm or 1.0 cm² pieces and arranged on MS basal solid medium amended with a battery of plant growth regulators at varying strengths so that callus initiation and proliferation could be triggered. Specifically, the amendments paired 6-Benzyl amino purine (BA) at 1.0 mg/l with Naphthalene acetic acid (NAA) at four levels: 0.0, 0.5, 1.0, and 2.0 mg/l.

Three leaf segments per jar were loaded, and every treatment was set up in five replicates. Across the three months in the dark at 25 ± 2°C the cultures were kept, with the media being refreshed monthly so that callus development could be supported. At the three-month mark, the survival fraction of the explants, the proportion forming callus, and callus fresh weight (g) were the parameters captured.

2.5- Maintenance and Elicitation of Callus Cultures

A. Elicitor Preparation

Following the protocol set out by Khan et al. (2019), elicitor preparation proceeded. A 1 µM salicylic acid stock was obtained by dissolving the compound in distilled water and stirring for 5 hours so that the elicitor went fully into solution. For the elicitation experiments, MS medium fortified with NAA at 2.0 mg/l, BA at 1.0 mg/l, and sucrose at 30 g/l received SA at 0, 0.5, 1.0, 2.0, and 4.0 mg/l. Each jar was seeded with about 0.5 g of callus. Across five replicates per dose, the jars were then sent to the growth room for a 30-day run.

B. Influence of salicylic acid (SA) on callus biomass

On a medium fortified with NAA at 2.0 mg/l plus BA at 1.0 mg/l, callus cultures were established and maintained without difficulty, in line with the present results. After three months of culturing, this callus stock was directed into the SA elicitation runs. Sub-culturing proceeded on MS medium fortified with the same plant growth regulators but with SA graded at 0, 0.5, 1.0, 2.0 and 4.0 mg/l (Sigma-Aldrich); the elicitor was first dissolved in ethanol, filter-sterilized through a 0.22-µm membrane, and then added to the medium under sterile conditions. Alongside, a control medium without any elicitor was run. Within the design, 0.5 g of callus per culture jar holding 40 ml of medium was the inoculum. After 30 days in culture, fresh and dry weight effects were scored, three cultures serving each treatment.

C. Extraction of callus for phytochemical analysis

Whitman filter paper was used to lift calli from the culture media at the 30-day point, with air-drying at room temperature for 20 minutes then removing the surface moisture. Further drying followed: each callus was weighed and then held at 48°C for 24 hours. A mortar and pestle were subsequently used to reduce the dried calli to a fine powder. For extract preparation, 0.1 g of the dry powder went into 0.5 ml of methanol; the mix was vortexed for 5 minutes, sonicated for 30 minutes, and finally centrifuged at 12,000 rpm for 15 minutes (El-Esawi et al., 2017).

D. Influence of salicylic acid on secondary metabolites in *Jatropha* extract

Through chemical assays performed on the dry callus material derived from the leaf segments, the active constituents of *Jatropha curcas* were profiled. All chemical work for the present study took place in the Department of Chemistry of Natural and Microbial

Products, Pharmaceutical Industry Division, National Research Center, sited in Dokki, Giza, Egypt.

D.1 Determination of Antioxidant Activity

1. Enzymatic Antioxidants content

In 5 ml of ice-cold 50 μ M phosphate buffer at pH 7.0 that contained 1% polyvinylpyrrolidone (PVP), fresh callus (0.5 g) was homogenized. The homogenate was then centrifuged at $12,000 \times g$ for 15 minutes at 4°C, with the supernatant retained for enzyme assays.

- The procedure of Beauchamp and Fridovich (1971) served for quantifying SOD activity.
- Aebi (1984) provided the procedure for evaluating CAT activity.
- With guaiacol as the substrate, POD activity was measured per Chance and Maehly (1955).
- The procedure outlined by Nakano and Asada (1981) was used to determine APX activity.

All enzyme activities were reported as U/mg protein.

D-1.1. Non-Enzymatic Antioxidants

1. Determination of total phenolic content

On the Folin–Ciocalteu reagent, the quantification of total phenolic compounds rested. To 2.5 ml of the diluted reagent and 2 ml of 7.5% Na_2CO_3 , an 0.5 ml extract aliquot was added. Following a 30-minute dark incubation, sample absorbance was registered at 765 nm on a spectrophotometer. Per the procedure of Strzelecka et al. (1987), outputs were expressed in mg gallic acid equivalents (GAE) per gram fresh weight (FW).

2. Determination of total flavonoid content

Through the aluminum chloride colorimetric procedure, total flavonoid levels were established. A 0.5 ml extract aliquot received 2% AlCl_3 and rested for 30 minutes. At 415 nm, absorbance was registered. Per the procedure of Chang et al. (2002), outputs were expressed in mg quercetin equivalents (QE) per gram fresh weight (FW).

D-1.2. Determination of total antioxidant activity

Through the DPPH radical scavenging procedure together with the FRAP assay, overall antioxidant performance was gauged.

1. DPPH radical scavenging assay

To 1 ml of DPPH solution (0.1 μ M), one milliliter of extract was added. Following a 30-minute dark incubation, absorbance was registered at 517 nm. Per the procedure of Blois (1958), activity was expressed as percent inhibition. As % radical scavenging, the DPPH-assay outputs were reported.

2. Determination of FRAP assay

With the FRAP reagent, reducing capacity was established; absorbance at 593 nm was the readout, per the procedure of Benzie and Strain (1996). The FRAP outputs were reported as $\mu\text{M Fe}^{2+}/\text{g DW}$.

D.2 Determination of total tannin content

Through the Folin–Ciocalteu reagent, total tannins were quantified. To 2.5 ml of diluted Folin–Ciocalteu reagent (1:10) and 2 ml of 7.5% sodium carbonate, an 0.5 ml extract

aliquot was added. The samples then rested in darkness at room temperature for 30 minutes, after which the absorbance was registered at 760 nm on a UV-Vis spectrophotometer. As the reference for the calibration curve, tannic acid or gallic acid served. Total tannin levels were expressed in mg/g FW (Makkar, 2003).

D.3 Determination of Alkaloids content

The procedure of Harborne (1998) governed the alkaloid determination. Specifically, 0.5 g of dried callus powder was extracted in 10 ml of 2N HCl, and Dragendorff's reagent was then applied to the resulting filtrate.

D.4 Determination of saponins content

For the saponin determination, the vanillin-sulfuric acid procedure of Obadoni and Ochuko (2001) was used. In 10 ml of 20% ethanol, a 0.5 g portion of dried material was extracted, and the absorbance read at 544 nm.

D.5 Determination of total anthocyanin content

As the reference standard for estimating total anthocyanin content in plant callus, cyanidin-3-glucoside chloride was used. From a certified chemical supplier (Sigma-Aldrich), the standard came at high purity ($\geq 95\%$). At a concentration of 1 mg/l, a stock standard solution of cyanidin-3-glucoside was made up in acidic methanol (methanol carrying 1% HCl, v/v). To prevent photodegradation, the solution was held in dark bottles at -20°C . Two uses for cyanidin-3-glucoside applied here: equation calculations under the pH differential method, or expression of outputs as mg cyanidin-3-glucoside equivalents per g FW. As an additive to the culture medium or to the plant, it was not introduced; its role was strictly as an analytical standard (Giusti and Wrolstad, 2001).

D.6 Determination of terpenoids content

In line with Ghorbanpour et al. (2020), terpenoid quantification proceeded. About 0.5 g of dried callus powder underwent a 24-hour methanolic extraction in 10 ml of solvent. Filtration, evaporation, and re-dissolution of the residue in 2 ml of methanol then followed. At 538 nm a spectrophotometric reading was taken, with terpenoid content reported as % of dry weight (DW).

D.7 Determination of total Steroid content

With the Liberman-Burchard reagent, steroid content was estimated spectrophotometrically. After 1 ml of extract had been evaporated to dryness, the residue was re-dissolved in 1 ml chloroform. To this solution, 2 ml of acetic anhydride and 2 ml of concentrated sulfuric acid were added. A 30-minute incubation at room temperature followed, after which absorbance was registered at 620 nm. With cholesterol serving as the reference standard, steroid concentration was determined, and the outputs were expressed as mg/g of dry weight (DW) (Harborne, 1998).

D.8 Determination of glycosides content

Through the Keller-Kiliani method (Harborne, 1998), glycosides were determined. In 5 ml of ethanol, 0.5 g of dried callus was extracted, with glycoside content calculated as % DW.

D.9 Determination of Phorbol Esters content

Following Devappa et al. (2018), quantification of phorbol esters rested on methanolic extraction paired with HPLC analysis. A C18 column carried out the separation. UV

detection at 280 nm was used. As the reference standard, phorbol-12-myristate-13-acetate served.

D.10 Determination of total sterol content

With the Liebermann–Burchard reagent, sterol levels were estimated. To two milliliters of extract, two milliliters of acetic anhydride were added; then, 0.5 milliliters of concentrated sulfuric acid was let down carefully along the tube wall. For 15–20 minutes at room temperature in the dark, the tube was left so that a bluish-green hue could develop. At 620 nm on a UV-Vis spectrophotometer the optical absorbance was registered. For the calibration curve, β -sitosterol was the reference compound. Reported in mg β -sitosterol equivalents g^{-1} FW, the total sterol content followed Harborne (1998).

E. Docking energetics across antioxidant and secondary-metabolism enzymes

E.1- Target selection and sequence retrieval

To match the biochemical endpoints captured in the in vitro work and to span both antioxidant defense and the entry steps of secondary metabolism, the panel of protein targets selected covered ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), phenylalanine ammonia-lyase (PAL), and chalcone synthase (CHS). From UniProt (<https://www.uniprot.org/>), protein identifiers along with their sequences were sourced. For cases where *Jatropha curcas* sequences or experimental structures could not be located, curated plant homologs closest in similarity were taken up so that structure-based analysis could go ahead (UniProt and/or PDB identifiers and source organisms. Sequences were sourced from UniProt (UniProt Consortium, 2023), with available structures/templates obtained from the RCSB PDB (Burley et al., 2021).

