Chelonian Conservation And Biology

Vol. 18 No. 2 (2023) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2023.11(2).1046.1062

"EFFECT OF DIFFERENT SALICYLIC ACID CONCENTRATIONS ON GUGGULSTERONE Z BIOSYNTHESIS IN *COMMIPHORA WIGHTII*"

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Abstract

Salicylic acid is an elicitor that has been widely used to enhance the production of secondary metabolites in plants. In this context, the present study aims to investigate the effect of salicylic acid on the production of Guggulsterone Z in the Guggul plant (*Commiphora wightii*). Guggulsterone Z is a bioactive compound with potent anti-inflammatory and cholesterollowering properties, which makes it a valuable pharmaceutical compound.

In this study, the Guggul plant was treated with different concentrations of salicylic acid. The results showed that the application of salicylic acid significantly elevated the Guggulsterone Z content in the Guggul plant, with the optimal concentration being 100 µm. The findings of this study provide valuable insights into the use of salicylic acid as an elicitor for enhancing the production of Guggulsterone Z of the Guggul plant.

Keywords:- Salicyclic acid, Guggulsterone Z, Guggul plant (*Commiphora wightii*)

Introduction:

Medicinal plants have been used for centuries to treat various ailments, and their therapeutic properties are attributed to the presence of secondary metabolites such as steroids, flavonoids, terpenoids, and phenolics (Abbasi et.al 2012). However, the production of these compounds is often low, making it challenging to obtain sufficient quantities for pharmaceutical applications. To overcome this limitation, various strategies have been developed to enhance secondary metabolite production in medicinal plants, including the use of elicitors (Ahlawat et.al 2017). Elicitors are compounds that can activate a plant's defense mechanisms, leading to the production of secondary metabolites. Salicylic acid is one such elicitor that has been extensively studied for its ability to

enhance secondary metabolite production in medicinal plants. Salicylic acid is a naturally occurring compound that is involved in plant defense against pathogens and environmental stresses (Baenas et.al 2014). Its ability to induce defense responses in plants makes it an attractive candidate for use as an elicitor. Several studies have shown that the application of salicylic acid can enhance the production of secondary metabolites in medicinal plants, including ginseng, ginkgo, and echinacea. Moreover, salicylic acid has been reported to increase the activity of key enzymes involved in secondary metabolite biosynthesis, such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and isoflavone synthase (IFS) (Dhiman et.al 2018). Despite the promising results, the use of salicylic acid as an elicitor is not standardized, and the optimal concentration and application method may vary depending on the plant species and the targeted metabolites (Corchete and Bru 2013). Therefore, it is crucial to develop standardized protocols for the use of salicylic acid as an elicitor to enhance secondary metabolite production in medicinal plants.

Guggul, also known as Commiphora wightii, is a resin extracted from the Mukul myrrh tree native to India. It has been a vital component of traditional Ayurvedic medicine for centuries due to its numerous therapeutic properties. Guggul is renowned for its potential to lower cholesterol levels, reduce inflammation, and aid in weight management. Its active compounds, called guggulsterones, have been studied for their impact on various health conditions. Guggulsterones have gained recognition for their cholesterol-lowering properties, believed to inhibit cholesterol synthesis and promote the breakdown of LDL ("bad") cholesterol. Additionally, they exhibit anti-inflammatory effects, making them valuable for conditions like arthritis. In the realm of weight management, guggulsterones may regulate metabolism and aid in fat loss. They also offer antioxidant benefits, protecting cells from oxidative damage. Rooted in traditional Ayurvedic medicine, guggulsterones have been used for centuries to address a range of health issues.A s demand for guggul-based products has increased due to their potential medicinal benefits, there has been a growing risk of overharvesting and depletion of the Commiphora wightii tree population in its native habitats, primarily in India. This overexploitation can have detrimental ecological consequences, including habitat disruption and loss.

