

IN VITRO MICROPROPAGATION, PHYTOCHEMICAL PROFILING AND MOLECULAR DOCKING OF *GUAIAECUM OFFICINALE* L. AGAINST A TARGET PROTEIN ASSOCIATED WITH INFLAMMATION

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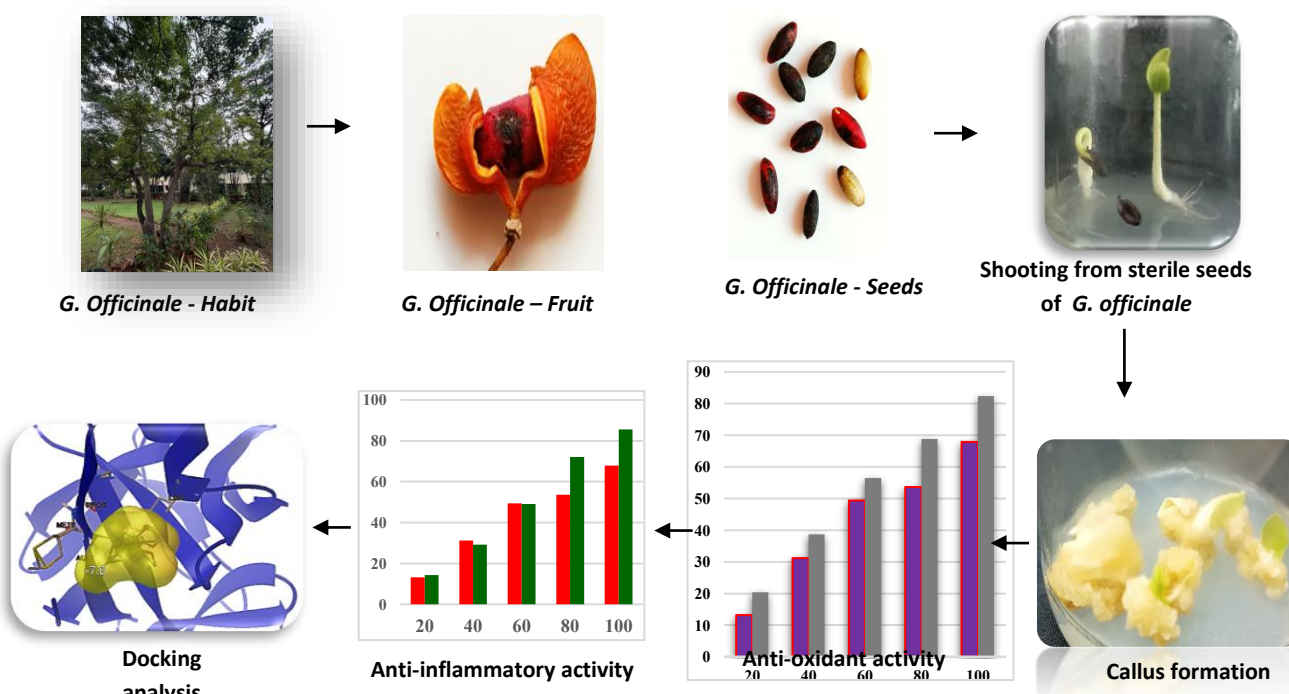
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ABSTRACT

Guaiacum officinale L., commonly known as lignum vitae, is a medicinal plant belonging to the family Zygophyllaceae and is recognized for its potential therapeutic properties. The plant is a promising source of phytochemicals that may possess antioxidant, anti-inflammatory and other biological activities. The present study aims to investigate the callus formation by micropropagation and its anti-inflammatory property by molecular docking analysis. Qualitative phytochemical analysis showed the presence of alkaloids, phenols, carbohydrates, flavonoids, proteins, while advanced analytical techniques such as gas chromatography-mass spectrometry help to identify individual chemical constituents present. Furthermore, molecular docking studies were employed to investigate the interactions of selected phytochemical compound with inflammation-associated protein targets and to predict their potential biological relevance. The integration of phytochemical characterization, biological evaluation, and computational analysis may provide valuable insights into the therapeutic potential of *Guaiacum officinale*.

KEYWORDS: *Guaiacum officinale*, phytochemical screening, anti-inflammatory activity, molecular docking, medicinal plants.

