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Research Article

Phytochemical profiling and therapeutic potential of *Miliusa velutina* var. *deviyarina*: Antioxidant, anticancer, antimicrobial, and α-amylase inhibitory activities

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Abstract
Miliusa velutina Hook. f. and Thomson var. *deviyarina* (Annonaceae) is a lesser-known medicinal tree traditionally used by tribal and local communities in Maharashtra, India. However, its pharmacological profile has not been systematically characterized. This study aimed to provide a comprehensive in vitro evaluation of the antioxidant, α-amylase inhibition, antimicrobial, antibiofilm and anticancer activities of different extracts of this variety. Bark, leaf, fruit and flower powders were successively extracted with water, methanol, ethanol, ethyl acetate and alcohol–water mixtures. Antioxidant activity was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, α-amylase inhibition by α-amylase inhibition, antimicrobial and antibiofilm activities by agar well diffusion and biofilm inhibition assays, and anticancer activity by sulforhodamine B (SRB) assays on MCF-7 and Vero cell lines. Methanol, ethanol and ethyl-acetate bark and leaf extracts showed strong DPPH scavenging with low IC_{50} values comparable to quercetin and vitamin C. Green and ripe fruit extracts produced up to 89.57% α-amylase inhibition, while bark extracts showed moderate inhibition (37.50–61.25%). Bark extracts exhibited pronounced antimicrobial and antibiofilm effects, with maximum biofilm inhibition of 95.45% and zones of inhibition comparable to penicillin and streptomycin at 50 µg/mL. The ethyl-acetate bark extract displayed potent cytotoxicity toward MCF-7 cells (GI_{50} < 10 µg/mL) with relatively lower effects on Vero cells, approaching the activity of Adriamycin. These in *vitro* findings highlight *M. velutina* var. *deviyarina* as a promising source of bioactive extracts and provide a multi-assay dataset to guide future phytochemical characterization and *in vivo* evaluation of this understudied medicinal plant.

Keywords: Anticancer, α-amylase inhibition, Antimicrobial, Antioxidant, *Miliusa velutina* Hook. F. and Thomson var. *Deviyarina*

INTRODUCTION

Medicinal plants have long served as an essential resource for human healthcare, offering a natural and holistic approach to disease prevention and treatment (Baillon, 1868; Brandis, 1906; Rates, 2001). Unlike synthetic medicines that often target specific symptoms or single biochemical pathways, medicinal plants contain a diverse array of bioactive compounds that work synergistically, providing broad-spectrum pharmacological benefits (Newman and Cragg, 2016). The increasing interest in plant-based therapeutics stems from their efficacy, lower toxicity, and reduced side effects compared to synthetic drugs, along with their ability to target multiple molecular pathways, which minimizes the risk of resistance development (Atanasov *et al.*, 2015; Gan *et al.*, 2022; Caughlin *et al.*, 2015).

Traditional medicinal uses of *M. velutina* are found throughout Southeast Asia, where it is valued both as a food source and an herbal remedy, notably for treating inflammation and bacterial infections (The Son *et al.*, 2019). *M. velutina* is broadly distributed across several Asian nations, including India, Laos, Myanmar, Cambodia, Thailand, and Vietnam. This species is notable for

its wide array of bioactive compounds including sesquiterpenes, acetogenins, alkaloids, flavonoids, phenylpropanoids, steroids, and terpenoids which collectively contribute to its remarkable bioactivity (Van *et al.*, 2022). Recent studies have identified novel alkaloids and a drimane sesquiterpenoid, miliutine C methyl ester from the stems of *M. velutina*, which exhibited strong anticancer activity against various cancer cell lines, including MCF-7, HepG2, HeLa, and NCI H460 (Ngo *et al.*, 2024). Extracts of *M. velutina* have demonstrated notable antioxidant and anti-inflammatory properties, along with antibacterial activity against acne-causing bacteria (Phrompanya, 2024). The multifaceted importance of *M. velutina* thus spans both cultural heritage and modern biomedical science, underscoring its value as a resource for the development of new pharmaceuticals and health promoting natural products.

M. velutina is a deciduous tree, 8–11 m tall, with dark grayish-brown rough bark, fibrous and soft brown inside, 1.5–2.5 cm thick. Young parts and branchlets are densely grey to golden tomentose. Leaves are simple, alternate, variable in shape (broadly ovate to obovate), 10–30 × 4–12 cm, aromatic, sub-coriaceous, dark green and velvety above, paler and densely tomentose beneath, with 12–16 lateral veins per side; petiole stout, tomentose, 0.2–0.7 cm. Flowers are complete, bisexual, pale yellow, 0.8–1 cm across, borne extra-axillary or terminally. Sepals 3, ovate to triangular; petals 6 in two whorls outer like sepals, inner larger, ovate, tomentose outside. Stamens many, with extrorse anthers; carpels many, velutinous with 2 ovules each. Fruits are bluish-purple, ovoid or subglobose, tomentose, 1.5 × 2.5 cm, with 1–2 seeds per carpel (Eflora of India, 2007)

M. velutina var. *deviyarin*a a lesser-known member of the Annonaceae family, was first described by Rajendran *et al.* (2003) in southern India, with its occurrence in Maharashtra being documented a decade later (Deshmukh *et al.*, 2013). This plant exhibits distinct morphological features, including bisexual, pale-yellow flowers that occur extra-axillary or terminally in clusters. The fruit, a monocarp, is bluish-purple upon ripening and contains one or two seeds, with a flowering period extending from March to May, followed by fruiting from June to October (Deshmukh *et al.*, 2013).

The aim of this study was to scientifically evaluate the therapeutic potential of the plant**,** integrating traditional ethnobotanical knowledge with contemporary scientific analyses. Specifically, the research assessed the efficacy of plant extracts against clinically relevant microbial pathogens and its potential to inhibit biofilm formation. The antioxidant capacity of the plant was determined by assessing its ability to mitigate oxidative stress. Furthermore, the study aimed to explore its potential anticancer effects through examination of cytotoxicity and underlying mechanisms in cancer cells. The investigation also included an assessment of α-amylase inhibition properties by examining the plant's impact on key processes related to glucose metabolism. Through these multifaceted evaluations, the research seeks to generate scientific evidence supporting the traditional therapeutic use of the plant and its potential for integration into contemporary healthcare strategies.

MATERIALS AND METHODS

Collection of plant material

The plant *M. velutina* var. *deviyarina* was collected from its natural habitat in Maharashtra, India and authenticated by a taxonomist and the plant herbarium was deposited to St. Xavier's College herbarium section, Mumbai.