E.2- Protein structure acquisition and modeling

From the RCSB Protein Data Bank (<https://www.rcsb.org/>), experimentally determined structures were downloaded when available. For targets where no suitable experimental structure could be sourced, homology models were generated on the SWISS-MODEL server (<https://swissmodel.expasy.org/>) with automated template selection. Through the SWISS-MODEL quality estimates (GMQE and QMEAN, for example), model quality was checked, and only those models whose confidence in the predicted pocket region was acceptable were carried forward into docking. The homology modeling step relied on SWISS-MODEL (Waterhouse et al., 2018).

E.3- Protein preparation

To prepare the receptors, non-relevant heteroatoms and crystallographic water molecules were stripped out, missing hydrogen atoms added, and standard protonation states assigned in line with near-neutral pH. Energy minimization then took place so that local steric clashes were relieved before docking. For all complex visualization and interaction analysis, BIOVIA Discovery Studio Visualizer was the tool of choice (Dassault Systèmes, BIOVIA, 2021).

E.4- Ligand preparation

The chemical structure of SA was obtained from a public compound repository (e.g., PubChem) and prepared by generating a low-energy 3D conformation, assigning an appropriate protonation state, and geometry minimization. The same prepared ligand was used for all receptors to ensure comparability. The ligand record was sourced from PubChem (Kim et al., 2023).

E.5- Molecular docking

On the SwissDock web server (<https://www.swissdock.ch/>), docking was carried out to generate candidate SA binding poses and rank them via the EADock DSS scoring scheme. So that reproducibility could be supported and a common scoring reference provided, the best-ranked pose for each receptor was additionally docked and/or rescored on AutoDock Vina. For Vina, the grid was a cubic 20 × 20 × 20 Angstrom box, exhaustiveness was set to 4, and the grid centers were receptor-specific. Per receptor, the top-scoring complex the one with the most favorable predicted affinity in kcal/mol was the one carried forward into interaction analysis. SwissDock served as the docking platform (Grosdidier et al., 2011).

E.6- Complex clustering and quality indicators

To assess pose convergence and obtain representative complexes, docking solutions were clustered and summarized using HADDOCK-derived cluster descriptors (cluster size, within-cluster RMSD, and energy terms). The top cluster and the best AutoDock Vina affinity for each receptor. Rescoring was carried out with AutoDock Vina (Trott and Olson, 2010).

E.7- Interaction analysis and figure preparation

Per selected complex, the receptor-ligand contacts hydrogen bonds, hydrophobic contacts, aromatic interactions (pi-pi and pi-cation), and ionic/salt-bridge contacts were annotated within Discovery Studio. To compose the publication figures, representative 3D pocket views were paired with 2D interaction diagrams; interface-RMSD plots were also produced so that pose dispersion against interaction energetics could be visualized.

3. Statistical Analysis

A completely randomized design was used for the experiment, with five replicates per treatment. Through the MSTAT computer tool, the top three results per treatment were statistically evaluated. Via Duncan's Many Range Test (1955), the means across multiple treatments were compared so that differences could be confirmed.

4. Results and Discussion

4.1- Sterilization of explants

The data presented in Table (1) and Fig. (1), illustrate the impact of surface sterilization using NaOCl at varying concentrations of 10%, 15%, and 20% for a duration of 20 minutes on the survival and contamination rates of *Jatropha curcas* leaf explants after a culture period of 6 weeks.

Notable variation across explant survival rates emerged from the findings. NaOCl applied at 20% gave the highest survival rate, at 87%. NaOCl at 10%, on the other hand, gave the lowest survival rate, at 10%.

In parallel, the data point to a marked difference in the contamination rates of the explants. NaOCl at 10% gave the highest contamination rate observed, namely 75%.

Table 1. Effect of surface sterilization by NaOCl on surviving and contamination percentage of *Jatropha curcas* leaf explants

Clorox concentrations	Leaves		
	Survival	Contamination	Mortality
10%	10 ^c	75 ^b	15 ^b
15%	35 ^b	41 ^a	24 ^a
20%	87 ^a	8 ^c	5 ^c

By contrast, the lowest contamination percentage 5% followed from NaOCl applied at 20%. Across plant tissue culture work, effective sterilization is indispensable to securing the desired outcomes. Selection of suitable chemicals and cleaning protocols therefore matters greatly. Straightforward and environmentally sustainable procedures have been studied so that the most effective approach to *J. curcas* tissue culture using leaf explants could be identified.

**Fig. 1. Preparation of the explants**

Of the sterilization regimes tested, 50% NaOCl was the most effective at decontaminating *Jatropha curcas* leaf explants and came through as the optimum choice for micro-propagation. Survival outcomes from this regime were the highest and most stable, signaling that the conditions on offer favor the survival and subsequent development of *Jatropha curcas* (Achten et al., 2008). At the four-week mark of field growth, leaves were collected, washed under running water, and given a one-minute 70% ethanol treatment ahead of a 10-minute immersion in a 6.0% NaOCl bath made up at a 2:8 ratio of NaOCl to distilled water (Khurana-kaul et al., 2010). For the leaves of *Coccinia abyssinica* together with *J. curcas*, 5% NaOCl produced satisfactory sterilization (Liu et al., 2016). On grapevine axillary buds, NaOCl at 1.3% for one hour gave a contamination rate of just 19.8% (Lazo-Javalera et al., 2016). Off the tree, young leaves of *J. curcas* were taken, washed under tap water, and then scrubbed with liquid soap. Within the decontamination procedure, two principal agents were tested H₂O₂ and NaOCl, both at 0%, 2.5%, 5%, and 10% over a 5-minute exposure. Three rinses with sterile distilled water then followed. Boonyanan and Ketudat-Cairns (2021) reported that the NaOCl-treated leaf explants gave the highest fraction of green, contamination-free explants, 51.2%.

4.2- Callus induction

Concerning leaf-segment survival, every NaOCl strength tested delivered a favorable survival rate. Per Table (2), the poorest survival, 70.0%, came from leaf explants set on MS medium amended with BA at 1.0 mg/l plus NAA at 0.5 mg/l. By contrast, the best outcomes 80% and 90% went to NAA at 1.0 mg/l and 2.0 mg/l, respectively, each combined with BA at 1.0 mg/l.

Concerning the callus induction percentage, Fig. (2) makes clear that the BA and NAA strengths exerted a pronounced influence on the induction rates. On MS medium amended with BA at 1.0 mg/l plus NAA at 0.5 mg/l, the poorest induction rate of 54.6% was recorded. Lifting the NAA strength from 1.0 to 2.0 mg/l favoured induction, and the peak rate of 95% emerged from the combination of NAA at 2.0 mg/l plus BA at 1.0 mg/l. As Table (2) makes clear, leaf-derived callus fresh weight was pronouncedly shaped by the NAA strength, with the cytokinin BA held fixed at 1.0 mg/l. Heavier calli followed higher NAA strengths. At NAA 0.5 mg/l, the poorest callus fresh weight (2.5 g/jar) was recorded; the heaviest callus (5.4 g/jar) followed from NAA at 2.0 mg/l alongside BA at 1.0 mg/l.

Table (2). Influence of varying NAA strengths combined with BA at 1.0 mg/l on callus induction in *Jatropha curcas* leaf explants

NAA concentrations (mg/l)	Survival %	Callus induction%	Callus fresh weight (g)
0.0	0.0 ^d	0.0 ^d	0.0 ^d
0.5	70.0 ^c	54.6 ^c	2.5 ^c
1.0	80.0 ^b	76.3 ^b	3.8 ^b
2.0	90.0 ^a	95.0 ^a	5.4 ^a

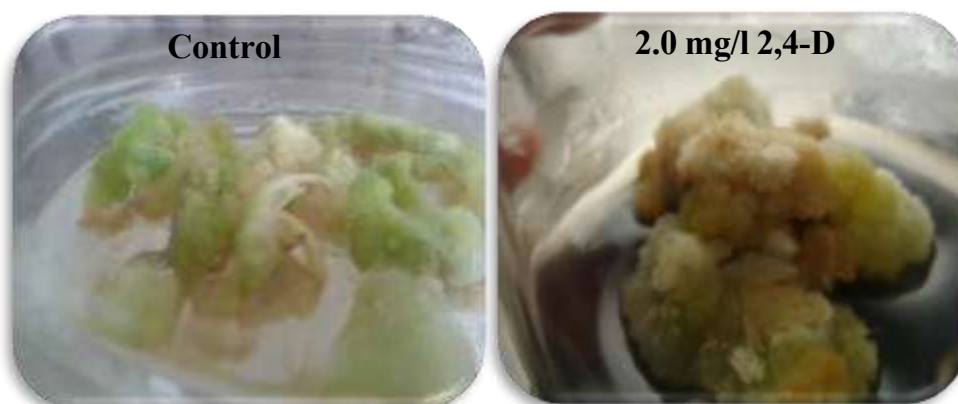


Fig. (2) Leaf-explant callus induction on MS medium carrying 1.0 mg/l BA + 2.0 mg/l NAA after three months in culture

For driving callus formation from *J. curcas* leaf explants, the regime identified as most favorable was MS medium amended with BA at 1.0 mg/l and NAA at 2.0 mg/l.

Consistency with a recent literature account is evident here: Gustian (2009) notes that auxins applied at low strengths typically favour callus development. Auxins together with cytokinins whether occurring within plant tissues or within the protoplasts of cultured cells also contribute substantially to callus induction on nutrient media (Akitha Devi et al., 2018). Among the auxins, NAA carries broad recognition as a key driver that backs cell division and quickens callus formation (Chen et al., 2019). Differences in callus colouration noted across the three plant parts may, beyond that, be tied to the actions of growth regulators supplied through the nutrient medium (Alwash et al., 2020).