Efforts are being made to promote sustainable harvesting practices and conservation initiatives to protect the Commiphora wightii and its ecosystem. These include regulations on harvesting, cultivation programs, and the promotion of ethical and environmentally-friendly sourcing of guggul resin. Salicylic acid, a naturally occurring plant hormone, plays a vital role in plant defense mechanisms against stressors like pathogens and environmental stress. When applied in controlled concentrations through tissue culture techniques, salicylic acid can enhance these defense mechanisms. It acts as a signaling molecule, triggering the plant's immune response and increasing the production of defensive compounds, such as phytoalexins and pathogenesisrelated proteins. This enhanced resistance to stressors makes salicylic acid an essential tool in tissue culture for propagating disease-resistant plant varieties and improving their overall health and resilience

However, the production of Guggulsterone Z in the Guggul plant is often limited, making it challenging to obtain sufficient quantities for pharmaceutical applications (Gadzovska et.al 2015). To overcome this limitation, various strategies have been developed to enhance the production of Guggulsterone Z in the Commiphora wightii, including the use of elicitors such as salicylic acid. Salicylic acid is a naturally occurring phytohormone that is involved in plant defense against biotic and abiotic stresses. Its ability to induce defense responses in plants makes it an attractive candidate for use as an elicitor to enhance Guggulsterone Z production (Jakovljevic et.al 2022).

In this context, the present study aims to investigate the effect of standardized concentrations of salicylic acid on the significant enhancement in Guggulsterone Z(Khare et.al 2020) in the Guggul plant through tissue culture techniques. The findings of this study may provide valuable insights into the use of salicylic acid as an elicitor for enhancing the production of secondary metabolites in medicinal plants (Esmaeilzadeh et.al 2014).

Materials and Methods:

Preparation of Explant for Callus Induction of Commiphora wightii.

Young fresh leaves of *Commiphora wightii* were collected from the medicinal and aromatic department GKVK Bengaluru. Explants were prepared according to the method described. Then leaf tips (about 1 cm) from the used as an explant for callus induction. *Commiphora wightii*. callus was induced and proliferated according to the method described by (Ali et al. 2016). Sterilized leaf explants were planted in MS (Murashige and Skoog) medium supplemented with auxin and cytokinen hormones combinations 2.0mg/l 2,4-D (Dichlorophenoxyacetic acid)+1.5 mg/l Kn (Kinetin) as plant growth regulator and were incubated at 25 ± 2º C and photo-period of 16/8h light/dark.

Maintaine of cell suspension

Fresh Callus samples were further taken out for the preparation of Cell suspension culture. Cell suspension culture maintenance Green color Callus culture with 15 days old age, were chosen to create cell suspension culture. Cell suspension culture media was prepared MS media (NH4NO3 825mg L^{-1} KNO3 950mg L^{-1} CaCl2.6H2O 220mg L^{-1}) containing growth regualators 24D 0.5 Kn 0.25 2iP- 6yy dimethyl Ally amino purine (1mg L^{-1}) (Ahlawat et.al 2017) and 3% Sucrose.

Salicyclic acid treatment

Salicylic acid treatment Well developed cell cultures were transferred to the Media treated with different concentration of Salicylic acid (SA) (25, 50, 75 and 100,125 mg/L) separately. Elicitor treatment was maintained at 25° ± 2°C, photoperiod of 16/8h light/dark for three weeks in Incubator – Shaker.

Estimation of Guggulsterone Z through High-performance liquid chromatography (HPLC)

Guggulsterone Z is a plant steroid and bioactive compound present in gum Guggul of *Commiphora wightii*. Plants have produced a wide variety of derivatives that have been identified and described. Therefore, it is evident that many peaks are comparable to various opiate derivatives that would be assessed in HPLC estimation. The presence or induction of steroids in our offerings was therefore predicted by combining the peaks in connecting a specific retention time (RT) based on the reference, Guggulsterone Z molecule (Sarma et al. 2002).

Statistical Analysis

The every part of experiments was performed here in triplicates (n = 3). The value in the table, text, and figures signifies mean value ± standard deviation (SD). The difference between control and treatment was examined statistically in means of the t-test, with the level of significance be $P < 0.01$ as shown in Fig 3.9.