The bark, leaf, stem, flowers, ripe and unripe fruits of *M. velutina* var. *deviyarin*a were airdried, powdered, and subjected to extraction using solvents in decreasing order of polarity: water, ethanol, methanol, ethyl acetate, and benzene. A 5 g sample of each plant material was added to 100 mL of solvent and left to stand for 48 hours with intermittent shaking for optimal extraction. For the alcohol water extract, alcohol and water were used in a 1:1 proportion. The extracts were centrifuged to remove any insoluble residues, and the supernatant was collected and evaporated to concentrate the extract. The final product was stored at 4°C to preserve its stability for future use as flower and fruits are seasonal and location of plant is limited to some parts of Maharashtra, India (Vasanthi *et al.*, 2022; Kausar *et al.*, 2023; Rajendran *et al.*, 2003).

Antioxidant activities by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay for antioxidant activity was performed following a modified method of Sharma and Bhat (2019) to evaluate the antioxidant potential of the extracts. A freshly prepared 0.1 mM DPPH solution was prepared in methanol and protected from light. Each extract stock solution was serially diluted in methanol to obtain concentrations of 500, 250, 100, 50, and 10 µg mL^{-1}. An aliquot of 1.0 mL of each extract concentration was mixed with 1.0 mL of DPPH solution and vortexed thoroughly. Methanol mixed with DPPH served as the control, while methanol alone was used as the blank.

Quercetin, a common antioxidant was used as a standard for comparison. After 30 minutes of incubation in the dark, the mixtures were measured spectrophotometrically at 517 nm. DPPH scavenging activity was expressed as a percentage using the following formula:

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Inhibition (%) = (Ac−At) / Ac ×100 Eq.1

where Ac and At represent the absorbances of the con-

trol and the sample, respectively (Kejariwal , 2014, 2016, 2021). The IC50 was calculated from the graph plotting scavenging percentage against test sample concentration (µg/mL).

Alpha-amylase inhibitory assay

The α-amylase inhibitory assay was performed following the method described by Rana *et al.* (2020). Briefly, 50 µL of plant extract (5 g in 100 mL of solvent) or acarbose (used as a standard α-amylase inhibitor), prepared in 0.2 M phosphate buffer (pH 7.0), was mixed with 50 µL of α-amylase enzyme solution (3 U/mL) and incubated at 37 °C for 15 min. Subsequently, 50 µL of starch solution (2 mg/mL) was added, and the reaction mixture was further incubated for 15 min. The reaction was terminated by the addition of 200 µL of HCl, followed by 300 µL of 1% iodine solution. Absorbance was measured at 660 nm using a spectrophotometer. The percentage inhibition of α-amylase activity was calculated using the following equation

Inhibition (%) = [1−Abs sample/Abs control] ×100 Eq.2

Biofilm inhibition assay

Glass test tubes were filled with 5 mL of sterile nutrient broth and inoculated with 20 µL of an overnight culture of *Staphylococcus aureus* (OD600 ≈ 0.6). Test samples (100 µL of extract at the indicated concentration series) were added. Negative control: bacterial culture + ethyl acetate as solvent at the same final volume as test wells. Positive control: bacterial culture + 100 µL of 50 µg/mL Penicillin G.Tubes were incubated without shaking at 37°C for 24 h. Biofilms were washed twice with sterile PBS, stained with 0.1% crystal violet for 15 min, washed, dye solubilized in 30% acetic acid and absorbance measured at 590 nm. Inhibitory rate (%) was calculated as per the formula:

Inhibitory rate (%) = [1−(OD (Sample)/OD (Positive control)] ×100 Eq.3

Where OD (Positive control) is untreated bacterial culture (vehicle only), unless otherwise specified. Each concentration was assayed in triplicate (technical replicates) and the experiment repeated on three independent days (biological replicates). A higher percentage of inhibition indicates a greater reduction in biofilm biomass (Mahmud *et al.*, 2023). The IC_{50} value was defined as the concentration required to inhibit 50% of biofilm formation.

Antimicrobial activity

Antibacterial activity was assessed using the Agar Cup Diffusion Method, as described by Nadeem *et al.* (2021). Mueller-Hinton agar plates were inoculated with bacterial suspension (0.5 McFarland). Wells (6 mm) were punched and 50 µL of test extract (50 µg/mL), solvent control (negative control — same solvent used to dissolve extract, e.g., methanol or water), and standard antibiotic solutions (positive controls: penicillin 50 µg/mL and streptomycin 50 µg/mL) were added. Plates were incubated at 37°C for 18–24 h and zones of inhibition measured in mm. Each test was performed in triplicate (technical) and repeated on three independent days (biological replicates). For each extract, three independent replicate measurements were used to calculate the mean inhibition zone and its associated standard error (SE = SD / √3). The mean ± SE values were plotted using bar charts generated in Python (matplotlib).

Cell line and cell culture

Human breast adenocarcinoma (MCF-7) cells and normal monkey kidney epithelial (Vero) cells from the Anti-Cancer Drug Screening Facility (ACDSF) at ACTREC at ACTREC (Tata Memorial Centre), Kharghar, Navi Mumbai, were used for cytotoxicity evaluation. Cells were maintained in appropriate culture medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine, and incubated at 37 °c in a humidified atmosphere containing 5% co_2.

Experimental procedure for sulforhodamine B (SRB) cytotoxicity assay

For cytotoxicity screening, cells were seeded at a density of 5,000 cells per well in 96-well microtiter plates and allowed to attach for 24 h. Bark extracts prepared using ethanol, ethyl acetate, and hydroalcoholic (ethanol: water, 70:30 v/v) solvent systems were used for the assay. Stock solutions of the extracts were prepared at 100 mg/mL, diluted to 1 mg/mL, and stored at −20 °C until use. Prior to treatment, extracts were further diluted with culture medium to obtain final concentrations of 10, 20, 40, and 80 µg/ml. Following 48 h of exposure, cells were fixed with 10% trichloroacetic acid (TCA), stained with 0.4% sulforhodamine B (SRB), and excess dye was removed by washing. The protein-bound dye was solubilized, and absorbance was measured at 540 nm using a microplate reader. Percent cell growth was calculated relative to untreated controls, and the GI_{50} value was defined as the concentration required to inhibit 50% of cell growth, according to the method described by Mishra *et al.* (2022).

Statistical analysis

All experiments were performed in triplicate technical replicates, and data are presented as mean ± standard deviation (SD). The IC_{50} and GI_{50} values were calculated using nonlinear regression analysis based on dose–response data in Microsoft Excel. Statistical comparisons between extracts were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p< 0.05$ considered statistically significant (Singh *et al.*, 2023). Non-linear regression and ANOVA were conducted in GraphPad Prism v10.