How NAA together with BA drives callus formation can be tied to their role in proton (H⁺) release and in triggering cell divisions events that subsequently re-tune specific

enzyme activities and reshape both metabolic flux and protein synthesis within callus cells (Mohammed and Yahya, 2018). For cotyledon-derived callus of the *J. curcas* plant, Yan et al. (2009) reported MS amended with BA at 2.0 mg/l plus NAA at 2.0 mg/l as the optimum. Mohanalakshmi et al. (2009), in their work, secured callus initiation by sub-culturing various *J. curcas* explants on MS medium delivering BA alone at 1.5 mg/l; meanwhile, Varshney and Johnson (2010) identified MS amended with IBA at 0.5 mg/l plus BA at 1.0 mg/l as the most efficient combination for inducing morphogenic callus from *J. curcas* immature embryos, with a response rate of 85.7%. A properly balanced cytokinin–auxin combination for *Jatropha* callus production has been highlighted across many recent reports (Biradar et al., 2012).

From the present data, considerable promise emerges for the use of BAP–NAA combinations in the in vitro initiation of *J. curcas* callus. Within one to two weeks, callus formation became visible on leaf explants set on MS basal medium amended with different plant growth regulator strengths. Callus initiation from leaf explants on MS basal medium amended with NAA at 1 mg/l plus BAP at 5 mg/l is highlighted in the work (Rajore and Batra, 2007), who likewise identified NAA as the most effective PGR for producing green, compact, and rapidly proliferating *Jatropha* callus. After testing an array of PGR combinations and strengths, Mohajer et al. (2012) reported that the BAP–NAA pairing came out ahead of BAP–IBA for callus production. Many investigators have stressed that the right combinations and strengths of cytokinins together with auxins are decisive for *Jatropha* callus production (Kumar et al., 2015).

BA paired with other PGRs, NAA at 1 mg/l in particular has, on this point, seen broad use in tissue culture work, frequently driving the proliferation of callus cells (Hossain et al., 2016). From hypocotyl explants taken off 15-day-old seedlings, callus cultures were initiated on MS basal medium amended with BA at 0, 2 or 4 mg/l alongside NAA at 0, 1 or 2 mg/l in different combinations. The best callus formation followed from medium amended with BAP at 2 mg/l plus NAA at 1 mg/l. With this particular PGR combination, a higher yield of white, friable calli was produced (Oliveira et al., 2017).

Across many plant species, callus formation has grown more systematic and more straightforward particularly so in herbaceous dicotyledons, in contrast with woody plants. The difficulties and slower reactions of woody plants in producing such cultures may account for this contrast. Establishing *J. curcas* callus cultures in the present study carries weight and value because of the economic standing of this plant. Production of valuable compounds and improved productivity relative to seed-grown plants may follow from this route (El- Sayed et al., 2020).

4.3- Maintenance and elicitation of callus cultures

Within plants, salicylic acid is recognized as a regulator of essential biochemical and physiological parameters (Rivas-San Vicente and Plasencia, 2011).

Biomass build-up both fresh and dry weight of *Jatropha* callus was scored once exposure to salicylic acid at 0, 0.5, 1.0, 2.0 and 4.0 mg/l had taken place, alongside a control treatment without elicitor. Harvesting of the callus cultures occurred on day 30 after inoculation.

Per Figure (3), salicylic acid exerted a marked effect on both fresh and dry weights of the *Jatropha* callus. From the untreated control (0 mg/l) came the poorest values, 3.2 g fresh weight and 0.61 g dry weight. With salicylic acid at 0.5 mg/l, both fresh and dry weights climbed sharply to 4.15 and 0.75 g, respectively. Peak fresh weight (5.30 g) and peak dry weight (1.05 g) emerged at 2.0 mg/l, which was therefore the most effective dose for driving callus growth and dry-matter deposition. On the other hand, when the SA strength was pushed to 4.0 mg/l, a gradual fall in callus fresh and dry weight relative to the optimum followed. Read together, the data indicate that the action of salicylic acid

scales with dose: at low to medium doses it favors callus growth, while at high doses it suppresses it. The same trend was reported by Fazal et al. (2016), whose work showed that elevated SA strengths held back callus proliferation an effect plausibly tied to the stress imposed by the heavier elicitor load.

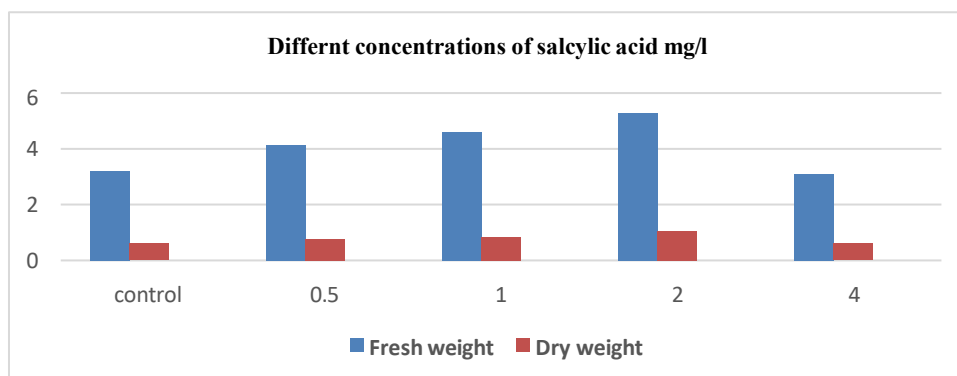


Fig. (3). Influence of salicylic acid on *Jatropha* callus growth at the 30-day mark in culture

Per Bulgakov et al. (2002), elevated SA strengths place substantial stress on cell growth, an effect that produces reduced biomass build-up in callus cultures of *Rauwolfia cordifolia*. In contrast, only a marginal SA influence on cell proliferation and biomass build-up was registered for callus cultures of grape and *Rosa* hybrid (Ram et al., 2013). A small decline in the proliferation of *Salvia miltiorrhiza* cell cultures was noted in earlier work under SA exposure (Li et al., 2003). Likewise, the growth of *Rubia cordifolia* callus cultures was shown to be substantially curbed as SA levels in the medium were lifted. With these earlier accounts the present results agree (Goyal et al., 2003).

Biomass build-up in *A. rosea* was studied once exposure to SA at 10, 20, 30, 40, 50 and 60 mg/l had taken place, alongside an elicitor-free control. On the 28th day after inoculation, callus cultures were harvested. Against control, biomass output climbed. With salicylic acid, peak biomass build-up, 281.25 g/l fresh weight and 14.75 g/l dry weight was registered at the 40 mg/l dose. At 60 mg/l SA, the poorest biomass output of 157.25 g/l was recorded. Higher SA strengths were shown to hold back callus growth. Such suppression can be tied to the stress brought on by the heavier elicitor load (Fazal et al., 2016). Under SA elicitation, both callus biomass and longevity were augmented. Improved callus traits in in vitro cultures under SA treatment have been documented (Nazir et al., 2021).

4.4- Influence of salicylic acid on secondary metabolites in *Jatropha* extract

On the callus cultures, three different SA elicitation regimes were trilled in this experiment, the design being guided by published reports (Singh et al., 2018). Through SA, a wide-ranging set of physiological and biochemical changes can be triggered in plants among them lifted output of secondary metabolites and antioxidants, fortified defense mechanisms, and improved tolerance to stress. Higher fresh and dry weights together with raised chlorophyll levels have been reported across many studies as outcomes of applying SA to in vitro cell cultures (Golkar et al., 2019). Compounds carrying considerable medicinal and commercial value can also be brought into production by the elicitor. Beyond that, SA lifts the action of antioxidant enzymes and can sharpen the stress resilience and longevity of cell cultures (Nechaeva et al., 2020). Shifts in gene expression and signaling routes are also brought about by SA switching on

a wide array of stress-responsive genes and prompting the synthesis of stress-related proteins. Through such reprogramming, the capacity of cell cultures to withstand external stresses salinity, drought, or pathogen attack can be strengthened (Miclea et al., 2020).

1- Determination of antioxidant content

Effects of salicylic acid on the antioxidant defense system of *Jatropha curcas* callus cultures are laid out in Tables (3, 4 and 5). Against the untreated control, both enzymatic and non-enzymatic antioxidant activities were pronouncedly lifted by SA application.

A. Determination of enzymatic antioxidants content

Per Table (3), activities of POD, APX, CAT and SOD in the control treatment (0 mg/l SA) were 14.2, 9.6, 18.3, and 11.5 U/mg protein, respectively. Once 0.5 mg/l SA had been applied, every enzyme activity climbed sharply to 20.4, 15.3, 25.7, and 18.8 U/mg protein, respectively. Peak activities of POD, APX, CAT and SOD, at 30.2, 28.2, 38.4, and 33.2 U/mg protein, respectively, equal to a 1.8–2.3-fold rise over the control was registered at 2.0 mg/l SA. Pushing the SA strength to 4.0 mg/l led to a slight dip in activities, to 19.5, 18.4, 22.9, and 20.1 U/mg proteins, respectively, though even these values stayed well above the control.