Results and discussion:

Several studies have investigated the use of salicylic acid (SA) as an elicitor to enhance the production of Guggulsterone Z in *Commiphora wightii* suspension cultures (Chodisetti et.al 2015). Here are some of the key findings from these studies:

This study shows the SA treatment resulted in a significant increase in the production of Guggulsterone Z in suspension cultures of salicylic acid 100µm concentration as seen in fig. 2.5. For instance, one study reported a 3-fold increase in the production of Guggulsterone Z in SAtreated cultures compared to control cultures (Anjum et.al 2017). The effect of SA on Guggulsterone Z production was found to be time-dependent, with maximum production observed at specific time points. In this study the maximum growth of Guggulsterone Z production was observed in 15 days. Similarly one study reported maximum Guggulsterone Z production at day 10 of SA treatment, while another study reported maximum production at day 15 (Danova and Pistelli 2022). SA treatment was also found to influence the production of other secondary metabolites in *Commiphora wightii* suspension cultures (Ahlawat et.al 2017).

The mechanism of action of SA in eliciting Guggulsterone Z production is not fully understood. However, it has been suggested that SA induces the expression of genes involved in the biosynthesis of Guggulsterone Z and other secondary metabolites (Biswas et.al 2018).

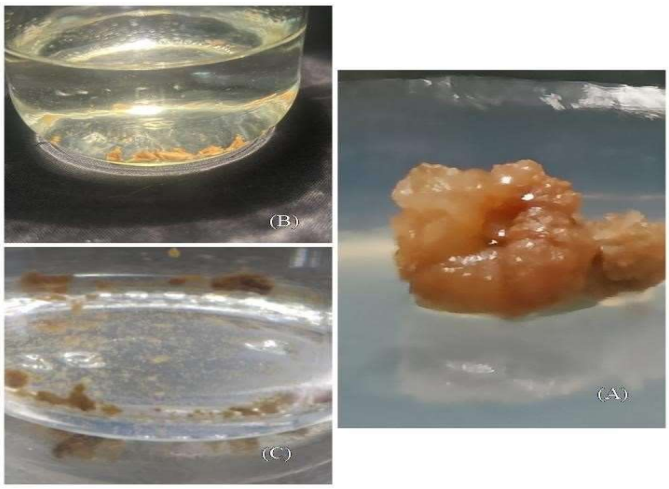


Fig 2.1 Control (A) callus (B) clumps of callus (C) cells generate after 15 days.

Fig 2.2: 25µm Salicyclic acid (A) callus (B) clumps of callus (C) cells generate after 15 days.

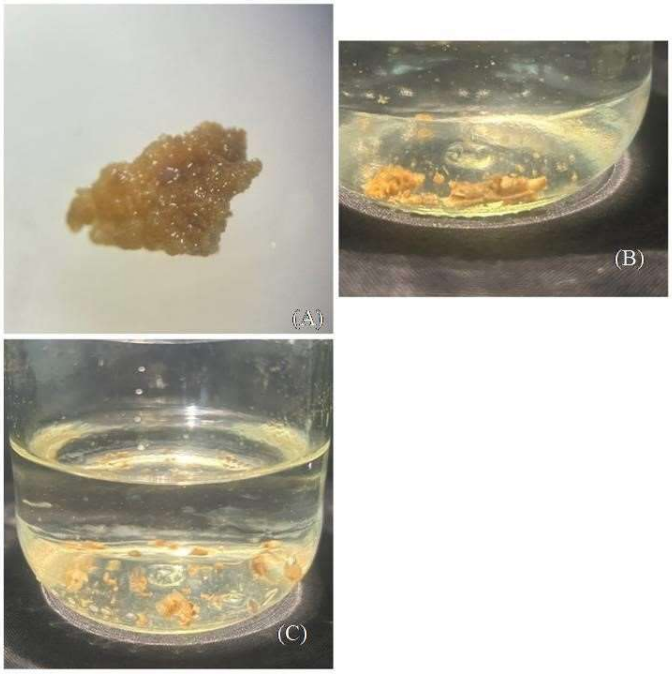


Fig 2.3: 50µm Salicyclic acid (A) callus (B) clumps of callus (C) cells generate after 15 days.