Graphical Abstract



INTRODUCTION

Medicinal plants have long been recognized as valuable therapeutic resource in traditional health care system and continue to play an important role in modern pharmacological research (Pendyala *et al.*, 2019). *Guaiacum officinale* L. commonly called as Lignum vitae belongs to the family Zygophyllaceae with its origin from West Indies, South America and also commonly found in Thailand (Pikulthong *et al.*, 2025; Sarkar *et al.*, 2014). This is a slow growing tree, reaching to a height of about 9 to 12 meters with hard wood, rough bark and dense rounded foliage (Pendyala *et al.*, 2019).

Phytochemical studies have revealed important constituents such as L-arginine, sitosterol, oleanolic acid, guaianins and guaiacins which are known for multiple pharmacological activities. Extract of the plant demonstrated antibacterial, anti-inflammatory, antioxidant and anti-HIV properties, while different plant parts are traditionally used as diuretics, tranquilizers and also in reproductive medicine (Azeem *et al.*, 2021; N & Jagannath, 2024). *Lingum vitae* was listed in the endangered species in IUCN 2019 due to over utilization of the species due to its various medicinal properties (Zhao *et al.*, 2017).

Conventional propagation of *Guaiacum officinale* through seeds is limited due to poor germination, seed dormancy, and slow growth rate. Therefore, plant tissue culture has emerged as an effective biotechnological approach for rapid multiplication, conservation, and sustainable utilization of endangered medicinal plants (World Conservation Monitoring Centre, 1998). Tissue culture techniques involve the aseptic cultivation of plant cells, tissues, or organs on nutrient media under controlled environmental conditions (Fay, 1992). However, research findings on conservation of the plant by micropropagation technique can be carried out using sterile explants inoculated in MS medium containing different hormone concentrations (Subbaiyan *et al.*, 2015).

Different parts of the plant contain important phytochemicals such as lignans, flavonoids, saponins, and phenolic compounds that exhibit antioxidant, anti-inflammatory, antimicrobial, and therapeutic activities. However, overexploitation, habitat destruction and slow natural regeneration have significantly reduced its population, leading to its classification as a threatened species in several regions (Rao, 2004). Phytochemical screening combined with advanced analytical techniques provides detailed information regarding the chemical constituents present in medicinal plants. Gas Chromatography-Mass Spectrometry (GC-MS) is one of the most reliable analytical techniques used for the identification and characterization of volatile and semi-volatile compounds (Sparkman *et al.*, 2011, Riaz *et al.*, 2021).

In recent years, molecular docking has become an important computational approach in drug discovery and medicinal chemistry. Molecular docking predicts the binding interaction between phytochemicals and target proteins, thereby helping to evaluate the therapeutic potential of plant-derived compounds (Morris *et al.*, 2009). This *in silico* technique provides insights into binding affinity, hydrogen bonding and molecular interactions with disease-related proteins such as cyclooxygenase-2 (COX-2), acetylcholinesterase and α -glucosidase. Integrating phytochemicals profiling with molecular docking analysis facilitates the identification of promising lead molecules for pharmaceutical applications.

Therefore, the present study aims to investigate the callus extract phytochemical screening, antioxidant, anti-inflammatory activity and molecular docking analysis of bioactive compounds from *Guaiacum officinale*. The study is expected to contribute toward the conservation of this medicinal species and provide scientific evidence for its pharmacological potential through phytochemical and computational analyses.

METHODOLOGY

Preparation of MS medium

MS medium was prepared using sucrose 30%, 0.1% meso-inositol, macronutrients, micronutrients and required plant growth regulators. pH was adjusted using 1N HCl or NaOH. The medium was solidified by addition of 8% agar and autoclaved at 15 lb pressure for 15 min at 121°C. After inoculation, the culture vials were kept under 16/8 h (light/ dark) photoperiod at 25± 2°C and observed at required time

interval for any growth change in the explants (Murashige and Skoog, 1962).

Surface sterilization of explants

Healthy fruits of *Guaiacum officinale* plant were collected from the campus of Women's Christian College, Chennai (Fig. 1) and washed thoroughly under running tap water for half hour without damaging the fragile tissues, followed by sodium hypochlorite (1.0%) and mercuric chloride (0.5 %) under different time duration. The seed coats were excised carefully and the seeds were transferred to culture medium under aseptic conditions.