RESULTS

Antioxidant activities by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay of *M. velutina* var. *deviyarina*

The DPPH radical scavenging assay revealed marked variations in antioxidant activity among different plant parts and extraction solvents. Overall, leaf extracts exhibited the highest antioxidant potential, followed by stem and bark extracts. Methanolic leaf extracts showed exceptionally strong activity with IC_{50} values below 10 µg/mL, while ethanolic and ethyl acetate leaf extracts demonstrated comparable potency, indicating a high abundance of polar antioxidant compounds in leaf tissue. The aqueous leaf extract showed moderate activity, whereas the benzene extract was weak, reflecting poor extraction of active constituents by non-polar solvents. Stem extracts displayed moderate to strong antioxidant activity, with the methanolic extract again showing the lowest IC_{50}, followed by ethanol, ethyl acetate, and water extracts, while benzene extracts exhibited reduced efficacy. In contrast, bark extracts generally showed lower antioxidant capacity, however, the methanolic bark extract retained notable activity, followed by aqueous and ethanolic extracts, whereas ethyl acetate and benzene extracts were largely inactive. Collectively, the antioxidant efficiency followed the order leaf > stem > bark and methanol > ethanol ≈ ethyl acetate > water > benzene, underscoring the importance of solvent polarity. Statistical analysis further supported these findings, as the IC50 values for methanol and ethanol extracts were significantly lower ($p < 0.05$) than those of other solvent extracts, indicating superior free radical scavenging capacity. Tukey's post-hoc analysis revealed a significant difference between the methanol extract and the ethyl acetate and benzene extracts, confirming the higher efficiency of the methanol extract. These results suggest that methanol and ethanol extracts, particularly from the bark, could serve as potent natural antioxidants (Fig. 1).

Antidiabetic activity test (α-amylase inhibition assay) of *M. velutina* var. *deviyarina*

The α-amylase inhibitory activity of different extracts of *M. velutina* var. *deviyarina* was evaluated to assess their potential to modulate carbohydrate digestion. The results showed marked variation in inhibition among the extracts. The green fruit extract exhibited the highest inhibitory activity, with inhibition ranging from 79.14% to 89.57%, followed by the ripe fruit extract (77.36%–88.96%) and the dried fruit extract (72.45%–89.57%). The bark extract showed moderate inhibitory activity, with inhibition values ranging from 37.50% to 61.25%, with the maximum inhibition recorded at 61.25%. In contrast, the leaf extract exhibited low inhibitory activity (12.50%–21.25%), while the flower extract showed negligible inhibition under the tested conditions.

Statistical analysis using one-way ANOVA indicated a highly significant difference in α-amylase inhibition among the different extracts ($F = 33.84$, $p < 0.001$). Post-hoc Tukey's analysis further confirmed that the green fruit extract had significantly higher inhibition compared to the other extracts ($p < 0.05$). The results indicate that the green fruit extract shows the highest potential for α-amylase inhibition, followed by ripe and dried fruit extracts, whereas the bark extract demonstrated moderate efficacy. Minimal inhibition observed in the leaf and flower extracts indicates their limited potential for α-amylase inhibition. These findings are visually represented in Fig. 2, which highlights the percentage inhibition across different extracts and conditions. Since the assay was performed at a single concentration, IC_{50} values were not determined.

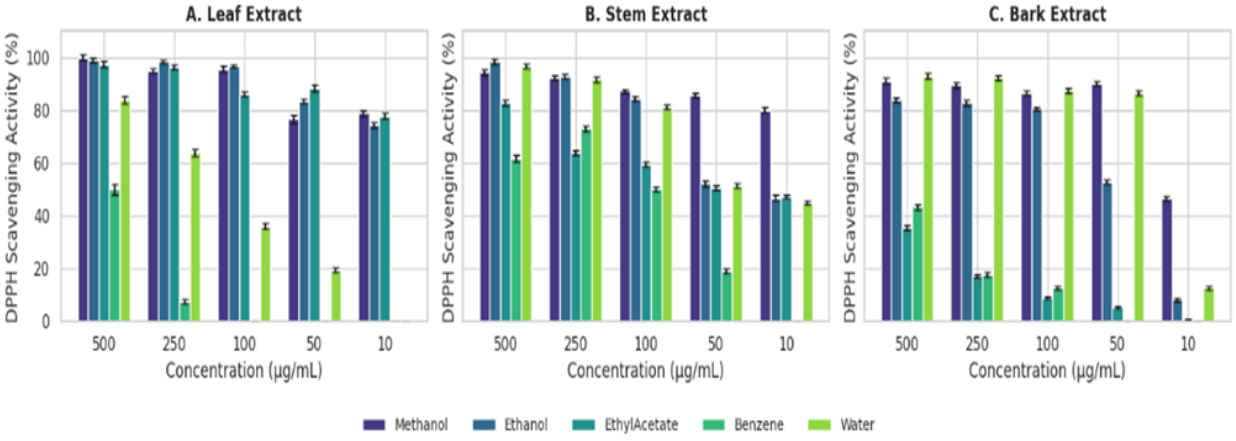


Fig. 1. *DPPH radical-scavenging activity of (A) leaf, (B) stem, and (C) bark extracts of M. velutina var. deviyarina at different concentrations (10–500 µg/mL). Bars represent mean ± SD (n = 3). Methanol, ethanol, ethyl acetate, benzene, and water extracts are compared. Methanolic and ethanolic extracts exhibited the highest antioxidant potential in a concentration-dependent manner*

Biofilm inhibition assay of *M. velutina* var. *deviyarina*

The biofilm inhibition activity of ethyl acetate extracts from the bark, leaf, and fruit of M. velutina var. deviyarina was evaluated against Staphylococcus aureus using varying concentrations of the extracts (500, 250, 125, 62.6, 31.25, and 15.6 µg/mL). In this assay, the negative control contains only sterile Nutrient broth without bacterial culture, whereas the positive control consists of the bacterial culture grown in the same medium to represent uninhibited biofilm formation. The results demonstrated that the bark extract exhibited the highest biofilm inhibition, with an inhibition rate of 95.45% at 500 µg/mL, which remained significant at 250 µg/mL (90.91%). A sharp decline was observed at 125 µg/mL, with inhibition dropping to 45.45%, while at lower concentrations (62.6 µg/mL and below), the inhibition reduced drastically to 18.18% and eventually 0% at the lowest concentration (Fig. 3).

In contrast, the green and dried fruit extracts exhibited moderate inhibition at 500 µg/mL and 250 µg/mL, both recording an inhibition of 36.36%. The inhibition decreased to 13.64% at 125 µg/mL and 9.1% at 62.6 µg/mL, while no inhibition was observed at 15.6 µg/mL. Similarly, the ripe fruit extract demonstrated lower inhibitory effects, peaking at 22.73% at 500 µg/mL, and declining gradually to 4.55% at 31.25 µg/mL. The leaf extract showed the least inhibitory effect, with a maximum inhibition of 18.18% at 500 µg/mL and progressively declining with decreasing concentration.

One-way ANOVA analysis revealed no statistically significant differences in biofilm inhibition across the extracts at different concentrations ($F = 2.18$, $p = 0.101$). Tukey's post-hoc test further confirmed that there was no significant difference between the extracts in terms of inhibition efficacy at the tested concentrations. This indicates that while the bark extract consistently showed higher inhibition, the differences observed among other extracts were not statistically significant.