Table (3). Influence of salicylic acid on the enzymatic antioxidant content in *Jatropha* extract

SA mg/l	POD (U/mg protein)	APX (U/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
0.0	14.2	9.6	18.3	11.5
0.5	20.4	15.3	25.7	18.8
1.0	25.7	22.1	30.5	26.3
2.0	30.2	28.2	38.4	33.2
4.0	19.5	18.4	22.9	20.1

Lifted levels of antioxidant metabolites, SOD, POD, CAT, APX, and PAL included have been documented. Among these, SOD is the enzyme that turns the superoxide radical into hydrogen peroxide (H_2O_2); subsequently, other antioxidant enzymes break this H_2O_2 down into water and oxygen (Zhao et al., 2021). For its part, POD contributes to the breakdown of H_2O_2 into water and oxygen, shielding cells from the harmful consequences of H_2O_2 build-up, which would otherwise trigger oxidative stress (Fall et al., 2023). Alongside, CAT catalyses the conversion of H_2O_2 into water and oxygen (Jannesari et al., 2023). PAL, an enzyme tied to the formation of phenolic compounds (central secondary metabolites of plants), operates within the phenylpropanoid route the same pathway from which these secondary compounds emerge. From up-regulated PAL activity may stem, in part, the rise in aervine together with the other secondary metabolites noted earlier (Medda et al., 2020). Ascorbate peroxidase (APX), in turn, is the enzyme that turns ascorbate (Vitamin C) into its oxidized counterpart (dehydroascorbate), while at the same time decomposing H_2O_2 into water and oxygen. Within plants and algae, APX serves a pivotal role, guarding chloroplasts against the oxidative damage that follows photosynthesis (Ishtiaq et al., 2023). The greenish hue noted on the CC after elicitation may therefore arise from elevated APX activity. One plausible mechanism for the upgraded antioxidant defense triggered by elicitation runs as follows: SA can elicit reactive oxygen species (ROS) production (Chang et al., 2021)

inside cells, with the resulting signal then switching on the expression of genes that encode antioxidant enzymes.

B. Determination of non-enzymatic antioxidants content

B-1 Determination of total phenolic content

Per Table (4), callus that received no salicylic acid carried the lowest total phenolic level (18.4 mg/g FW). With SA at 0.5 mg/l, the phenolic content climbed sharply to 20.7 mg/g FW. Peak total phenolic content (33.5 mg/g FW) emerged at 2.0 mg/l SA; pushing the dose to 4.0 mg/l brought the value down to 15.0 mg/g FW.

B-2 Determination of total flavonoid content

Per Table (4), the pattern paralleled that of flavonoids, with values reaching 9.2 mg/g FW in the control treatment. With SA added at 1.0 and 2.0 mg/l, the content climbed in stepwise fashion to 18.6 and 23.5 mg/g FW, respectively. At 4.0 mg/l SA, on the other hand, the flavonoid level dropped to 6.2 mg/g FW.

Table (4). Influence of salicylic acid on the non-enzymatic antioxidant content in *Jatropha* extract

SA mg/l	Phenolic mg/g FW	Flavonoids mg/g FW
0.0	18.4 ^d	9.2 ^{cd}
0.5	20.7 ^{bc}	12.0 ^c
1.0	25.3 ^b	18.6 ^b
2.0	33.5 ^a	23.5 ^a
4.0	15.0 ^e	6.2 ^e

C. Determination of total antioxidant capacity (DPPH and FRAP)

After elicitation, the DPPH scavenging percentage climbed. Through in vitro cell cultures, elicitation can lift the production of antioxidant-bearing secondary metabolites phenolic compounds and flavonoids included (Anjum and Abbasi, 2016). These substances act as competent scavengers of free radicals such as DPPH, thereby shielding the plant against oxidative stress (Kedare and Singh, 2011). Per the work of Sharifi et al. (2019), salicylic acid can lift DPPH scavenging by engaging directly with DPPH and donating an electron or hydrogen radical, an interaction that lowers the radical's absorbance. Antioxidant performance of salicylic acid follows a dose-dependent pattern, with heavier doses translating into stronger DPPH scavenging. These observations match the present results, in which a higher SA dose of 100 μ M gave better DPPH scavenging (Mahdavian, 2022).

Per Table (5), DPPH radical scavenging activity of the callus climbed from 41.2% in the control to 55.8% under 0.5 mg/l SA, and reached 72.6% at 2.0 mg/l SA. Likewise, FRAP values climbed from 210 μ M Fe²⁺/g DW in the control to 298 μ M Fe²⁺/g DW under 0.5 mg/l SA, and peaked at 425 μ M Fe²⁺/g DW under 2.0 mg/l SA. At 4.0 mg/l SA, both DPPH and FRAP values dipped a little to 49.5% and 260 μ M Fe²⁺/g DW, respectively an outcome that confirms how excess SA produces partial oxidative stress.

Table (5). Influence of salicylic acid on the non-enzymatic antioxidant content in *Jatropha* extract

SA mg/l	DPPH inhibition %	FRAP $\mu\text{M Fe}^{2+}$ g/l DW
0.0	41.2 ^e	210 ^e
0.5	55.8 ^c	298 ^c
1.0	68.9 ^{ab}	401 ^b
2.0	72.6 ^a	425 ^a
4.0	49.5 ^d	260 ^d

As the present data show, both enzymatic and non-enzymatic antioxidant systems of *Jatropha curcas* callus cultures are clearly lifted by salicylic acid. Under moderate SA dosing (50–100 μM), pronounced rises in the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) emerged, alongside higher total phenolic, flavonoid, and overall antioxidant capacity values as registered by the DPPH and FRAP assays. At 100 μM SA, peak antioxidant performance was reached, marking that level as the most favorable for triggering defense responses without producing excessive oxidative stress (Rai et al., 2022).

Stronger enzymatic antioxidants can be traced to the role of SA as a signaling molecule that drives a regulated burst of reactive oxygen species (ROS). Through this mild oxidative pressure, antioxidant enzymes are switched on to keep redox balance in check. Via SOD, superoxide radicals are dismutase into hydrogen peroxide; this hydrogen peroxide is subsequently neutralized by CAT and APX. POD contributes to cell wall fortification alongside further ROS detoxification. Through this orchestrated enzymatic response, the capacity of the callus cells to weather oxidative stress is upgraded (Ghorbanpour et al., 2020).

Non-enzymatic antioxidants total phenolics and flavonoids in particular likewise climbed appreciably under SA exposure. As ROS scavengers, these compounds shield cellular components against oxidative injury. The parallel rise in non-enzymatic antioxidants tracks closely with the observed gains in secondary metabolite build-up, terpenoids, alkaloids, saponins, and glycosides included. From this, the implication is that SA-driven activation of the antioxidant system underpins secondary metabolism by holding cellular redox equilibrium steady (Gill and Tuteja, 2010).

As gauged by DPPH and FRAP, total antioxidant capacity lends further support to these conclusions. At 100 μM SA, both assays registered a marked rise a reflection of the joint contribution of enzymatic and non-enzymatic antioxidants in shielding the cells from oxidative pressure. A small dip in antioxidant activity at 150 μM SA points to the possibility that heavier doses produce excess ROS, overwhelming the defense system and partially curbing antioxidant enzyme function. Dose-dependent behavior of this kind tracks with earlier reports across other medicinal and oil-producing species (Abdelgadir et al., 2021).

Read together, the data point to SA as a potent elicitor capable of fortifying the antioxidant defense apparatus of *Jatropha curcas* callus an outcome that not only sharpens stress resilience but also drives the build-up of valuable secondary metabolites. At the 100 μM SA optimum, peak activities of both enzymatic and non-enzymatic antioxidants were reached, alongside the strongest rises in DPPH and FRAP values confirmation of SA's effectiveness as a lever for in vitro metabolic boosting (Rai et al., 2021).

Adding salicylic acid to the tissue culture medium markedly drove the deposition of secondary compounds in the callus relative to the untreated control, as the present data show. With salicylic acid dose the response varied, the effect being favorable up to a threshold and then dropping back at heavier doses.

Table (6). Influence of salicylic acid on the tannin, alkaloid, saponins and anthocyanins content in *Jatropha* extract

SA (mg/l)	Tannin (mg g/l FW)	Alkaloid (mg g/l DW)	Saponins (mg g/l DW)	Anthocyanins (mg/g FW)
0.0	2.53 ^c	0.69 ^e	0.8 ^e	0.48 ^e
0.5	3.64 ^b	1.3 ^c	1.9 ^c	0.71 ^c
1.0	3.92 ^{ab}	2.1 ^b	2.8 ^b	1.02 ^b
2.0	4.21 ^a	2.7 ^a	3.4 ^a	1.50 ^a
4.0	2.40 ^d	1.0 ^{cd}	1.5 ^d	0.76 ^{cd}

2- Determinate of total tannin content

Per Table (6), against the control, total tannin content responded favorably to salicylic acid addition. From the untreated callus came the poorest tannin level (2.53 mg g/l FW). Once SA at 0.5 mg/l had been added, tannin content climbed steadily to 3.64 mg g/l FW; the peak value of 4.21 mg g/l FW emerged at 2.0 mg/l SA. By contrast, pushing the dose to 4.0 mg/l SA drove tannin content back down to 2.40 mg g/l FW.

Once salicylic acid elicitation had been applied, the total phenolic content of *Jatropha curcas* callus climbed pronouncedly against the untreated control, as the present data make clear. As key secondary metabolites linked with plant defense, antioxidant performance, and stress-tolerance routes, phenolic compounds are produced chiefly through the phenylpropanoid route a pathway strongly responsive to elicitor cues such as SA. Pronounced rises in total phenolic content alongside antioxidant activities under SA elicitation have been demonstrated (Elbouzidi et al., 2025).

Acting as a signaling molecule, salicylic acid switches on phenylalanine ammonia-lyase (PAL), the rate-limiting enzyme of phenolic biosynthesis. Through PAL up-regulation, the elicitor channels metabolic precursors more strongly toward the formation of phenolic compounds, with raised accumulation of TPC and accompanying flavonoids as the outcome. Within *Pelargonium graveolens* callus cultures, SA treatment lifted TPC pronouncedly above controls, with peak TPC emerging under the lower elicitor dose (25 μ M) and accompanied by higher levels of individual phenolic constituents kaempferol and rutin among them (Bahadori et al., 2025). The elicitation impact of SA is also reflected in lifted antioxidant activity that tracks with raised phenolic levels. Within the same *P. graveolens* cultures, SA-elicited extracts produced stronger antioxidant profiles for example, in DPPH and total antioxidant capacity assays signaling that SA-induced phenolic synthesis lifts not only the quantity but also the functional activity of phenolic compounds (Wang et al., 2023). Evidence from advanced fed-batch adventitious root cultures of *Oplopanax elatus* showed, in addition, that an optimum SA elicitation regime 100 μ M for set durations, for instance produced a substantial uplift in both total phenolic and flavonoid content, with corresponding gains in antioxidant enzyme activities. Through these findings, SA elicitation is confirmed as a driver of phenolic pathways across diverse plant systems, reinforcing the mechanistic link between SA signaling and phenolic biosynthesis (El-Sayed and Mohamed, 2024). Past quantitative gains, SA exposure often re-tunes the phenolic profile of callus tissues, channeling secondary routes and producing variable raises in particular phenolic subclasses. Through these shifts the overall antioxidant potential of cultured tissues can be lifted, with meaningful implications for pharmaceutical and nutraceutical use, where richer phenolic content translates into stronger bioactivity and broader health benefits (Al-Mahtab and Hassan, 2020).