Fig.1. Habit of *Guaiacum officinale*

Shoot and Callus initiation: Sterilized seeds were inoculated on MS basal medium without hormones and medium supplemented with different concentrations of 2,4-D growth hormone (0.5-2.0 mg/L).

Extraction: Callus (5g) from *in vitro* leaf explant of *G. officinale* was extracted using 50 mL of methanol and filtered after 72 h soaking. The filtrate was condensed and the condensed sample of 3.2 g was used for further analysis.

Qualitative analysis

The formulated cream was tested for their presence of alkaloids (Abubakar & Haque, 2020), carbohydrates (Yadav & Agarwala, 2011), proteins (Narasimhan, 2014), phenolic compounds, flavonoids (Rao, 2016), saponins and glycosides (Gul *et al.*, 2017).

DPPH Radical Scavenging Assay

The antioxidant activity of the callus extract of *G. officinale* was evaluated using the DPPH Assay Szabo *et al.*, 2007. Different concentrations of the extract (20–100 µg/mL) were prepared and mixed with 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark for 30 min at room temperature, and the absorbance was measured at 517 nm. Quercetin was used as the standard antioxidant. The percentage inhibition of DPPH radicals was calculated using the following equation:

Scavenging effect % = {[control absorbance-sample absorbance]/control absorbance} *100

Anti-inflammatory assay

The anti-inflammatory activity of callus extract of leaves of *G. officinale* was assessed by *in vitro* HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline. To 1 mL of HRBC suspension, callus extract in different concentrations 20–100 µg/mL was added. The assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm (James *et al.*, 2009).

% of inhibition = {[control absorbance-sample absorbance]/control absorbance} *100

Docking Analysis

Molecular docking was performed for the plant compound with protein target of anti-Inflammatory. The docking of the ligands on the receptors was performed with Auto Dock Vina (Forli *et al.*, 2016). The crystal structure of the protein targets was obtained from the Protein Data Bank (PDB). The structure of the ligand target was processed by removing any docked ligands, and water molecules from the PDB crystal structure and also addition of polar hydrogen atoms. The PDBQT file of potential protein targets was generated from the PDB files using Auto Dock Tools (Morris *et al.*, 2009).

RESULT

Shoot Initiation: Healthy seeds from *G. officinale* plant were used for the study (Fig. 2). After sterilization techniques, the seeds were placed on the basal MS medium devoid of plant growth regulators.



Fig.2. Healthy seeds of *G. officinale*

After 7 days of incubation, slight initiation of shoot was observed. The shoots initiated were transferred to MS medium supplemented with BAP (1.0 and 2.0 mg/L). After 25th day of culture, well grown *in vitro* plantlets were observed in the medium containing BAP 2.0 mg/L (Fig. 3).

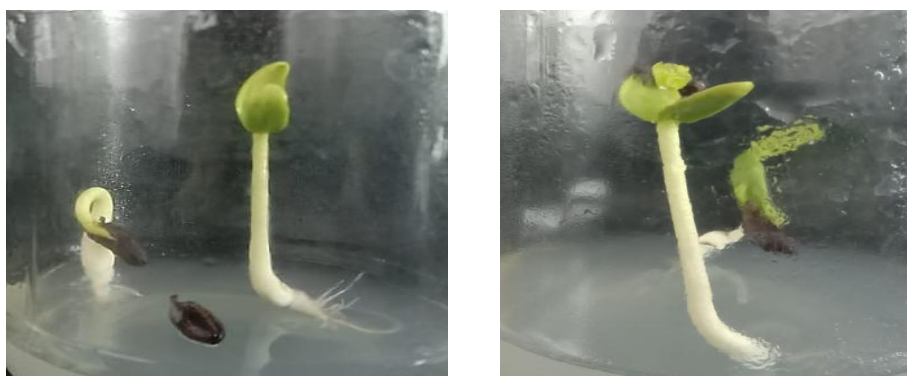


Fig. 3. *In vitro* plantlet formation from sterile seeds of *G. officinale* in MS medium

Callus Induction: The *in vitro* grown plantlets showed 86% callus growth on MS medium supplemented with 2,4-D 2.0 mg/L. Fresh weight of the callus was 2.74g and after drying the callus weight was recorded as 286 mg. Different concentrations and combinations showed minimal callus induction when compared which is represented in fig. 4, table.1. The callus obtained was collected and stored at room temperature in an amber bottle for further studies.