The bark extract demonstrated the most effective biofilm inhibition potential, indicating strong anti-biofilm activity against *S. aureus* (IC_{50} = 160 µg/mL). The green and dried fruit extracts exhibited moderate inhibitory activity, while ripe fruit and leaf extracts showed comparatively lower inhibition. The results suggest that higher concentrations are required for optimal biofilm suppression, with bark extract being the most promising candidate for further antimicrobial research. These findings are visually represented in Fig. 3, highlighting the inhibition trends across various extracts.

Antimicrobial activity of *M. velutina var. deviyarina*

The antimicrobial potential of leaf, stem, and bark extracts of *M. velutina* var. *deviyarina* was evaluated against six bacterial strains to evaluate the antimicrobial activity of *M. velutina* var. *deviyarina* using the agar cup diffusion method. The strains were biochemically identified and maintained at the Department of Microbiology, Swami Vivekanand College, Mumbai. The extent of antimicrobial activity varied depending on the plant part and solvent used, as illustrated in Fig. 4 and 5.

Among the tested plant parts, bark extracts exhibited the most pronounced antimicrobial activity, followed by stem and leaf extracts. Methanolic and ethanolic bark extracts produced larger zones of inhibition against both Gram-positive and Gram-negative bacteria, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The inhibition patterns observed for these extracts were comparable to those produced by the standard antibiotics for certain bacterial strains.Leaf extracts demonstrated moderate antibacterial activity, primarily in methanolic and ethanolic preparations, with observable inhibition against *Staphylococcus aureus* and *Proteus vulgaris*. In contrast, aqueous and ben-

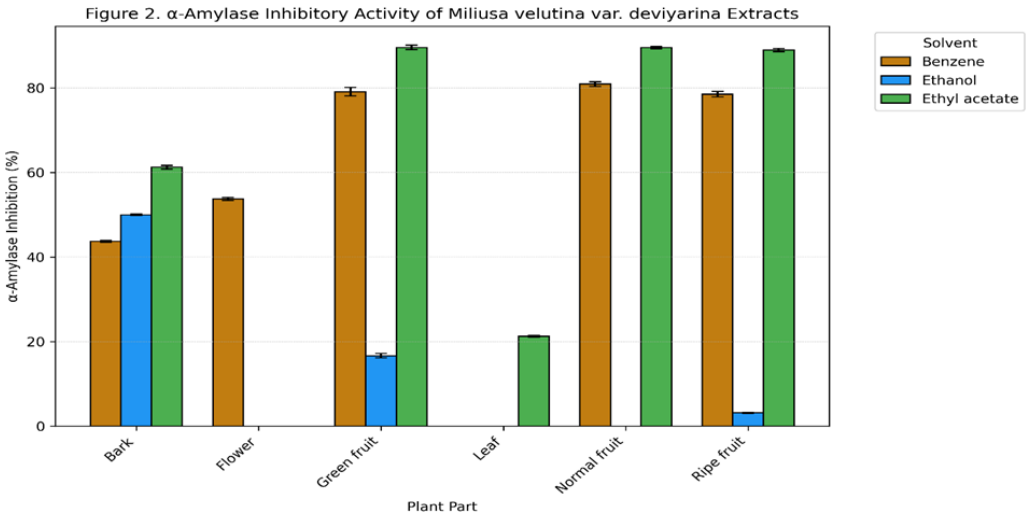


Fig. 2. α-Amylase inhibitory activity of different extracts of *M. velutina* var. *deviyarina*.

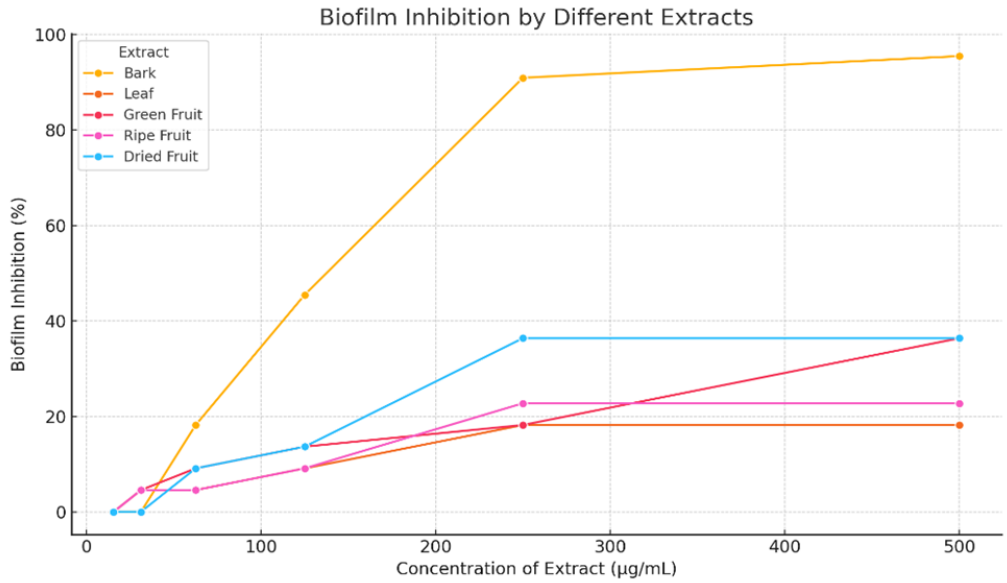


Fig. 3. *Biofilm Inhibition (Bark extract exhibiting significant antibiofilm activity against Staphylococcus aureus)*

