# Association of miR-181a-5p with *Lantana camara* leaf extract-mediated inhibition of proliferation, survival, and migration in luminal A-type MCF-7 cells and triple-negative type MDA-MB-231 cells

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**Abstract**

*Ethnopharmacological relevance:* Breast cancer has a high mortality with increasing chemoresistance and recurrence. *Lantana camara* has diverse medicinal properties, including anti-cancer potential; however, its role in breast cancer is not well-explored.

*Aim of the study:* The study aims to elucidate the role of *Lantana camara* leaf extract in miR-181a-5p-mediated control of proliferation, survival, and migration of breast cancer cells.

*Material and methods:* Real-time PCR was employed for RNA expression studies. Bioinformatic tools were used for identifying miR-181a-5p target mRNAs and seed regions in the 3 prime untranslated region of target mRNAs. miR-181a-5p was overexpressed in cells using transfection method. Flow cytometry was employed for cell cycle and apoptosis assays. Scratch assay was performed to study cell migration. The 3 prime untranslated region of *CDKN3* was cloned, and site-directed mutagenesis was employed to mutate miR-181a-5p seed region. Western blot analysis was employed for a reporter assay.

*Results: L. camara* leaf extract upregulated the expression of miR-181a-5p with concomitant decrease in mRNAs of *BCL-2*, *MCL-1*, and *CDKN3* in breast cancer cells. Overexpression of miR-181a-5p induced cell death in MCF-7 and MDA-MB-231 cells, and cell death increased in the presence of leaf extract. miR-181a impeded migration in both cell lines in the presence of the extract. Reporter assay confirmed the interaction of miR-181a-5p with 3 prime untranslated region of the *CDKN3* mRNA.

*Conclusion:* Our study suggests that the extract may play an important role in regulating miR-181a-5p in MCF-7 and MDA-MB-231 cells, and may have important therapeutic potential for breast cancer therapy.

**Keywords:** Breast cancer, *Lantana camara*, miR-181a-5p, cell death, migration

## 1. Introduction

Breast cancer is one of the most commonly reported cancers in females worldwide, and it also accounts for the majority of cancer-related deaths in females. Several factors like genetics, physiological condition, lifestyle habits, Smoking, and alcohol consumption may increase the risk of breast cancer. Women in their 50s are more prone to developing breast cancer; however, it can occur at any age. Depending on the severity of the breast cancer, it is subdivided into different subtypes- luminal A, luminal B, HER2–enriched, and triple-negative breast cancer (TNBC)(Al-thoubaity, 2020). Among different types of breast cancer, luminal A type contributes to almost 58.5% of the breast cancer burden, whereas TNBC accounts for 16% of all cases(Al-thoubaity, 2020). Luminal A-type breast cancer is slow growing, has a low grade, and has the best prognosis. In contrast, TNBC is the most aggressive, highly proliferative, and has high mutation rates in DNA repair genes, resulting in high genomic instability. TNBC has a very poor prognosis and a high relapse rate(Orrantia-Borunda et al., 2022). There are different treatment strategies for breast cancer depending on molecular subtypes, e.g., surgery, chemotherapy, radiation therapy, hormonal therapy, and targeted therapy. For treating luminal A-type breast cancer, drugs like tamoxifen and fulvestrant are used (Xiong et al., 2025). For TNBC, chemotherapeutic drugs like doxorubicin and taxanes are being used. Despite the success, chemotherapeutic agents have several limitations, like toxicity, drug resistance, varying patient outcomes, etc. Moreover, TNBC has higher tumor heterogeneity, a high relapse rate. Targeted therapies are also limited for TNBC (Obidiro et al., 2023). To overcome the limitations of conventional therapies, the discovery of more effective and safe treatment options is the utmost necessity. The plant world is a huge source of bioactive compounds, and only 10% of it is known till now (Iqbal et al., 2017). Different plant parts, such as stems, bark, leaves, flowers, and roots, are sources of metabolites like terpenoids, flavonoids, glycosides, saponins, and lignans. These compounds have the potential to act like anti-tumor agents. Some examples include: taxols, genistein, berberine, curcumin, etc (Iqbal et al., 2017).