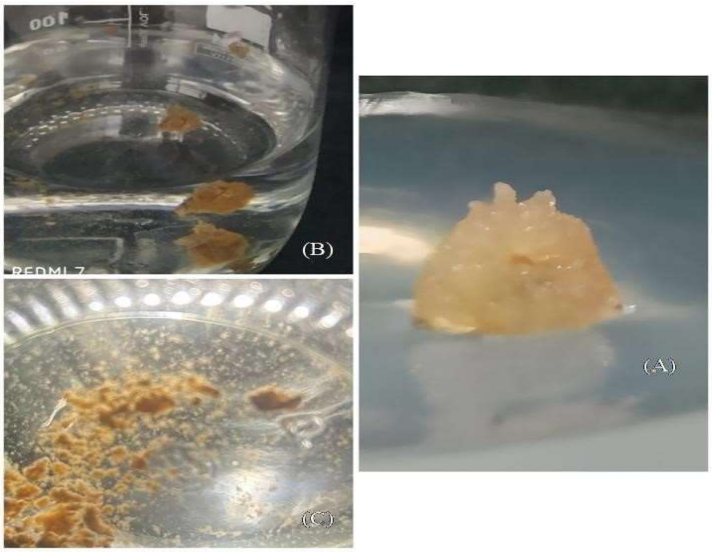


Fig 2.4: 75µm Salicyclic acid (A) callus (B) clumps of callus (C) cells generate after 15 days.

Fig 2.5: 100µm Salicyclic acid (A) callus (B) clumps of callus (C) cells generate after 15 days.

Fig 2.6: 125µm Salicyclic acid (A) callus (B) clumps of callus (C) cells generate after 15 days.

HPLC graphs of *Commiphora wightii*. suspension culture extracts at 25µM, 50 µM, 75 µM,100µM and 125 µM.

The estimation of isolated secondary metabolites required the use of high pressure liquid chromatography (HPLC). Overall, the results of this study suggest that SA treatment can be an effective strategy for enhancing the production of Guggulsterone Z and other secondary metabolites in *Commiphora wightii*. Further studies are needed to elucidate the underlying mechanisms and optimize the conditions for SA treatment in order to maximize the production of Guggulsterone Z.

The study investigated the effect of Salicylic acid (SA) treatment as an elicitor on the accumulation of Guggulsterone Z steroid in suspension cultures. Different concentrations of Salicylic acid were applied, and the levels of Guggulsterone Z were analyzed using highperformance liquid chromatography (HPLC).

The HPLC analysis revealed that the accumulation of Guggulsterone Z in the suspension cultures was significantly increased at specific concentrations of Salicylic acid as seen in below tables.

The concentrations of 25 µM, 50 µM, 75 µM, and 100 µM of Salicylic acid resulted in significant increases in Guggulsterone Z accumulation compared to the control (194.70 ppm) of untreated suspension cultures. Specifically, the concentrations of 25 µM, 50 µM, 75 µM,100µM and 125 µM of Salicylic acid corresponded to Guggulsterone Z accumulations of 675.65 ppm, 856.58 ppm, 502.38 ppm,1110.17ppm and 397 ppm, respectively. The highest accumulation of Guggulsterone Z in suspension cultures was observed at the concentration of 100 µM of Salicylic acid, surpassing the control as shown in table 1.1,1.2,1.3,1.4,1.5,1.6 and 1.7.

These findings indicate that Salicylic acid, when used as an elicitor, plays a role in enhancing the concentration of the secondary metabolite Guggulsterone Z in the cells of Commiphora wightii. The results suggest that Salicylic acid treatment can be an effective strategy for increasing the production of Guggulsterone Z steroid in Commiphora wightii cultures.