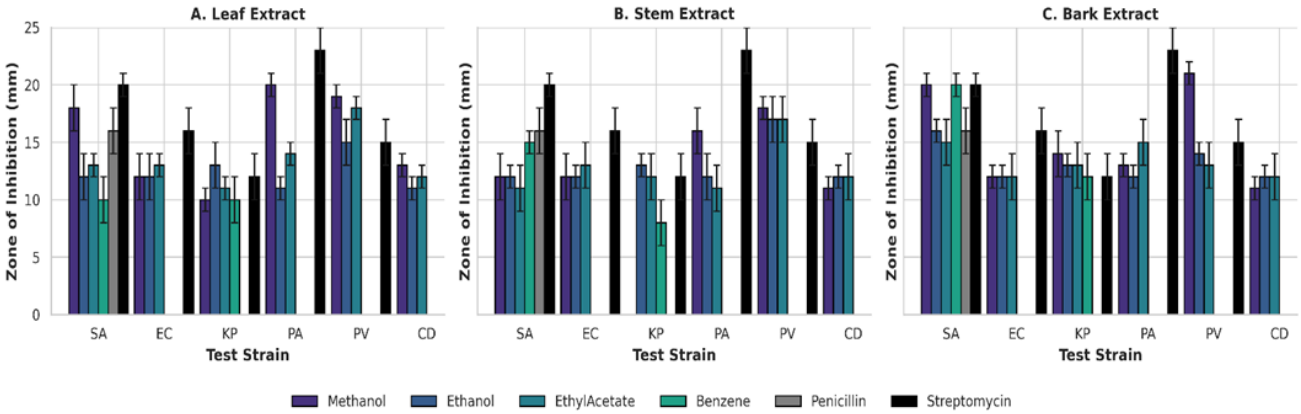


Fig. 4. *Quantitative representation of antimicrobial activity of leaf, stem, and bark extracts of M. velutina var. deviyarina against six bacterial strains determined by agar cup diffusion assay. Bars represent the mean zone of inhibition (mm ± SD, n = 3) for extracts prepared in different solvents. Penicillin and streptomycin were used as positive controls, while corresponding solvents without plant extract served as negative controls. SA, Staphylococcus aureus; EC, Escherichia coli; KP, Klebsiella pneumoniae; PA, Pseudomonas aeruginosa; PV, Proteus vulgaris; CD, Corynebacterium diphtheriae*

zene leaf extracts showed little to no inhibitory effect against most test organisms.Stem extracts exhibited selective antimicrobial activity, with methanol and ethanol extracts inhibiting the growth of *Staphylococcus aureus*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. However, activity against *Escherichia coli* and *Klebsiella pneumoniae* remained limited.

Across all plant parts, aqueous and benzene extracts consistently showed minimal or no antimicrobial activity, indicating poor extraction of bioactive compounds in these solvents. Ethyl acetate extracts showed intermediate activity against selected bacterial strains. Solvent controls did not exhibit any zones of inhibition, confirming that the observed antimicrobial effects were attributable to the plant extracts (Fig. 4 and 5).

Statistical analysis of inhibition zones using one-way ANOVA followed by Tukey's post-hoc test revealed significant differences ($p< 0.05$) among the solvent extracts for several test strains. Methanol and ethyl acetate extracts showed notably higher antibacterial activity compared to other extracts.

Cytotoxicity analysis using SRB Assay in *M. velutina* var. *deviyarina*

The cytotoxic potential of plant extracts was evaluated using the Sulforhodamine B (SRB) assay on MCF-7 (Human Breast Cancer Cell Line) and Vero (Normal Monkey Kidney Cell Line). The method involved treating cells with different concentrations (10, 20, 40, and 80 µg/mL) of the extracts, followed by fixation with trichloroacetic acid (TCA) and staining with 0.4% SRB. The absorbance was measured at 540 nm, and the

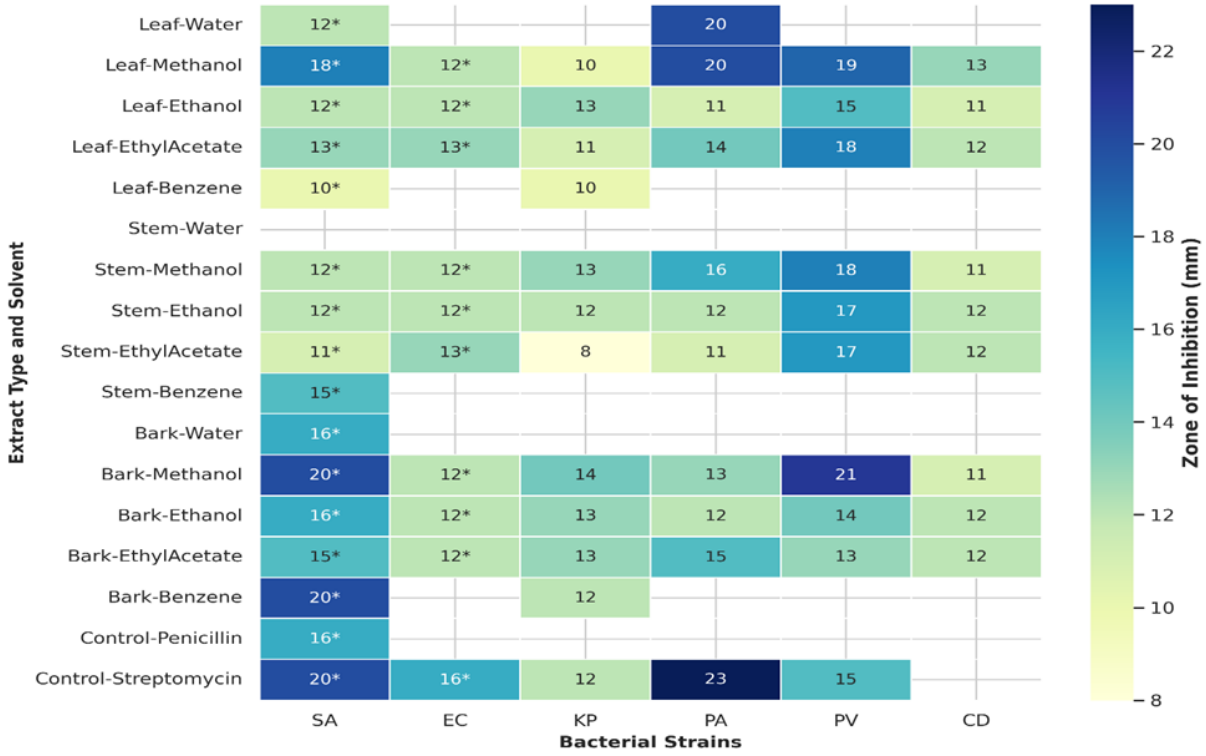


Fig. 5. *Comparative heatmap showing antibacterial activity (zone of inhibition, mm) of leaf, stem, and bark extractsincluding penicillin and streptomycin controls of M. velutina* var. *deviyarina* against *SA, Staphylococcus aureus; EC, Escherichia coli; KP, Klebsiella pneumoniae ; PA, Pseudomonas aeruginosa; PV, Proteus vulgaris; CD, Corynebacterium diphtheriae. Higher color intensity indicates stronger antibacterial effect. Numerical values represent mean inhibition zones, and asterisks (*) denote extracts showing statistically significant differences (p*< 0.05) according to one-way ANOVA followed by Tukey's HSD test

percentage of cell growth inhibition was calculated. GI50 values (concentration required to reduce cell growth by 50%) were used to assess anticancer activity.

Cytotoxic effect of bark extracts of *M. velutina* var. *deviyarina* on MCF-7 Cells

The cytotoxic potential of bark extracts of *M. velutina* var. *deviyarina* was evaluated using the SRB assay against MCF-7 human breast cancer cells. Cells were treated with increasing concentrations (10–80 µg/mL), and growth inhibition was expressed in terms of GI_{50} values (Table 1).Among the tested extracts, the ethyl acetate bark extract exhibited the highest growth inhibitory effect, with a GI_{50} value of <10 µg/mL, comparable to the standard drug Adriamycin (ADR). In contrast, ethanol and alcohol–water bark extracts showed limited cytotoxic activity, with GI_{50} values exceeding 80 µg/mL.