*Lantana camara* is a flowering ornamental plant that belongs to the Verbenaceae family, also known as red or wild sage. Its nomenclature is verified via the World Flora Online (WFO) Plant List (https://wfoplantlist.org/). This plant was first introduced in the 19$^{th}$ century in India (Wagh et al., 2023). There are 650 different types of plants found in over 60 other countries. Different parts of the plants, including flowers, leaves, and roots, have been used in traditional medicine to treat various ailments like fever, pox, flu, stomach ache, wounds, etc. Lantadene – A, B, C, and

oleanolic acid isolated from the plant have attracted attention in drug research for their anti-cancer, antibacterial, AIDS, and anti-inflammatory activities (major) (Wagh et al., 2023).In various studies, including our own, it has been reported that extracts from the *Lantana* plant exhibit anti-tumor effects on different cancer cell lines, including those of breast cancer. Studies by Han et al. demonstrated that the ethanolic extract of *L. camara* could induce apoptosis in MCF-7 cells (Han et al., 2015). Another report by Shamsee et al. showed that Lantadene B, isolated from the methanolic extract of the plant, is capable of inducing cytotoxicity and G1 phase cell cycle arrest in MCF-7 cells (Shamsee et al., 2019). *Lantana camara* root extract-derived root nanoparticle exhibited cytotoxicity on MDA-MB-231 cells (Ramkumar et al., 2017). El-Din et al. also reported that the methanolic extract of *L. camara* leaves could exert cytotoxic effects on MCF-7 and MDA-MB-231 breast cancer cell lines (El-Din et al., 2022). However, the detailed mechanism of cytotoxicity on MDA-MB-231 cells was not explored. In our previous study, it was reported that the ethanolic leaf extract of *Lantana camara* is cytotoxic to both MCF-7 and MDA-MB-231 cell lines (Pal et al., 2024). Moreover, the extract could induce the G0/G1 phase cell cycle arrest, cell death, and decreased migration potential of MDA-MB-231 cells (Pal et al., 2024). These findings make *Lantana camara* leaf extract a potential candidate for breast cancer therapy and call for further exploration of the anti-tumor effect of the extract.

Plant-derived compounds can affect various intracellular processes, like epigenetic alteration, microRNA regulation, which are vital players in cancer development and progression, including breast cancer. A study by Hargraves et al. revealed that phytochemicals derived from cruciferous vegetables and sweet wormwood plant could up-regulate the expression of tumor suppressor miR34a, resulting in down-regulation of target gene CDK4 in MCF-7 and T47D cell lines (Hargraves et al., 2016). Polyphenol-Enriched Blueberry Preparation has also been reported to downregulate miR-210 in MDA-MB-231 and 4T1 cells and increase miR145 expression (Mallet et al., 2021). In an elaborate review by Ahmed et al., it has been mentioned that various plant-derived compounds like EGCG from tea, DIM from cruciferous can modulate miR-16, miR-21, and miR-92a. Other plant-derived compounds, Glyciollins, increased the expression of miR-181c and miR-181d, and many other microRNAs, and have a tumor suppressive role (Ahmed et al., 2020). Quercetin, a plant-derived flavonoid, is also known to regulate various miRNAs involved in apoptosis and cancer metastasis. A few of them include miR-16, 34a, 26b, miR-146a/b, 194, etc(Ahmed et al., 2020). Therefore, it would be no surprise for *Lantana camara* leaf extract to

102 show a similar kind of epigenetic regulation. However, there are no reports available to date
103 showing *Lantana camara*-mediated epigenetic regulation through miRNA in cancer.