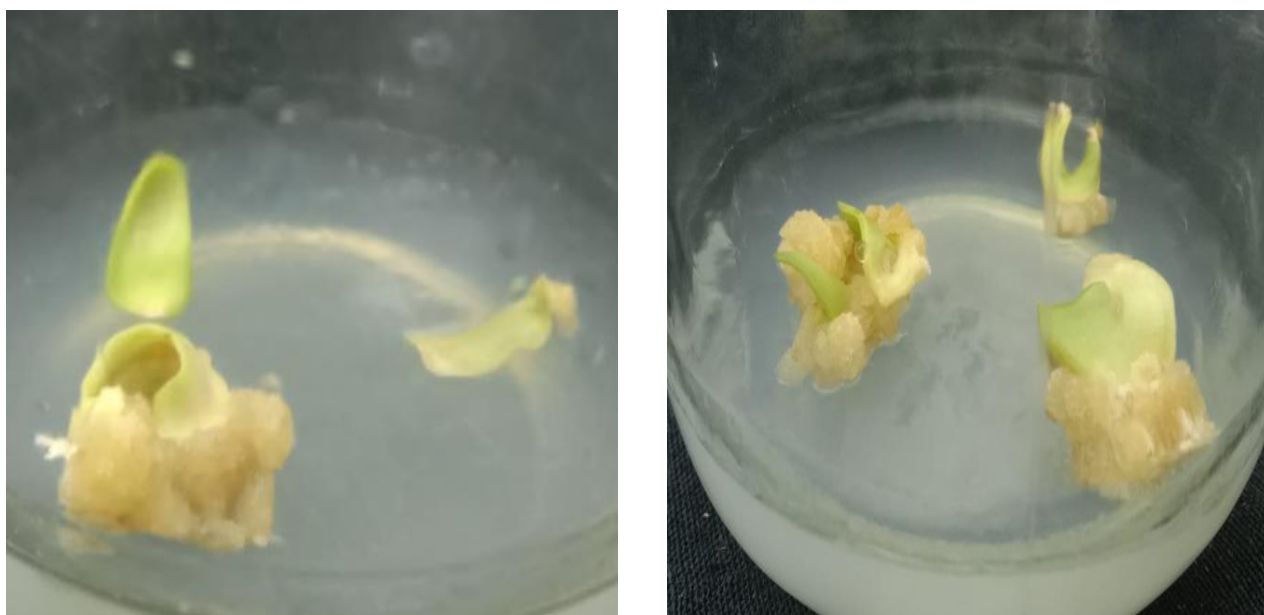


Fig. 4. Callus formation from in vitro grown plantlets of *G. officinale* in MS medium supplemented with 2,4-D (2.0 mg/L)

Hormones	Concentration (mg/L)	Callus Induction (%)
2,4-D	0.5	33.79±1.49
	1.0	56.41±2.17
	1.5	67.73±0.97
	2.0	86.30±0.79

Values represent mean ± S.D

Table 1.: Effect of different concentrations of 2,4-D on callus induction from leaf explants of *G. officinale*
Qualitative Analysis

The phytochemical analysis of callus extract of *G. officinale* showed the presence of alkaloids, phenols, carbohydrates, flavonoids, proteins.

Antioxidant activity

DPPH radical scavenging activity of the methanolic callus extract of *G. officinale* compared with standard quercetin at 517 nm. Maximum dose-dependent radical scavenging activity of 67.83% was observed at 100 µg/mL and compared with the standard showing maximum percentage of inhibition of at 100 µg/mL (Fig.5, Table.2). IC₅₀ value of methanolic callus extract was observed as 60 µg/mL and standard as 53 µg/mL.