S. No.	Retention Time	Area	Concentration (ppm)
1.	11.31	2945.05	194.7
2.	11.31	2892.56	191.23
3.	11.31	2981.65	197.12

Table 1.1 HPLC analysis of suspension cell culture Control

S. No.	Retention Time	Area	Concentration
1.	11.312	10131.3	675.65547
2.	11.312	10095.76	673.28
3.	11.312	10171.78	678.35

Table 1.2 HPLC analysis of suspension cell culture 25 µM

S. No.	Retention Time	Area	Concentration
1.	11.312	12834.7	856.58262
2.	11.312	12785.1	853.27
3.	11.312	12869.61	858.91

Table 1.3 HPLC analysis of suspension cell culture 50 µM

S. No.	Retention Time	Area	Concentration
1.	11.317	7542.28	502.38248
2.	11.317	7513.3	500.45
3.	11.317	7639.11	508.83

Table 1.4 HPLC analysis of suspension cell culture 75 µM

S. No.	Retention Time	Area	Concentration
1.	11.319	16623.8	1110.17164
2.	11.319	16780.42	1120.63
3.	11.319	16458.33	1099.12

Table 1.5 HPLC analysis of suspension cell culture 100 µM

S. No.	Retention Time	Area	Concentration
1.	11.304	5972.4	397.31731
2.	11.304	6059.88	403.13
3.	11.304	5917.23	393.64

Table 1.6 HPLC analysis of suspension culture 125 µM

S. No	STANDARD CONCENTRATION (PPM)	AREA	RETENTION TIME
1	1	45.27123	11.314
2	10	227.42294	11.314
3	20	466.95285	11.314
4	50	957.78363	11.314
5	100	1897.39648	11.314

Table 1.7: Overview table of calibration peaks of Guggulsterone Z

Chromatogram

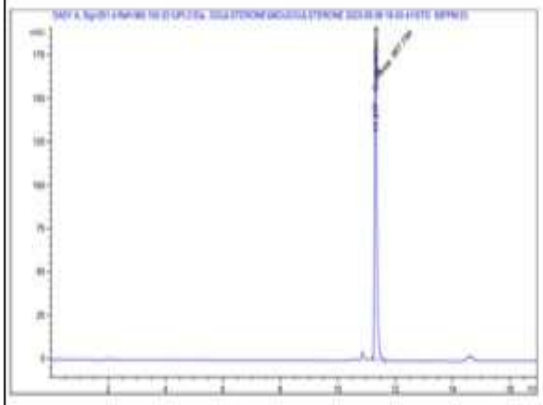


Fig 3.1: HPLC analysis of a Guggulsterone Z standard (Table 2)

Chromatogram

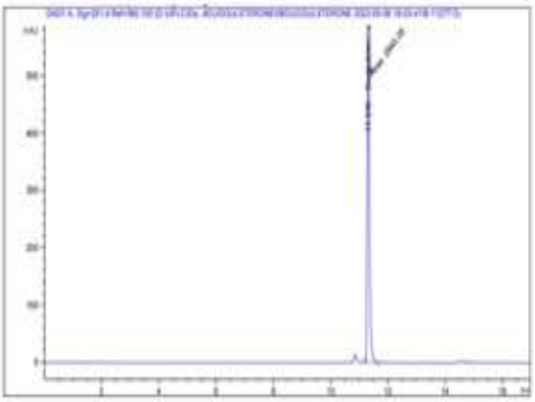


Fig 3.2: HPLC analysis of suspension culture control µM (Table 1)

Chromatogram

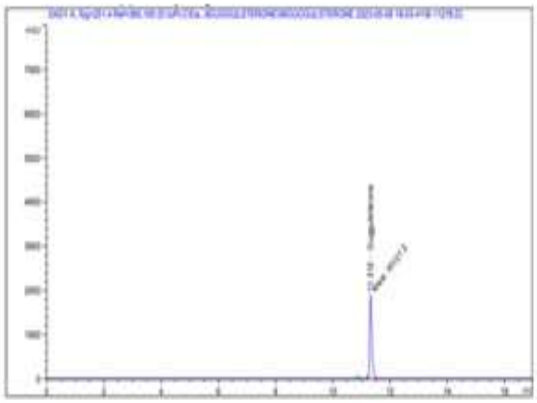


Fig 3.3: HPLC analysis of suspension culture 25µM (Table 1)