On the present evidence, salicylic acid acts as an effective elicitor of phenolic build-up in *Jatropha curcas* callus most plausibly via activation of the phenylpropanoid route and uplift of key enzymatic activities. This elicitor-based approach lifts not only the total phenolic yield but also the biological activity of the phenolic extract, marking SA elicitation as a worthwhile tool in plant biotechnology for producing phenolic-rich biomass.

3- Determinate of total alkaloid content

Per Table (6), against the control, the salicylic-acid-treated calli registered a pronounced rise in alkaloid content. From a value of 0.69 mg g/l DW in the control treatment, alkaloid content climbed to 2.1 and 2.7 mg g/l DW at 1.0 and 2.0 mg/l SA, respectively, before receding to 1.0 mg g/l DW at 4.0 mg/l SA.

Against the control, the alkaloid content of the SA-treated callus climbed pronouncedly evidence of SA's elicitor role in driving the formation of secondary compounds, alkaloids in particular, which rank among the most biologically active classes and carry antioxidant and defensive functions. Per recent reports, SA switches on biochemical routes responsible for alkaloid build-up in plant cells in vitro (Singh et al., 2022). Stronger alkaloid accumulation can be ascribed to SA's stimulation of key enzymes along the alkaloid synthesis route tyrosine decarboxylase, strictosidine synthase, and N-methyltransferases included which channel amino acids into specialized, stable alkaloids. Research has shown, in addition, that SA up-regulates the gene expression of these enzymes, with alkaloid accumulation in tissue cultures driven up accordingly (Kaur et al., 2021). Moderate SA doses, in addition, were the most effective at driving alkaloid build-up, while heavier doses may bring on partial production inhibition owing either to oxidative stress or to the redirection of metabolism toward other defensive routes. This pattern echoes findings across a variety of plants, where the balance between dose and exposure time is treated as decisive for reaching the optimum alkaloid yield (Patra and Das, 2020).

Once SA had been applied, alkaloid build-up was pronouncedly favored. Such a rise may be tied to SA-driven oxidative signaling, which switches on shikimate-derived routes and channels more nitrogen into secondary metabolism. Parallel responses have been registered for alkaloid-producing species such as *Rauvolfia serpentina* and *Catharanthus roseus* (Rai et al., 2022). For their part, alkaloids serve a key role in plant defense, acting as antimicrobial and anti-insecticidal compounds and aiding adaptation to environmental stress. Stronger alkaloid build-up in *Jatropha curcas* callus under SA influence therefore mirrors a fortified defensive response together with improved metabolic equilibrium within the cells (Isah, 2019). Read together, these data confirm that salicylic acid at the right dose is an effective lever for raising alkaloid output in plant tissue cultures, thereby lifting the pharmacological and biotechnological value of *Jatropha curcas* callus (Singh et al., 2022).

4- Determinate of total saponin content

Per Table (6), against the control, the saponin content of the salicylic-acid-treated callus climbed pronouncedly. Within the control, the saponin value was 0.8 mg/g DW. Under 0.5 mg/l SA, the content climbed to 1.9 mg/g DW, and reached its peak value of 3.4 mg/g DW at 2.0 mg/l SA. By contrast, at the 4.0 mg/l SA dose the content dropped to 1.5 mg/g DW.

Per recent literature, biological and physical stimulation of in vitro plant culture systems has been shown to clearly lift the build-up of valuable saponins, signaling the capacity of elicitors to drive the metabolic routes responsible for the synthesis of these secondary compounds via the switching on of defensive signals inside plant cells (Jiang et al.,

2021), against the control, treating *Jatropha curcas* callus with salicylic acid produced a pronounced rise in saponins content. Such uplift points to SA's elicitor role in secondary metabolism, given its known capacity to switch on plant defense routes and to drive the synthesis of phenolic and terpenoid compounds terpenoid saponins among them, a class that ranks among the most biologically active and physiologically meaningful in plants (Rivas-San and Plasencia, 2011).

Within *Bacopa floribunda*, applying inducers such as SA and JA produced a pronounced rise in triterpenoid saponins glycoside content, lending support to the view that elicitor-based biotechnological strategies can effectively raise saponin yields in callus systems and in in vitro plants (Patil and Park, 2023). Beyond this, other reports have pointed out that combinations of multiple elicitors JA, proline, and nutrient substrates among them produce favorable outcomes for raising specialized saponins in adventitious root cultures, stressing the need to design an optimized elicitation strategy for stronger build-up of these compounds (Sarropoulou et al., 2023).

Beyond this, work on *Agave salmiana* points to salt stress as a trigger for higher saponin output, lending support to the view that environmental cues and electors can act together to drive the synthesis of these bioactive compounds (Puente-Garza et al., 2021). Per a study on *Panax ginseng*, electors are not solely chemical agents such as SA and MeJA but also factors of another kind elemental combination among them that can step up saponin (ginsenoside) accumulation. The implication is that grasping the action of electors and managing their dose may be central to raising output in in vitro plant systems (Song et al., 2017).

Stronger saponin accumulation in the callus can be tied to the catalytic capacity of salicylic acid to switch on key enzymes along biosynthetic routes β -amyrin synthase and UDP-glycosyltransferases in particular which channel precursor compounds into more stable and potent saponins. Per recent reports, saponin lifts the gene expression of these enzymes, with saponin production in plant cell cultures driven up accordingly (Patil and Park, 2023). Low to moderate doses of saponin, in addition, were the most effective at driving saponin accumulation, while heavier doses produced a relative dip in saponin content. Such a drop can be ascribed to the excess cellular stress brought on by heavy saponin doses, which redirects metabolism toward basal defense routes rather than saponin biosynthesis. This trend tracks with findings across other plants, where the balance between dose and exposure duration to the elicitor is treated as decisive for stronger build-up of secondary compounds (Sarropoulou et al., 2023). Saponins, in addition, serve a key role in shielding plant cells from oxidative pressure. Acting as partial antioxidants and contributing to membrane stability, they aid callus adaptation under chemically stimulated conditions. From this comes the importance of using SA as an inducer in plant tissue cultures so that the highest possible build-up of beneficial saponins is reached (Zhang and Mahalingam, 2021).

5- Determinate of total anthocyanin content

Per Table (6), against the control, salicylic acid produced a clear rise in anthocyanin content. From the untreated callus came a value of 0.48 mg/g FW; under 0.5 mg/l SA the content climbed to 0.71 mg/g FW, and reached its peak of 1.5 mg/g FW at 2.0 mg/l SA. At 4.0 mg/l SA, the content fell back to 0.76 mg/g FW.

Against the control, treating *Jatropha curcas* callus with salicylic acid produced a pronounced rise in anthocyanin content. From this, the implication is that salicylic acid operates as an effective catalyst for the build-up of phenolic compounds and anthocyanins, which serve key roles in plant defense, protection against oxidative pressure, and the regulation of growth and senescence within plants (Sudha and Ravishankar, 2003). Stronger anthocyanin accumulation can be tied to SA-driven

stimulation of flavonoid biosynthesis routes particularly the gene expression of key enzymes such as chalcone synthase (CHS), chalcone isomerase (CHI), and UDP-glucose: flavonoid 3-O-glucosyltransferase (UGT), which channel intermediate compounds into stable anthocyanins that can be stored in cell vacuoles (Chen et al., 2022). Moderate SA doses proved the most effective at lifting anthocyanin content, while heavier doses may partially constrain build-up through redirection of metabolism toward alternative defensive routes or via the consumption of phenolic compounds in detoxification. These observations match earlier work across a variety of plants, where SA was seen to drive anthocyanin build-up in a manner that depends on dose and exposure duration (Ali et al., 2020).

Beyond this, anthocyanin build-up serves a key role in lifting the resilience of plant cells against oxidative pressure. Acting as potent antioxidants, anthocyanins scavenge oxygen free radicals, with membrane stability preserved and callus vitality supported accordingly (He et al., 2021). Read together, these findings confirm that salicylic acid at the right dose is an effective lever for driving anthocyanin production in *Jatropha curcas* callus, with tissue defense fortified and the physiological and pharmacological value of the phenolic compounds lifted as a result (Zhang et al., 2021).

Table (7). Influence of salicylic acid on the terpenoid, steroid, glycoside, phorbol ester and sterol content in *Jatropha curcas* callus

SA mg/l	Terpenoids (mg g/l DW)	Steroids (mg/g DW)	Glycosides (mg g/l DW)	Phorbol esters (mg g/l DW)	Sterols (mg g/l FW)
0.0	1.1 ^e	1.4 ^d	1.2 ^e	0.15 ^d	0.25 ^d
0.5	2.4 ^c	1.8 ^c	2.5 ^c	0.32 ^c	0.39 ^c
1.0	3.2 ^b	2.6 ^b	3.3 ^b	0.47 ^b	0.43 ^{ab}
2.0	3.9 ^a	3.5 ^a	4.2 ^a	0.56 ^a	0.55 ^a
4.0	2.1 ^{cd}	1.2 ^{de}	1.5 ^d	0.11 ^d	0.14 ^e

10- Influence of salicylic acid on terpenoid build-up

Per Table (7), against the control (1.1 mg/g DW), salicylic acid produced a pronounced rise in terpenoid content. At the 2.0 mg/l SA dose, the peak terpenoid value of 3.9 mg/g DW emerged; at 4.0 mg/l SA, the content fell back to 2.1 mg/g DW.