Conc. µg/mL	% of inhibition	
	Callus Extract	Std- Quercetin
20	13.15	20.34
40	31.21	38.67
60	49.42	56.45
80	53.61	68.79
100	67.83	82.34

Table 2.: DPPH activity of callus induction from leaf explants of *G. officinale*

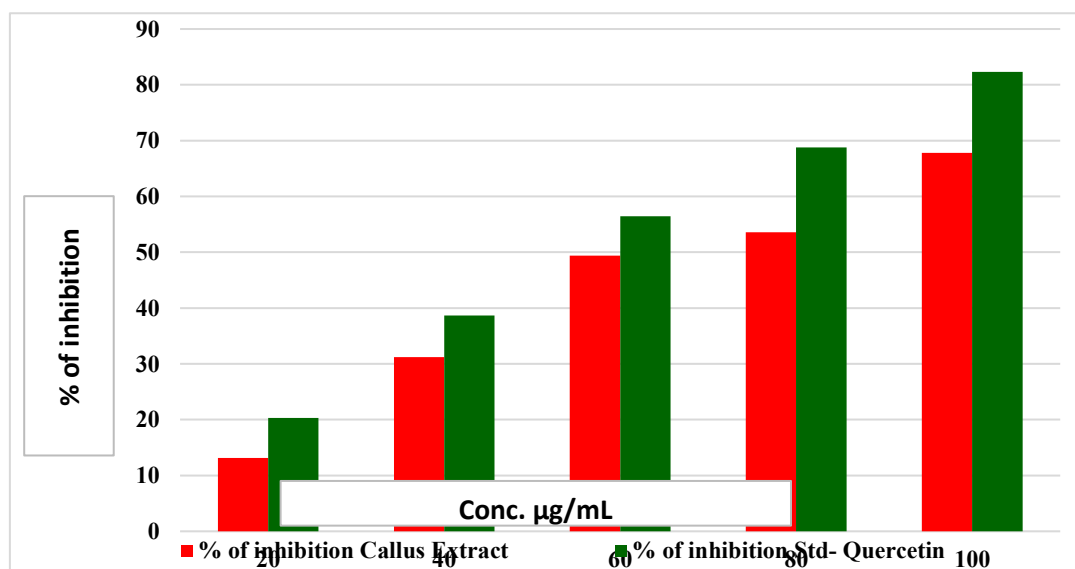


Fig.5. Antioxidant activity of methanolic callus extract of *G. officinale*

Anti-inflammatory activity

Anti-inflammatory activity of the methanolic callus extract of *G. officinale* by HRBC method was carried out. The extract showed significant anti-inflammatory activity with increasing concentration. Maximum inhibition was observed at 100 µg/mL, indicating potent anti-inflammatory potential comparable to the standard drug. The highest inhibition percentage was observed at 100 µg/mL with 79.5% inhibition, which was comparable to the standard Diclofenac sodium (Fig.6, Table.3).

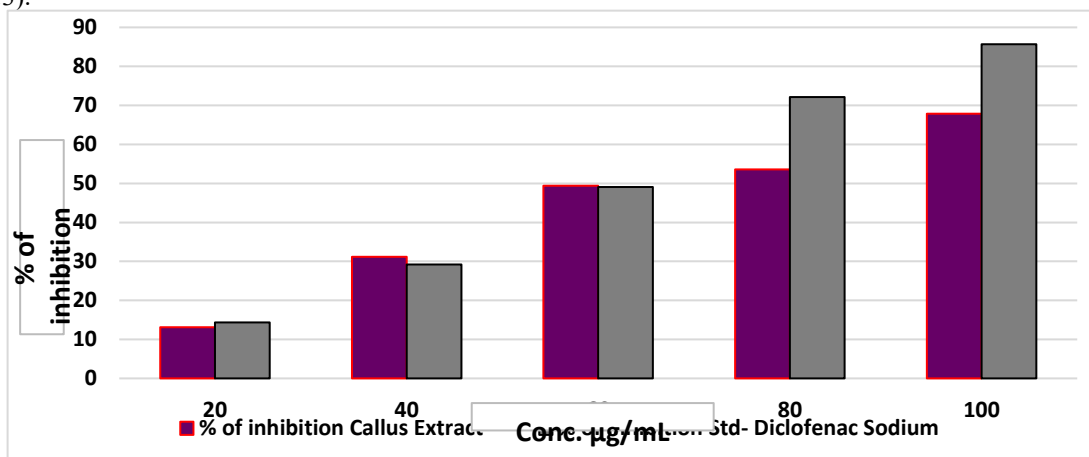


Fig.6. Anti-inflammatory activity of methanolic callus extract of *G. officinale*