Microscopic examination revealed concentration-dependent morphological alterations in MCF-7 cells treated with ethyl acetate bark extract, including reduced cell density, cell rounding, and loss of adherence, relative to untreated controls (Fig. 6). Ethanol and alcohol–water extracts induced comparatively minor morphological changes.

Table 1. Anticancer activity of bark extract against MCF-7 Cells

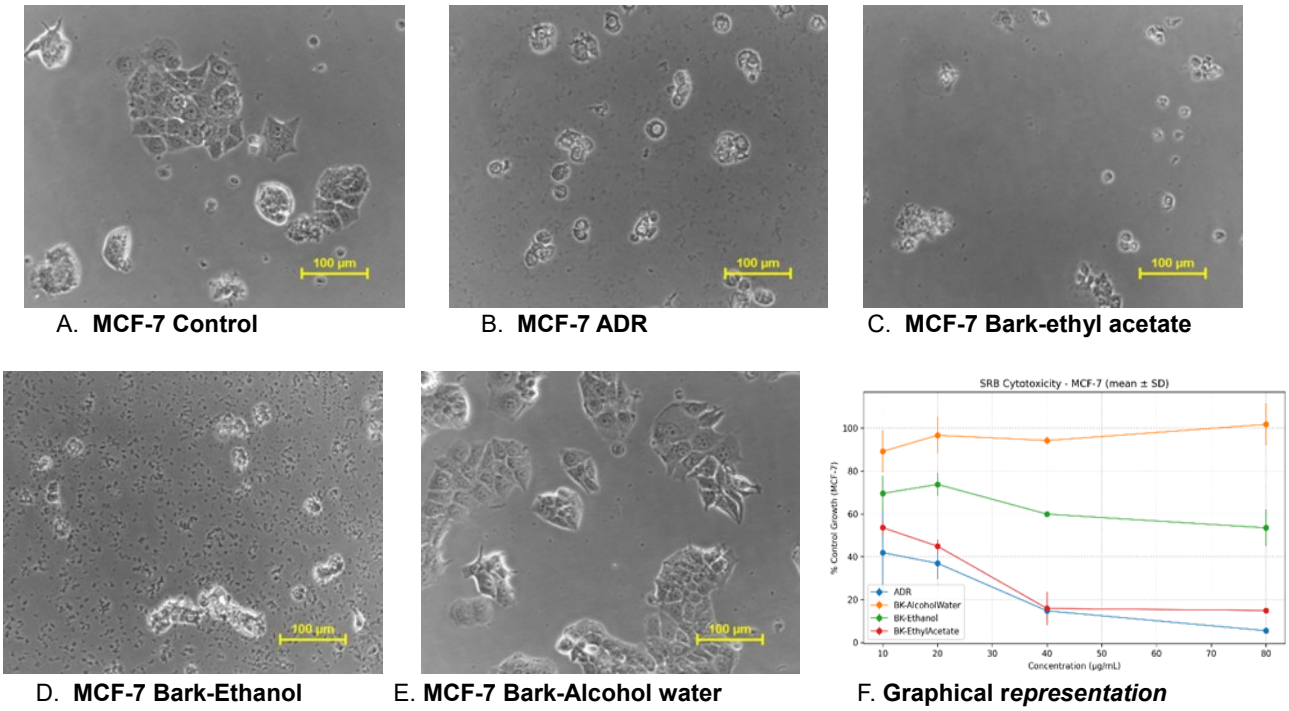
Extract type	GI50 (µg/mL)
Bark (ethanol)	>80
Bark (ethyl acetate)	<10
Bark (alcohol-water)	>80
ADR (control)	<10

Selectivity against normal cells (Vero cell line)

To examine cytotoxic response in normal cells, ethanol bark and leaf extracts were evaluated against the Vero cell line using the SRB assay. Both extracts exhibited minimal growth inhibition, with GI_{50} values exceeding 80 µg/mL, whereas ADR demonstrated marked cytotoxicity (GI_{50} < 10 µg/mL) (Table 2). These observations indicate lower in vitro cytotoxic response in Vero cells under the tested conditions.

The ethyl acetate bark extract exhibited strong cytotoxicity toward MCF-7 cells, while both bark and leaf ethanol extracts showed low toxicity in normal Vero cells (GI_{50} > 80 µg/mL), indicating selective anticancer potential.

To determine the statistical significance of the observed cytotoxic effects, One-way ANOVA was per-



A. **MCF-7 Control** B. **MCF-7 ADR** C. **MCF-7 Bark-ethyl acetate**

D. **MCF-7 Bark-Ethanol** E. **MCF-7 Bark-Alcohol water** F. **Graphical r*epresentation***

Fig. 6. *Representative microscopy images (A-E) and graphical representation (F) of MCF-7 human breast cancer cells treated with bark extracts of M. velutina var.* deviyarina *using the SRB assay. Treatments include: untreated control, Adriamycin (ADR; positive control), bark-ethanol extract, bark-ethyl acetate extract, and bark-alcohol water extract. The bark-ethyl acetate extract shows pronounced cytotoxic morphological changes, consistent with its strong activity (GI_{50} < 10 µg/mL). Images were captured 48 h post-treatment.*

Table 2. Anticancer activity of ethanol bark and leaf extract against human breast cancer cell line MCF-7 and monkey derived normal renal cell line **vero**

Extract type	GI50 (µg/mL)
Leaf (ethanol)	>80
Bark (ethanol)	>80
ADR (control)	<10

formed, followed by Tukey's post-hoc test to compare differences between extracts. Results showed a significant difference ($p < 0.05$) between the bark ethyl acetate extract, the ethanol and alcohol-water extracts in MCF-7 cells. For Vero cells, there was no significant difference ($p > 0.05$), confirming that bark and leaf ethanol extracts were non-toxic to normal cells. These findings indicate that ethyl acetate bark extract exhibits potent anticancer activity against MCF-7 cells, while ethanol and alcohol-water extracts are less effective (Fig. 7). The extracts also show selective toxicity, as they do not significantly affect normal Vero cells. This selectivity highlights the potential therapeutic value of ethyl acetate bark extract for further drug development.

DISCUSSION

While several studies have investigated individual bioactivities of *Miliusa* species and *M. velutina*, the unique contribution of the present work is the simultaneous assessment of antioxidant, α-amylase inhibitory, antibiofilm, antimicrobial, and anticancer activities on the deviyarina variety, and the identification of the ethyl-acetate bark extract as a particularly promising fraction for antibiofilm and anticancer follow-up studies (Fig. 1-7 and Table 1-2).