Against the untreated control, treating *Jatropha curcas* callus with salicylic acid (SA) gave rise to a pronounced increase in terpenoid content, as the present data show an outcome that signals SA's effectiveness as an elicitor that switches on secondary metabolic routes within plants. Spanning a broad class monoterpenes, sesquiterpenes, and polyterpenes among them terpenoids serve key roles in plant defense, defense signaling, and the response to environmental pressure. Chemical stimulants have been documented to lift the build-up of these compounds within in vitro plant culture systems (Razzaq et al., 2025). On the mechanistic side, the rise in potential terpenoids involves switching on key biochemical routes such as the MEP and MVA (2-C-methylerythritol 5-phosphate and mevalonate) pathways, which deliver isoprene production and subsequently its terpene derivatives, together with the activation of rate-limiting enzymes inside these routes. Per recent reviews, terpenoid synthesis is regulated at both the gene expression level and through key enzymes that respond to cues such as SA an arrangement that lifts the output of these secondary compounds (Lu et al., 2016). Beyond this, recent work indicates that SA can drive the build-up of particular terpenoid compounds for example; plant systems treated with SA gave rise to higher triterpenoid accumulation in *Pulsatilla chinensis* tissues, the inducer there

contributing to the fine-tuning of gene expression along central terpenoid routes (Dong et al., 2023).

Within plants, terpenoids serve key physiological and biological roles. Past their action as defense compounds against pests and pathogens, they are deployed to strengthen responses to environmental pressure, to draw in pollinators, and to govern the interaction between the plant and its surroundings. Stronger terpenoid build-up in callus may therefore upgrade the plant's capacity to handle stimulating conditions and to keep metabolic equilibrium efficient (Zhao et al., 2023). Read together, these results confirm that salicylic acid at an appropriate dose can act as a worthwhile lever for driving terpenoid build-up in *Jatropha curcas* callus consistent with recent work that draws on elicitation as an effective biotechnological strategy for raising the yield of secondary compounds of industrial and medical value (Razzaq et al., 2025).

As an effective lever for sharpening the defense mechanisms of plants, elicitation lifts phytochemical output from in vitro cultures. Beyond this, it is deployed to gauge the influence of various agents on plants through in vitro cell culture technology (Yue et al., 2016). The deployment of in vitro culture technologies for rapid, large-scale production of secondary metabolites is gathering pace. Driving this trend is the growing industrial appetite for bioactive compounds sourced from medicinal plants (Isah et al., 2017). For boosting productivity, elicitation comes through as the most widely recognized and effective approach (Alvarado et al., 2019).

6- Determination of steroid content

Per Table (7), against the control treatment lacking SA, supplementing the culture medium with salicylic acid clearly influenced the total steroid content within *Jatropha* callus. From callus that received no salicylic acid came the poorest steroid value, 1.4 mg/g DW. By contrast, an SA dose as low as 0.5 mg/l lifted steroid content sharply to 1.8 mg/g DW. At a 2.0 mg/l SA dose, the peak total steroid value (3.5 mg/g DW) emerged, well ahead of every other treatment. Pushing the SA strength to 4.0 mg/l brought about a relative dip in steroid content, even though the level still stayed above the control treatment. Broadly read, salicylic acid at low to medium doses drove steroid accumulation within *Jatropha* callus, while at heavy doses a partial inhibitory effect may set in.

Against the control, treating *Jatropha curcas* callus with salicylic acid gave rise to a pronounced increase in steroid content, as the present data show an outcome that signals the active role of salicylic acid as an elicitor of secondary metabolism within plant tissue cultures. Among the central signaling molecules tied to plant defense responses, salicylic acid switches on several metabolic routes that produce secondary compounds of physiological and pharmacological importance (Khan et al., 2021). At low and medium doses of salicylic acid, the pronounced uplift in steroid content can be ascribed to its capacity to switch on the mevalonate route (MVA), the primary pathway that produces terpenoid and steroid compounds within plant cells. Salicylic acid has been documented to sharpen the activity of rate-limiting enzymes such as HMG-CoA reductase, which in turn lifts the build-up of steroids and sterols within cell cultures and callus (Nazar et al., 2020). At a 1.0 mg/l salicylic acid dose, peak steroid content emerged the level that marks the optimum for metabolic stimulation without producing excessive cellular stress. With this, recent reports stand in agreement moderate SA doses drive metabolite production more efficiently than heavier doses, which can disrupt intracellular metabolic balance (Javadian et al., 2022). By contrast, a heavier salicylic acid dose of 2.0 mg/l gave a relative dip in steroid content an outcome that can be tied to the induction of excess oxidative pressure that channels cellular metabolism toward basic defense mechanisms rather than the synthesis of metabolites. Several

reports have indicated that heavy SA levels may hold back callus growth and curb the efficiency of biosynthesis of certain metabolites (Rady et al., 2021).

Steroid build-up may, in addition, be tied to the physiological role of steroids in keeping cell membrane stability and in regulating permeability especially under induced stress conditions. Some plant steroids, beyond this, serve as precursors of brassinosteroids, which carry a key role in regulating cell division and differentiation within plant tissue cultures and therefore underline the worth of maximizing steroid build-up within callus (Vardhini and Anjum, 2019). Read together, these results confirm that salicylic acid at an appropriate dose offers an effective strategy for raising steroid output within *Jatropha curcas* callus, paving the way for future biotechnological uses aimed at raising the efficiency of producing pharmaceutically and industrially valuable bioactive compounds (Sharma et al., 2023).

5- Determination of total glycosides content

Per Table (7), against the control, salicylic acid contributed to a rise in the total glycoside content of the callus. From the control treatment came 1.2 mg/g DW. Under 1.0 mg/l SA, the content climbed to 2.5 mg/g DW, before reaching its peak of 4.2 mg/g DW at 2.0 mg/l SA. At 4.0 mg/l, the content fell back to 1.5 mg/g DW.

Once salicylic acid had been applied, glycoside content within *Jatropha curcas* callus climbed pronouncedly, as the present data show evidence that confirms its role as a catalyst for the synthesis of glycosides tied to plant defense mechanisms. Per the reports, salicylic acid drives glycoside build-up within tissue cultures by switching on phenolic routes and by regulating metabolic equilibrium within plant cells (Falaki et al., 2021). The uplift in glycosides can be ascribed to the activation of UDP-glycosyltransferases (UGTs) the enzymes that attach sugars to secondary compounds and thereby raise their stability and intracellular storage. Per recent work, exposure to chemical stimulants such as salicylic acid lifts the gene expression of UGTs within plant cell cultures (Ross et al., 2001).

Glycoside build-up under low and medium SA doses suggests that such doses favor biosynthesis without inducing excess cellular pressure, while heavier doses may channel glycosides into detoxification and oxidative-stress mitigation (Naik and Al-Khayri, 2019). Within plant cells, glycosides serve a key role in upgrading the capacity to adapt under stress conditions. Acting as non-toxic reservoirs of bioactive compounds, they support the intracellular antioxidant system particularly so within tissue cultures (Sharma et al., 2020).

8- Determination of phorbol ester content

Per Table (7), supplementing the callus medium with salicylic acid pronouncedly influenced the Phorbol content. Under moderate doses, greater accumulation of the compound was registered; under heavy doses, the content fell back. From the untreated callus (control) came the poorest Phorbol value, 0.15 mg/g DW. Under 0.5 mg/l SA, the Phorbol content climbed to 0.32 mg/g DW. At 2.0 mg/l SA the peak build-up of 0.56 mg/g DW emerged a 127% gain against the control. At the heaviest dose tested, 4.0 mg/l SA, the Phorbol content fell to 0.11 mg/g DW.

Per recent reports, phorbol esters are diterpenoid compounds derived from the phorbol basic structure. As prominent secondary compounds within some members of the Euphorbiaceae family *Jatropha curcas* among them they fulfill biological roles and serve defensive functions, despite a degree of toxicity within some industrial applications (where they act, for example, as tumor promoters within biological models) (Abou-Arab and Abu-Salem, 2010). Branched terpenoids such as phorbol esters take shape via the primary terpenoid routes (MEP/MVA) the same routes that chemical cues such as

salicylic acid are thought to act on by regulating the genes and enzymes responsible for the biosynthesis of these compounds. Direct studies on SA's influence on phorbol ester build-up within callus remain limited; nevertheless, recent literature signals that environmental cues and stimuli switch on the gene expression of diterpenoid routes an effect that can translate into altered phorbol levels within plant tissues (Phasukarratchai et al., 2017).

Per recent genetic and metabolomic studies on *Jatropha curcas*, phorbol ester build-up varies with tissue type and with the gene expression level of diterpenoid routes an outcome that marks environmental cues and stimuli as decisive factors in setting the concentrations of these bioactive compounds. The responses registered may stem from metabolic re-tuning rather than simply from a uniform rise in overall output (Bueso et al., 2016). Viewed physiologically, phorbol ester build-up represents a strategic plant defense response against biotic and abiotic pressure. On this reading, modulating phorbol ester levels via cues such as SA can reprogram the biosynthesis of secondary compounds within the plant's integrated defense network. Even though phorbol esters can be toxic within some settings, grasping and regulating their build-up within tissue culture may aid in steering culture quality and in tailoring secondary components to their intended use (whether medical or industrial) (Zhang et al., 2024). Drawing on these arguments, applying salicylic acid as a signaling modulator within *Jatropha* may directly or indirectly steer phorbol ester build-up through the redirection of metabolic flow within the diterpenoid routes. Further targeted experimental work within tissue cultures will, however, be required so that the specific influence of dose and treatment duration can be verified.