Table 3.: Anti-inflammatory activity of callus induction from leaf explants of *G. officinale*

Conc. µg/mL	% of inhibition	
	Callus Extract	Std- Diclofenac Sodium
20	13.15	14.32
40	31.21	29.21
60	49.42	49.05
80	53.61	72.12
100	67.83	85.64

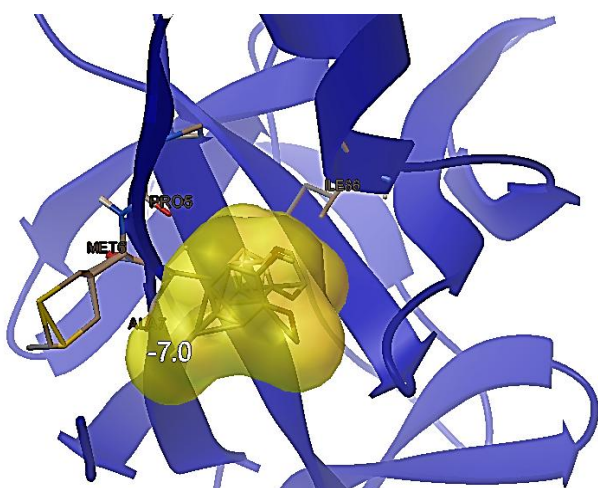
Docking analysis

The docking analysis of guaiacin with the inflammatory protein target (5BOW) exhibited a binding affinity of -7.0 kcal/mol, indicating a stable interaction between the ligand and protein. The ligand formed a carbon hydrogen bond with the GLY A:63 residue, which contributes to the stabilization of the ligand within the active site. Additionally, several amino acid residues including TYR A:9, TRP A:152, MET A:6, PRO A:5, ALA A:7, GLU A:87, LEU A:45, ILE A:86, and GLY A:64 were involved in van der Waals interactions, supporting ligand accommodation inside the binding pocket. These interactions suggest that guaiacin possesses favorable binding characteristics and may exhibit potential anti-inflammatory activity against the selected protein target (Table. 4, Fig.7 a,b).

Table 4. Molecular docking analysis of Guaiacin and 5BOW (PDBID) from *G. officinale*

Protein (ID)	Ligand	Binding Affinity (kcal/mol)	Binding Interaction Residues
5BOW	Guaiacin	-7.0	1. Carbon hydrogen bond interaction with GLY A:63. 2. Van der Waals interactions with TYR A:9, TRP A:152, MET A:6, PRO A:5, ALA A:7, GLU A:87, LEU A:45, ILE A:86, and GLY A:64 residues.

a. Binding interaction of guaiacin-A with Interleukin-38 (PDB ID: 5BOW)



b. 2D structure of the protein–ligand interaction profile

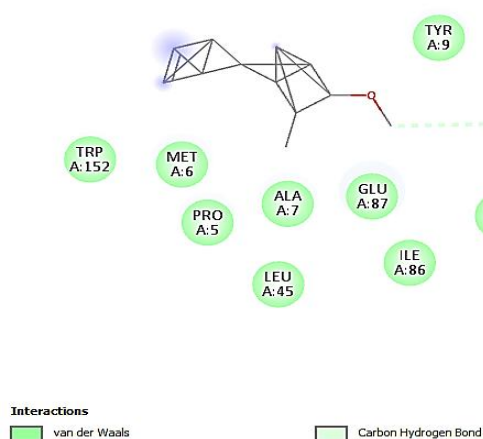


Fig.7. Molecular docking analysis of Guaiacin and 5BOW (PDBID) from *G. officinale*

DISCUSSION

The present investigation demonstrated the successful *in vitro* propagation and bioactivity evaluation of *Guaiacum officinale* L. through shoot induction, callus induction, antioxidant, anti-inflammatory and molecular docking studies. In the current study, healthy seeds cultured on basal MS medium without plant growth regulators exhibited initial shoot emergence within 7 days, indicating the suitability of MS medium for early seed germination and establishment. Subsequent transfer of shoots to MS medium supplemented with BAP resulted in enhanced shoot proliferation, with 2.0 mg/L BAP producing well-developed plantlets after 25 days. Similar findings were reported by recent studies on medicinal plants where BAP significantly promoted shoot organogenesis due to its cytokinin-mediated stimulation of cell division and apical meristem activity. The superior response observed at 2.0 mg/L suggests that this concentration provided an optimal hormonal balance for shoot multiplication in *G. officinale* (Bhatia, Sharma, & Verma, 2023)