Chromatogram

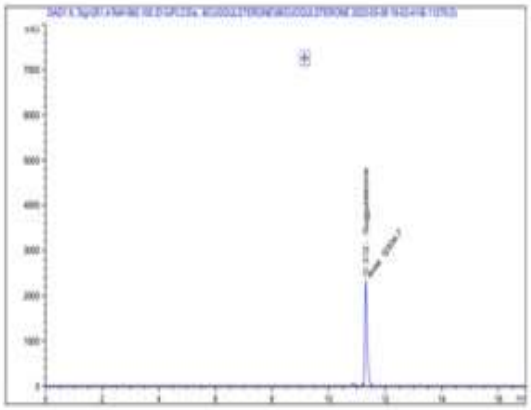


Fig 3.4: HPLC analysis of suspension culture 50µM (Table 1)

Chromatogram

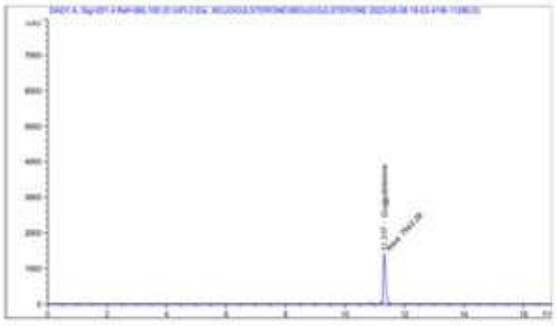


Fig 3.5: HPLC analysis of suspension culture 75µM(Table 1)

Chromatogram

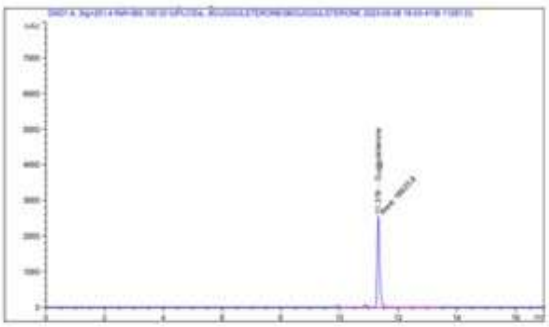
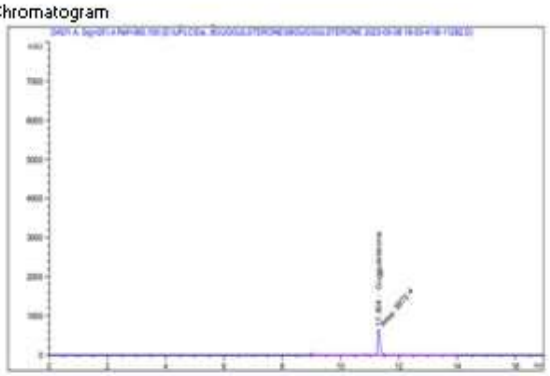


Fig 3.6: HPLC analysis of suspension culture 100µM (Table 1)

Chromatogram



3.7: HPLC analysis of suspension culture 125µM (Table 1)