3- Determinate of total sterol content

Per Table (7), the total sterol content showed a pronounced improvement once salicylic acid had been applied. Within the control, the sterol value was 0.25 mg/g FW. Under 1.0 and 2.0 mg/l SA, values climbed to 0.43 and 0.55 mg/g FW, respectively. At 4.0 mg/l SA, the content fell to 0.14 mg/g FW.

As core constituents of plant cell membranes, sterols fulfill a pivotal role in regulating membrane fluidity, in stabilizing membrane proteins, and in transmitting cellular signals. Within salicylic-acid-treated callus, the elevated sterol content can be traced to activation of the mevalonate route, which delivers isoprenoid synthesis plant sterols among them (Du et al., 2022). Salicylic acid plays into the induction of gene expression of key enzymes along this route 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), for instance channeling more carbon flows toward sterol synthesis. Salicylic acid's role in sharpening the efficiency of the antioxidant system may, in addition, aid in preserving cell membrane integrity, curbing sterol degradation and lifting sterol build-up (Benveniste, 2004).

Under heavy salicylic acid doses, the relative dip in sterol content may stem from metabolic imbalance, or from the redirection of metabolic energy toward the production of other defensive compounds phenols or specialized terpenoids, for instance at the expense of structural sterols. Such observations match earlier reports indicating that salicylic acid drives isoprenoid build-up at low to moderate doses, while heavier doses produce inhibitory effects on plant growth and metabolism (Vardhini, 2017).

4.5- Docking energetics across antioxidant and secondary-metabolism enzymes

AutoDock Vina rescoring indicated that SA binds most strongly to CAT (top score: -6.041 kcal/mol), followed by APX, CHS, and SOD (each -4.776 kcal/mol), then POD (-3.567 kcal/mol), while PAL showed the weakest predicted affinity (-2.796 kcal/mol) (Table 8). Across targets, the best solutions were supported by sizable dominant clusters and low

within-cluster RMSD values for most receptors, indicating convergence toward a preferred binding mode (Table 8).

4.5-1 Pose convergence and interface-RMSD patterns

Interface-RMSD scatter plots revealed receptor-dependent dispersion of docking poses, with compact clusters for several targets and broader distributions for others, suggesting differences in pocket definition and ligand accommodation. These patterns are consistent with the clustering statistics reported in Table (9) and support selection of a representative complex per target for contact mapping.

4.5-2 Receptor-ligand interaction fingerprints

In Figures, (4, 5, 6 and 7) show the interaction mapping showed that SA is stabilized by recurrent polar contacts involving its carboxyl and hydroxyl groups, complemented by hydrophobic packing within the pocket. Representative 3D poses and corresponding 2D interaction maps are presented for APX-SA, CAT-SA, and CHS-SA (Fig. 4) and for PAL-SA, POD-SA, and SOD-SA (Fig. 5). Across complexes, hydrogen-bonding networks frequently involved His, Ser, Glu/Asp, and backbone amide groups, while hydrophobic contacts were observed with Leu, Ile, Val, and aromatic residues.

Through this *in silico* analysis, a mechanistic link between SA elicitation and the observed gains in antioxidant capacity and secondary metabolite accumulation within *Jatropha* callus cultures gains support. Among the targets examined, CAT registered the most favorable predicted affinity evidence that, within the tested enzyme panel, CAT may stand as a plausible direct interaction partner of SA. More broadly, SA's capacity to settle into stable, convergent poses within the pockets of antioxidant enzymes (CAT, APX, POD, and SOD) furnishes a structural basis for how SA could re-tune ROS handling and thereby drive a controlled redox signal that switches on defense-linked routes. Such redox signaling tracks with the coordinated increases registered experimentally for enzymatic antioxidants and for non-enzymatic antioxidant capacity. Alongside, docking of SA against PAL and CHS offers a route for indirect coupling of SA signaling with phenylpropanoid and flavonoid pathway activation, lending support to the measured gains in phenolic and related secondary metabolites. Limitations include the reliance on homologous plant proteins where *Jatropha*-specific structures were unavailable, the static nature of docking, and the approximate character of scoring functions; the docking outputs should therefore be read as hypothesis-generating. Future work would benefit from incorporating *Jatropha*-specific models once sequences become available, from molecular dynamics simulations to evaluate pose stability, and from targeted enzyme assays to test whether SA directly re-tunes catalytic activity at biologically relevant doses. As a central plant defense signal, SA re-tunes redox balance and antioxidant systems (Vlot et al., 2009, Poór et al., 2020, Shoala et al., 2021), and SA-linked changes in stress enzyme activities have been reported within *Jatropha curcas* (Soares et al., 2016).

Table 8. Top HADDOCK cluster per receptor + best AutoDock Vina affinity

Receptor	PDB ID/ Uniprot	Source	Z-score	HADDOCK score	Cluster size	RM SD	E _{vdW}	E _{elec}	E _{desolv}	Restraints viol.	BSA	Vina top score (kcal/mol)
APX	Q42	Arabid	-	274.	17	0.3	-	-	-3.6	306	32	-4.776
	564	opsis thaliana	1.4	3 ± 2.6	8.0		6.3	11 3.3		8.4	7.2	
CAT	P25819	Arabidopsis thaliana	- 1.2	346.8 ± 20.4	38.0	0.3	- 4.8	- 91.3	-2.4	372 2.1	32 2.1	-6.041
CHS	9JL6	Physcomitrella patens	- 0.7	284.0 ± 25.1	10.0	0.2	- 9.9	- 55.2	-0.9	305 8.9	29 2.0	-4.776
PAL	P35511	Solanum lycopersicum	- 1.3	376.6 ± 13.4	11 1.0	0.5	- 10.2	- 23.4	-1.8	393 3.1	29 4.2	-2.796
POD	F4H VJ3	Arabidopsis thaliana	- 1.2	-6.6 ± 2.0	15 0.0	1.5	- 3.9	- 11 4.5	-2.3	224 .0	25 8.6	-3.567
SOD	7BJK	Arabidopsis thaliana	- 1.5	204.6 ± 27.0	9.0	0.2	- 1.9	- 56.2	-0.6	218 3.0	28 2.6	-4.776

Table 9. AutoDock Vina parameters for each receptor with ligand

Receptor	Center _x	Center _y	Center _z	Size _x	Size _y	Size _z	Exhaustiveness
APX	-1.0	-18.0	2.0	20.0	20.0	20.0	4.0
CAT	4.0	1.0	-3.0	20.0	20.0	20.0	4.0
CHS	111.0	108.0	102.0	20.0	20.0	20.0	4.0
PAL	0.0	37.0	35.0	20.0	20.0	20.0	4.0
POD	280.0	217.0	210.0	20.0	20.0	20.0	4.0
SOD	218.0	2.0	139.0	20.0	20.0	20.0	4.0

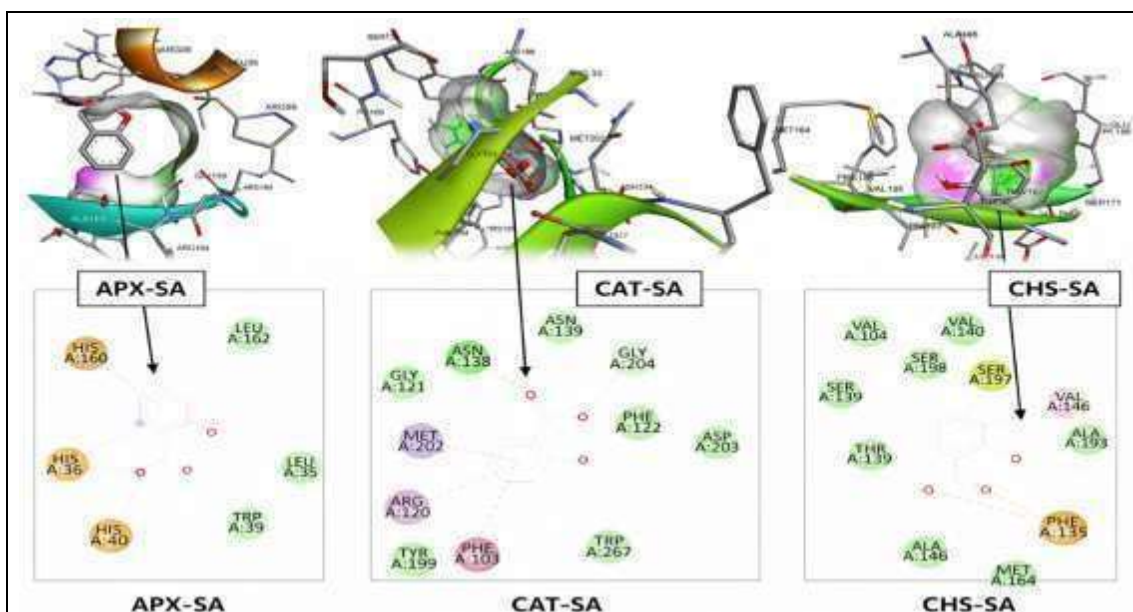


Fig. 4. For each complex (APX-SA, CAT-SA, CHS-SA), the top panel shows the ligand bound in the receptor pocket, with the ligand rendered as sticks (colored by element) and the receptor shown as cartoon/sticks with a semi-transparent pocket surface; key contacting residues are labeled, and the bottom panel provides a 2D interaction map summarizing receptor-ligand contacts, where residues are labeled by amino-acid name and sequence number (with chain ID) and interaction types are encoded by line/symbol style: dashed connectors indicate hydrogen bonds (with distances in Å when annotated), hydrophobic contacts are shown as contact lines/arcs, aromatic interactions (π - π stacking and π -cation) are shown with aromatic connectors, and ionic interactions/salt bridges are depicted between oppositely charged groups (water-mediated contacts indicated where present); only interactions meeting the analysis cutoffs used in the workflow are displayed.