Callus induction was highest (92%) on MS medium supplemented with 2,4-D at 2.0 mg/L, whereas other hormonal combinations showed comparatively lower responses. Auxins such as 2,4-D are widely recognized for their ability to induce cellular dedifferentiation and rapid callus proliferation in medicinal plant cultures. Recent reports on woody medicinal species demonstrated that 2,4-D at moderate concentrations effectively enhances friable callus formation and biomass accumulation, which is consistent with the present findings. The high percentage of callus induction observed in this study indicates the effectiveness of 2,4-D in promoting active cell proliferation and suggests its potential application in large-scale production of bioactive metabolites from *G. officinale* cultures (Pachipala *et al.*, 2023) (Suresh, Devi, & Rajalakshmi, 2023).

The methanolic callus extract exhibited strong dose-dependent antioxidant activity in the DPPH assay, with maximum radical scavenging observed at 100 µg/mL. This activity may be attributed to the presence of phenolics, flavonoids, and other secondary metabolites synthesized during in vitro culture conditions. Similar antioxidant potential has been reported in callus-derived extracts of medicinal plants where enhanced accumulation of bioactive compounds contributed to effective free radical scavenging activity. The observed IC₅₀ value further confirms the antioxidant efficiency of the extract when compared with standard quercetin, suggesting that callus cultures of *G. officinale* can serve as a sustainable source of natural antioxidants. (Priya, Kannan, & Arumugam, 2022) (Venkatesh, Rani, & Mahalakshmi, 2022).

The anti-inflammatory activity assessed by the HRBC membrane stabilization assay revealed significant inhibition of hemolysis with increasing concentration of the extract. Maximum inhibition of 79.5% at 100 µg/mL demonstrated strong membrane stabilization ability comparable to Diclofenac sodium. This effect may be associated with the capacity of phytoconstituents to inhibit lysosomal enzyme release and stabilize erythrocyte membranes under stress conditions (Venkatesh, Rani, & Mahalakshmi, 2022). Furthermore, molecular docking analysis supported the biological activity of guaiacin against the inflammatory protein target 5BOW, showing a binding affinity of -7.0 kcal/mol. The interaction of guaiacin with residues such as GLY A:63, TYR A:9, and TRP A:152 through hydrogen bonding and van der Waals interactions suggests stable ligand binding within the active site. These findings collectively indicate that *G. officinale* possesses promising antioxidant and anti-inflammatory potential, supported by both experimental and computational approaches (Singh, Kumar, & Yadav, 2024).

CONCLUSION

The current investigation effectively showcased the capabilities of *Guaiacum officinale* for in vitro propagation, callus formation and an initial assessment of its antioxidant and anti-inflammatory properties. The addition of 2.0 mg/L BAP to the MS medium promoted significant shoot growth, while 2.0 mg/L 2,4-D resulted in the highest callus induction rate of 92%, emphasizing the crucial role of plant growth regulators in refining tissue culture conditions. The methanolic extract from the callus exhibited a dose-dependent antioxidant effect in the DPPH assay, and the HRBC membrane stabilization test revealed a maximum hemolysis inhibition of 79.5% at a concentration of 100 µg/mL. In addition, molecular docking studies indicated a binding affinity of -7.0 kcal/mol for guaiacin with the inflammatory protein target 5BOW, providing computational support for a potential interaction with the target. Overall, these results imply that the callus of *G. officinale* exhibits considerable potential. These findings suggest that *G. officinale* callus cultures may serve as a promising source for investigating bioactive phytochemicals with antioxidant and anti-inflammatory potential. Further studies involving phytochemical characterization, compound isolation, mechanistic investigations and in vivo validation are necessary to establish the therapeutic relevance, safety and efficacy of the identified bioactivities.

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Conflict of Interest: The author declared no conflict of interest.

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