The findings demonstrate notable antioxidant activity, enzyme-based antidiabetic potential and antimicrobial and anticancer activities, thereby providing preliminary scientific support for its traditional medicinal applications (Fig. 1-7 and Table 1-2) (Al Fahad *et al.*, 2020; Balachandran and Chakrabarty, 2011; Ban, 2000; Chuakul and Sornthornchareonon, 2003; Fahad *et al.*, 2020). Previous research on the Annonaceae family has highlighted their diverse bioactive compounds, including alkaloids, flavonoids and sesquiterpenoids, which contribute to their pharmacological efficacy (Bin Chen *et al.*, 2003; Caughlin *et al.*, 2015).

According to previous work, *M. velutina* contains flavonoids, alkaloids, tannins, and glycosides, contributing to its medicinal potential. Its flower extract shows significant antioxidant activity in DPPH and FRAP assays, indicating its role in combating oxidative stress. Additionally, the extract inhibits α-amylase suggesting antidiabetic properties (Anh *et al*, 2021). The isolated compounds from the *Miliusa* genus contain various functional groups and exhibit diverse bioactivities (Hoang Le *et al*, 2022). Moreover, compounds like goniothal-

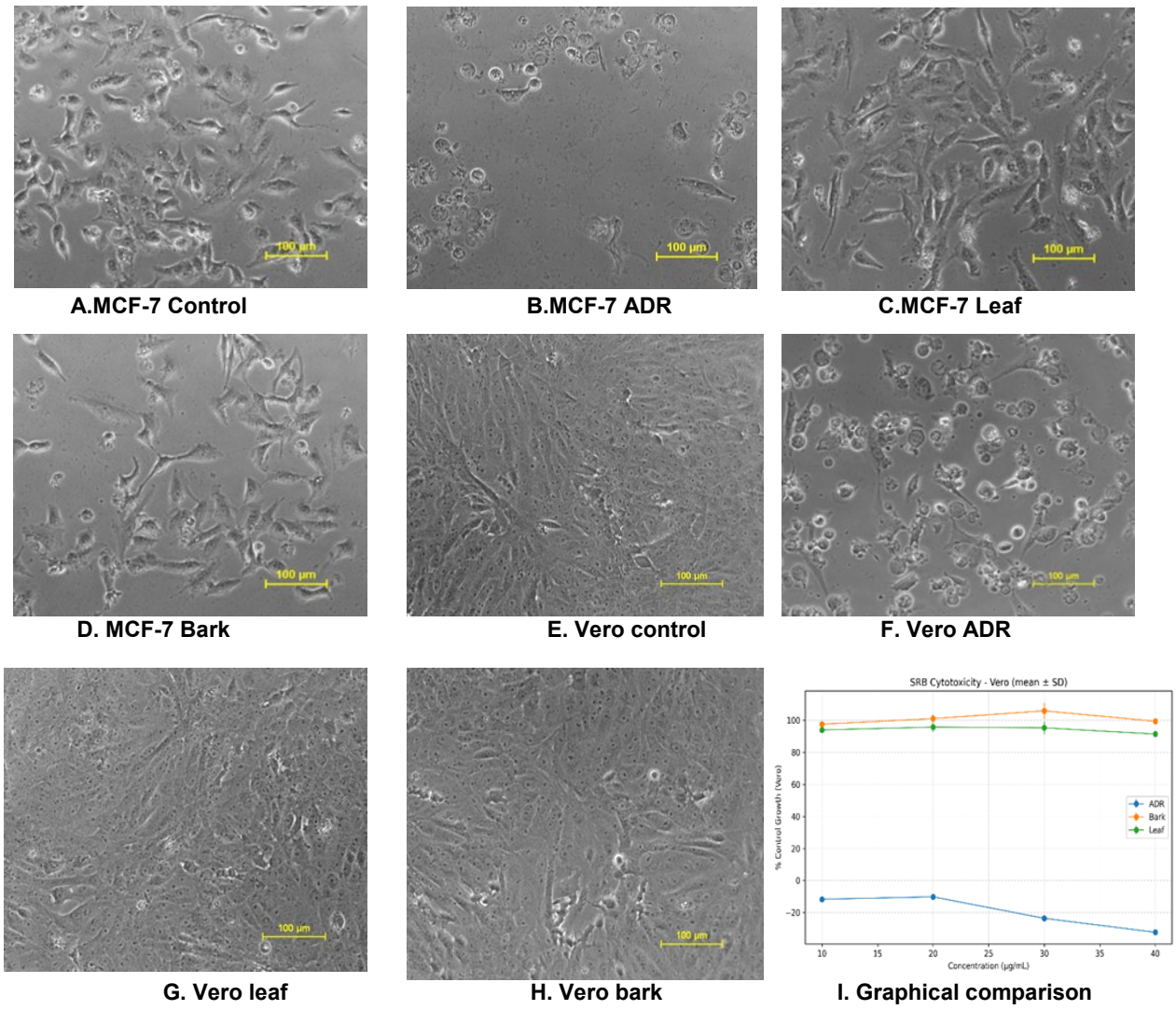


Fig. 7. *Representative microscopy images (A-H) and graphical comparison (I) of the cytotoxic activity of bark and leaf extracts of M. velutina var. deviyarina against human breast cancer (MCF-7) and normal monkey kidney (Vero) cell lines, evaluated using the Sulforhodamine B (SRB) assay.*

amusin isolated from the plant have demonstrated cytotoxic and antibacterial activity, which opens up its potential as an antimicrobial agent (Jumana *et al*, 2000). Previous chromatographic studies on *M. velutina* fruits and flowers had led to the isolation of rare homogentisic acid derivatives, which demonstrated antimalarial, antimycobacterial, cytotoxic and moderate antibacterial activities (Trinop Promgool *et al*, 2019).

The DPPH assay for *M. velutina* had previously shown the presence of tannins and saponins, along with limited antibacterial and antifungal activity, suggesting the need for further investigation of its remaining bioactive constituents (Jogi, 2017). The current study on *M. velutina var. deviyarina* demonstrated that antioxidant activity varied with plant part and solvent, with leaf extracts showing the highest activity (below 10 µg/mL), followed by stem and bark. Methanolic extracts exhibited the lowest IC_{50} values across all tissues, while benzene extracts were least effective (Fig. 1). Such antioxidant activity is often associated with the presence of hydrogen-donating compounds, including phenolic substances such as polyphenols and flavonoids, which in many plant extracts are known to contribute to free radical neutralisation and protection against oxidative stress (Forni *et al*, 2019). Antioxidants from *M. velutina* are applied in both food and medical fields. However, the utilization of this plant is restricted because certain species within the *Miliusa* genus are considered endangered or critically endangered (Trang *et al*, 2024).