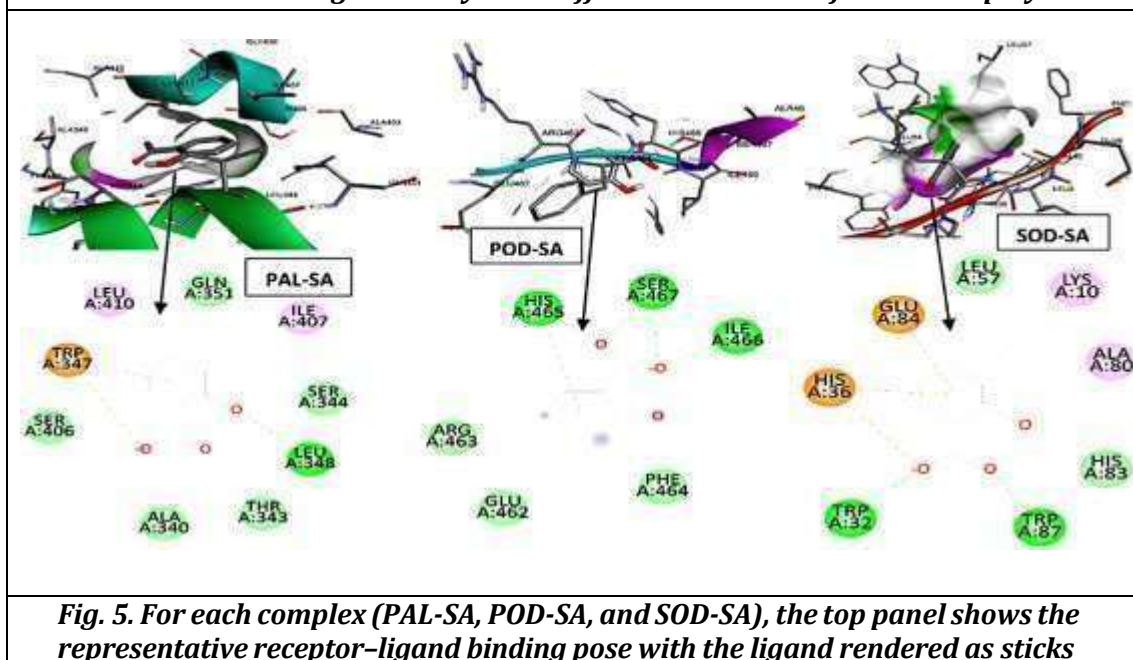


Fig. 5. For each complex (PAL-SA, POD-SA, and SOD-SA), the top panel shows the representative receptor-ligand binding pose with the ligand rendered as sticks

(colored by element) in the receptor pocket (receptor shown as cartoon/sticks with key residues labeled), while the bottom panel presents the corresponding 2D interaction map summarizing residue–ligand contacts; receptor residues are labeled by amino-acid name and sequence number (with chain identifier), and interaction types are encoded by line/symbol style such that dashed connectors denote hydrogen bonds (with distances in Å when reported), hydrophobic contacts are shown as contact lines/arcs, aromatic interactions (π – π stacking and π -cation) are indicated by aromatic connectors, and ionic interactions/salt bridges are depicted between oppositely charged groups (water-mediated contacts indicated where present), with only interactions meeting the predefined analysis cutoffs included

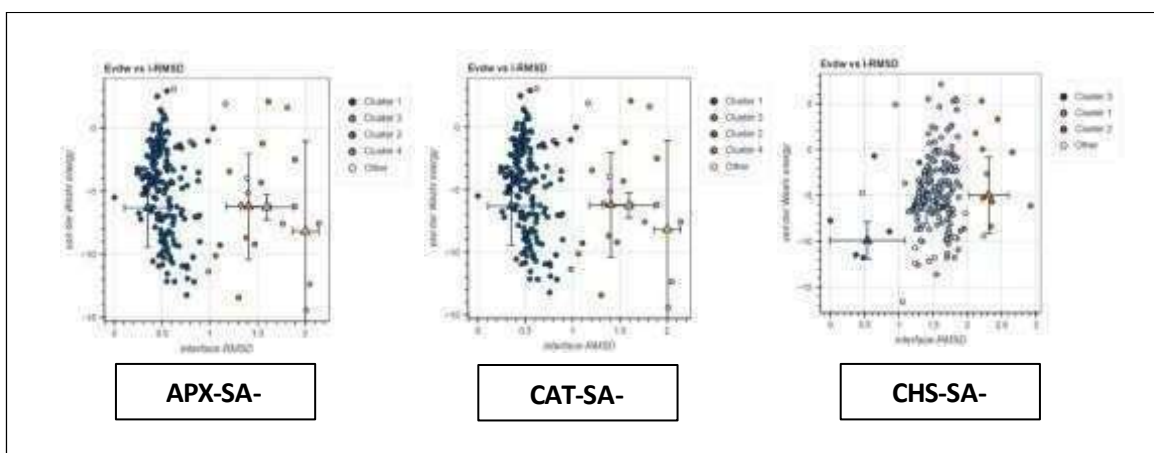


Fig. 6. Receptor–ligand interaction legend: The ligand is shown as sticks (colored by element), and interacting receptor residues are labeled by residue name/number (chain ID where applicable). Noncovalent contacts between the ligand and receptor are depicted as: hydrogen bonds (dashed lines), hydrophobic contacts (arc/spoke contacts), salt bridges / ionic interactions (dashed contacts between oppositely charged groups), π - π stacking (aromatic–aromatic interactions), π -cation interactions (aromatic ring with cationic group), and water-mediated hydrogen bonds (contacts bridged by a water molecule, where shown). Interaction distances are reported in Å when annotated. Surface rendering highlights the binding pocket surrounding the ligand.

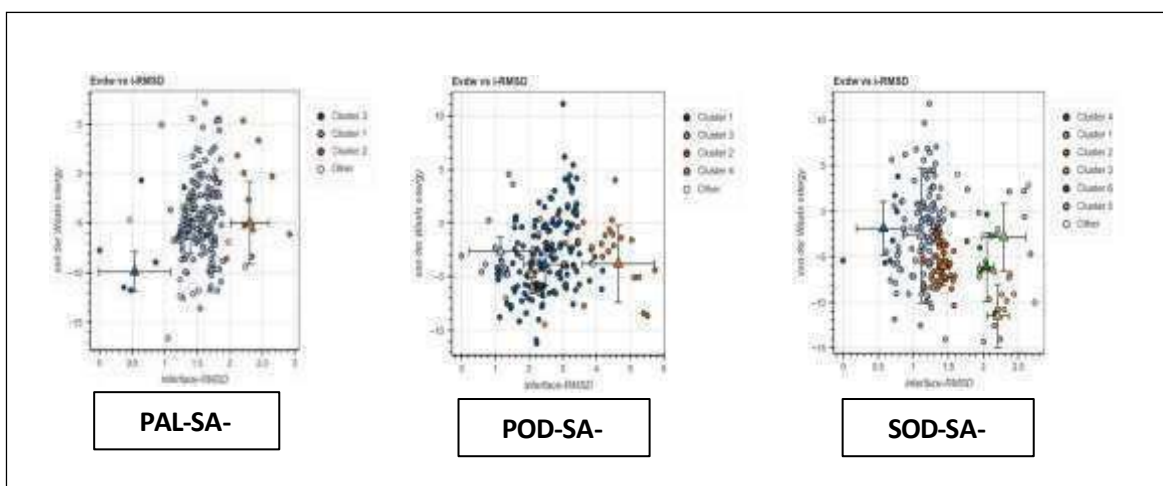


Fig. 7. Receptor–ligand interaction legend: The ligand is shown as sticks (colored by element), and interacting receptor residues are labeled by residue name/number (chain ID where applicable). Noncovalent contacts between the ligand and receptor are depicted as: hydrogen bonds (dashed lines), hydrophobic contacts (arc/spoke contacts), salt bridges / ionic interactions (dashed contacts between oppositely charged groups), π – π stacking (aromatic–aromatic interactions), π –cation interactions (aromatic ring with cationic group), and water-mediated hydrogen bonds (contacts bridged by a water molecule, where shown). Interaction distances are reported in Å when annotated. Surface rendering highlights the binding pocket surrounding the ligand.

5. Conclusion

Per the present work, salicylic acid acts as a potent driver of growth regulation, antioxidant defense mechanisms, and secondary metabolite synthesis within *Jatropha* callus cultures. Both physiological and biochemical responses of the callus were pronouncedly shaped by salicylic acid, with moderate doses sharpening metabolic activity without producing severe growth inhibition. From these outcomes follows a basis for fine-tuning stimulator-based strategies within plant tissue culture systems, with the goal of sustainable production of bioactive compounds carrying pharmaceutical and industrial value.

Once salicylic acid had been applied, pronounced activation followed for both enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) among them and non-enzymatic antioxidants such as total phenols and flavonoids. This antioxidant response served a key role in preserving cellular redox equilibrium and in shielding callus cells from oxidative pressure. As a result, the improved antioxidant standing supported stronger accumulation of valuable secondary metabolites terpenoids, alkaloids, saponins, glycosides, sterols, anthocyanins, and phorbol esters among them. Among the doses tested, 2.0 mg/l of salicylic acid came through as the optimum, delivering the peak antioxidant enzyme activity, the peak total antioxidant capacity (DPPH and FRAP), and the strongest drive of secondary metabolite production.

Through the joint in vitro and in silico analyses, salicylic acid stands out as a substantial driver of antioxidant capacity and secondary metabolite accumulation within *Jatropha curcas* callus. Molecular docking outputs furnish mechanistic evidence for the interaction of salicylic acid with key antioxidant and phenylpropanoid/flavonoid pathway enzymes, lending support to its regulatory function at the molecular level. From these findings, the promise of salicylic acid as an effective elicitor for sharpening the biosynthesis of valuable secondary metabolites is underlined, and a sharper grasp of the molecular basis of metabolite uplift within plant tissue culture systems is provided.

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