Chromatogram

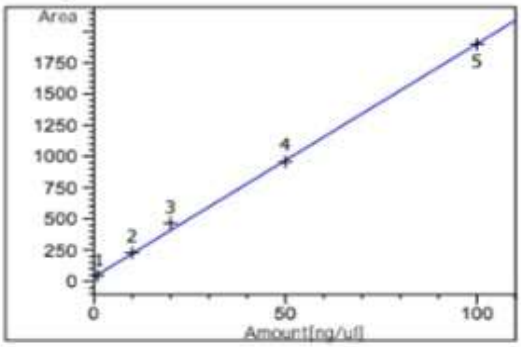
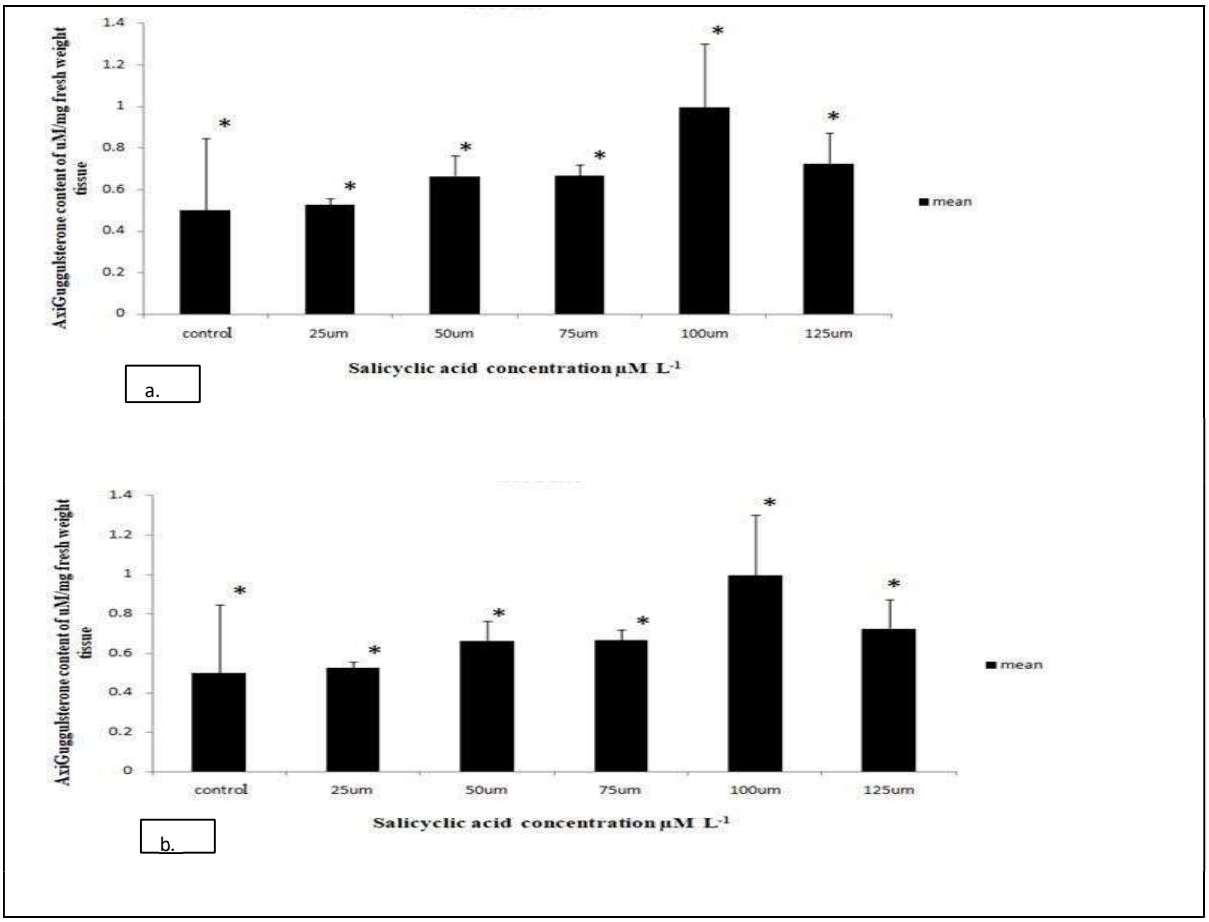


Fig 3.8: Callibration curve (Peak sum linear curve) (Table 1.2)


AxiGuggulsterone content of uM/mg fresh weight tissue
1.4
1.2
1
0.8
0.6
0.4
0.2
0
control
25um
50um
75um
100um
125um
mean
Salicyclic acid concentration µM L-1
a.
AxiGuggulsterone content of uM/mg fresh weight tissue
1.4
1.2
1
0.8
0.6
0.4
0.2
0
control
25um
50um
75um
100um
125um
mean
Salicyclic acid concentration µM L-1
b.