These findings align with previous studies on *M. velutina*, where methanolic extracts exhibited potent antioxidant properties comparable to quercetin and vitamin C (Anh *et al.*, 2021). *M. velutina* methanolic extracts showed strong antioxidant, anti-inflammatory, and broad-spectrum antibacterial activities (Tragoolpua *et al*, 2024). Notably, our study revealed statistically sig-

nificant differences in IC50 values, supporting the efficacy of these extracts as natural antioxidants (Brophy *et al.*, 2004).The essential oils of *M. velutina* have also been reported to exhibit antioxidant activity, reinforcing the potential of this species as a source of bioactive compounds (Brophy *et al.*, 2004). A unique class of bicyclic lactones, velutinones A–H, and dimeric styrylpyrones, velutinindimers A–C, along with known compounds like kawapyrone and yangonin, were isolated from *M. velutina* leaves. These compounds had shown antimalarial activity and cytotoxicity against cancer cell lines (Wongsa *et al*, 2017). Past research on the stem bark of *M. velutina* had resulted in the isolation of four compounds and showed significant antioxidant, cytotoxic, thrombolytic, and antibacterial activities in various extract fractions (Fahad *et al*, 2020) which is also supported in bark extract of *M. velutina var. deviyarina* (Fig. 1-7).

The green, ripe, and dried fruit extracts of *M. velutina var. deviyarina* exhibited strong α-amylase inhibitory activity indicating high potency (Fig. 2). The bark extract showed moderate inhibitory activity. In contrast, the leaf and flower extracts displayed low to negligible inhibition. Similar antidiabetic activities have been reported in *M. velutina* extracts, where potent enzyme inhibition and antioxidant effects were observed in vitro (Anh *et al*, 2021).

The moderate activity observed in the bark extract (Fig. 2) further supports its potential role in diabetes management, though the minimal activity in leaf and flower extracts indicates variability in bioactive compound distribution. These results align with prior research highlighting the α-amylase inhibition of Annonaceae plants, particularly due to their flavonoid and alkaloid content (Hasan *et al.*, 2000; Gan *et al.*, 2022).

Bark extract showed significant antibiofilm activity (IC_{50} = 160 µg/mL) (Fig. 3), whereas other extracts displayed substantially lower activity. This suggests a strong antibiofilm potential, which could be beneficial in combating persistent bacterial infections (Connolly *et al.*, 2003; Ngo *et al.*, 2024; Olawuwo *et al.*, 2022).

Bark extracts showed the strongest antimicrobial activity (Fig. 4-5), followed by stem and leaf extracts, with ethanolic and ethyl acetate solvents exhibiting greater potency than aqueous and benzene extracts. Previous studies have confirmed the antimicrobial activity of *M. velutina*, with methanol and ethanol extracts from the bark and leaves showing inhibition zones comparable to standard antibiotics such as penicillin and streptomycin (Huong *et al.*,2005). The presence of alkaloids, essential oils, and sesquiterpenoids is likely responsible for these antimicrobial effects (Bin Chen *et al.*, 2003; Chaowasku *et al.*, 2014; Damthongdee and Chaowasku, 2018). The ethanol leaf extract of *M. velutina* had previously shown the presence of tannins and saponins, along with limited antibacterial and antifungal activity, suggesting the need for further investigation of its remaining bioactive constituents (Jogi, 2017). Antioxidants from *M. velutina* are applied in both food and medical fields. However, the utilization of this plant is restricted because certain species within the *Miliusa* genus are considered endangered or critically endangered (Trang *et al*, 2024).

The SRB assay revealed that ethyl acetate extract exhibited strong cytotoxic activity against the MCF-7 breast cancer cell line (Fig. 6-7), with GI50 value of <10 µg/mL comparable to Adriamycin (ADR), a standard chemotherapy drug (Harrigan *et al.*, 1994). These findings suggest that *M. velutina var. deviyarina* contains bioactive compounds capable of inducing cytotoxic effects in cancer cells. Previous reports have identified alkaloids and drimane sesquiterpenoids in *M. velutina*, further supporting its potential as a natural source of anticancer agents (Bin Chen *et al.*, 2003). The Annonaceae family has been extensively studied for its cytotoxic properties, particularly in relation to its acetogenins and flavonoid-rich extracts (Caughlin *et al.*, 2015) supporting its claim to categories as good liver tonic (Tewari *et al*, 2021; Trang *et al.*, 2020). While the SRB assay-derived GI_{50} values and qualitative morphological observations provide preliminary evidence of cytotoxic activity, the absence of full dose–response curves with error bars represents a limitation of the present study. Additionally, as the findings are based solely on in vitro assays, factors such as bioavailability, metabolism, and tumour microenvironment interactions cannot be fully addressed. Therefore, the observed cytotoxic effects should be interpreted as indicative of *in vitro* anticancer potential, warranting further validation through detailed dose–response analyses and *in vivo* studies.

The phytochemical composition and associated bioactivities of *M. velutina var. deviyarina* are likely influenced by several environmental and seasonal factors. Studies have shown that variations in soil composition, altitude, rainfall, temperature, and light intensity can markedly affect the biosynthesis and accumulation of secondary metabolites, including phenolics, flavonoids, terpenoids, and alkaloids in medicinal plants (Khoddami *et al.*, 2013; Yang *et al.*, 2018; Wink, 2015). Seasonal changes during plant growth and harvest also modulate metabolite concentrations, which in turn influence the antioxidant, antimicrobial, and anticancer efficacy of plant extracts (Singh *et al.*, 2021). Since the present study utilized plant material collected from a single natural habitat in Maharashtra, India, future comparative analyses across different geographic regions and collection seasons would be valuable to for validation to establish the reproducibility and chemotypic stability of this species' phytochemical and pharma-

cological profiles.

Conclusion

The present study demonstrated that *M. velutina* var. *deviyarina* dispalys noteworthy antioxidant, antibacterial, and anticancer activities. Methanolic and ethanolic extracts of leaf, stem, and bark showed the highest DPPH radical scavenging potential, while the methanolic bark extract displayed broad-spectrum antibacterial activity, particularly against *S. aureus* (SVC–01) and *P. aeruginosa* (SVC–04). *In vitro* assays also revealed notable anticancer potential of the extracts, suggesting the presence of cytotoxic bioactive compounds. Statistical analyses (ANOVA and Tukey's HSD) confirmed significant differences among extracts ($p < 0.05$). Overall, the findings highlight *M. velutina* var. *deviyarina* as a promising source of multifunctional phytoconstituents with potential therapeutic applications. Future studies should focus on isolating and characterizing the bioactive compounds responsible for the observed effects, along with mechanistic evaluations such as membrane integrity, enzyme inhibition, and efflux pump modulation assays. Additionally, optimizing *in vitro* cultivation could enhance the production of pharmacologically active compounds for applications in medicine, nutraceuticals, and functional foods. *In vivo* studies are essential to confirm efficacy and safety.

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Conflict of interest

The authors declare that they have no conflict of interest.

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