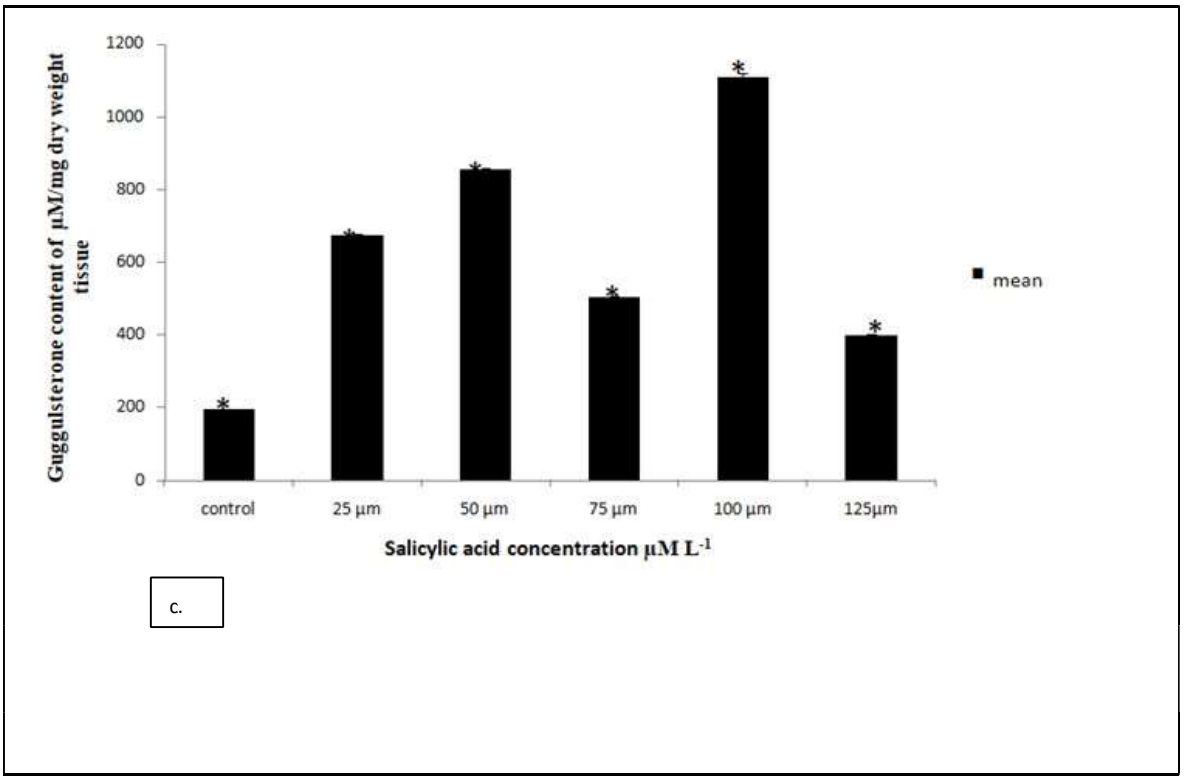


Figure 3.9 a)Fresh weight callus of *Commiphora wightii* to obtain guggulsterone content underneath Salicyclic acid concentration values be means of three replicates in adition to bar explain standard deviation(SD)by performing Student t test and statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control(*p<0.01).b) Dry weight callus of *Commiphora wightii* to obtain guggulsterone content underneath Salicyclic acid concentration values be means of three replicates in adition to bar explain standard deviation(SD) by performing Student t test and.statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control(*p<0.01).c) Guggulaterone content of *Commiphora wightii* to obtain guggulsterone content underneath Salicyclic acid concentration values be means of three replicates in adition to bar explain standard deviation(SD) by performing Student t test and.statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control(*p<0.01).

Conclusion:

Salicylic acid (SA) has been shown to be an effective elicitor for enhancing the production of Guggulsterone Z in Commiphora wightii into suspension cultures. SA treatment resulted in a significant increase in Guggulsterone Z production, with the optimal concentration of 100µm. The effect of SA on Guggulsterone Z production was also time-dependent, with maximum production observed at specific time points at 15th day. It has been suggested that SA induces the expression of genes involved in secondary metabolite biosynthesis.

Overall, the use of SA as an elicitor for Guggulsterone Z production in *Commiphora wightii* into suspension cultures has immense potential for the pharmaceutical and nutraceutical industries. The findings from this study can serve as a basis for further optimization of SA treatment conditions to maximize the production of Guggulsterone Z and other secondary metabolites in medicinal plants.

Acknowledgement

Authors are thankful to Hon'ble Chairperson JV'n Mithilesh Garg and Hon'ble Founder and Advisor JV'n Vedant Garg, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan) for their kind cooperation, encouragement, and providing the facilities of University Innovation Center and other laboratories.

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