104 Due to these reasons, in our current study, we were interested in exploring the effect of *Lantana*
105 *camara* leaf extract on microRNA regulation in MCF-7 and MDA-MB-231 cells. For the study,
106 miR-181a-5p was selected. miR-181a-5p is a member of the miR181 family, which is evolutionary
107 conserved, indicating its importance (Yang et al., 2017). This miRNA is known for playing a dual
108 role in breast cancer progression and regulation (Yang et al., 2017). Various reports have also
109 shown the dual role of miR-181a-5p in breast cancer. Liu et al. have shown that miR-181a can
110 induce the proliferation and invasion of breast cancer cells by targeting KLF6 and KLF15,
111 respectively (Liu et al., 2020). Another report by Zhang et al. demonstrated that the upregulation
112 of miR-181a-5p under hypoxia reduces epirubicin sensitivity in breast cancer cells by inhibiting
113 EPDR1/TRPC1, thereby activating the PI3K/AKT signalling pathway (Y. Zhang et al., 2024). On
114 the other hand, Lin et al. have shown that overexpression of miR-181a in HS578T breast cancer
115 cells can reduce their proliferation, migration, and also induce autophagy (Lin et al., 2021). miR-
116 181a is also predicted to target *BCL-2*, *MCL-1*, and *CDKN3* mRNAs, which are vital genes for cell
117 survival and proliferation. The interaction of miR181a with *BCL-2* and *MCL-1* is already well
118 established in the literature (Ouyang et al., 2012). However, no report is available on the interaction
119 between *CDKN3* and miR-181a. Similarly, the role of *Lantana camara* in the regulation of
120 miR181a and its combined effect on breast cancer cells is also not explored. Hence, this study may
121 reveal a new avenue to breast cancer therapy mediated through miR-181a and *Lantana camara*
122 extract.

123 **2. Materials and methods**

124 **2.1. Cell culture**

125 The MCF-7 and MDA-MB-231 cell line was purchased from the National Centre for Cell Science
126 (NCCS), Pune, India. All cell lines were maintained in glucose-rich Dulbecco's Modified Eagle
127 Medium (DMEM) (HiMedia, India), which is supplemented with 10% (v/v) Fetal Bovine Serum
128 (FBS) (HiMedia, India), Penicillin and Streptomycin antibiotic solution (HiMedia, India). Cells
129 were grown at 37ºC in a humidified incubator with 5% $CO_2$. 1X Trypsin-EDTA solution (HiMedia,
130 India) was used for cell harvesting during sub-culturing and experiments.

131

132 **2.2. Collection of *Lantana camara* leaves and ethanolic leaf extract preparation**

133 Healthy and fresh leaves of *Lantana camara* were collected from the Indian Institute of Science
134 Education and Research Kolkata (latitude-22°57′50″N and longitude-88°31′28″E) campus and
135 surroundings. The leaves of the plant were identified and authenticated by an expert botanist of
136 the Central National Herbarium, Botanical Survey of India, West Bengal, India. A voucher
137 specimen was deposited at their herbarium with a voucher/reference number IISER/AP/01. A
138 detailed protocol for plant extract preparation is described and published in our last published
139 paper on MDA-MB-231 (Pal et al., 2024). Briefly, fresh and healthy leaf of the *Lantana camara*
140 plant is collected, washed, and shade-dried. After that, dried leaves are crushed to a fine powder
141 and mixed with 100% molecular grade ethanol (Merck, Germany) in 1:10 ratio overnight in an
142 orbital shaker. The mixture is filtered using filter paper and dried using a rotary evaporator, and
143 the dry powder was reconstituted using 100% molecular grade ethanol (Merck, Germany) at the
144 desired concentration and filtered using a syringe filter (0.2 Micron) and used for experiments. As
145 the extract has been prepared in 100% ethanol, 0.8% (v/v) ethanol has been used as a solvent
146 control in all the experiments.

147 **2.3. Selection of treatment doses for the study**

148 For treating breast cancer cells with the extract, different doses of the extract were selected based
149 on the $IC_{50}$ values of the extract for MCF-7 and MDA-MB-231 cells, already reported in our
150 previous publication (Pal et al., 2024). As the $IC_{50}$ value of the extract for MCF-7 ($IC_{50}$: 91.66
151 µg/mL) was lower than MDA-MB-231 cells ($IC_{50}$: 111.33 µg/mL), low treatment doses of the
152 extract have been chosen for MCF-7 cells (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL,
153 120 µg/mL) compared to MDA-MB-231 (40 µg/mL, 60 µg/mL, 80 µg/mL, 120 µg/mL,180
154 µg/mL) cells. However, the 24h treatment time is constant for both cell lines. As the treatment
155 time is fixed, for observing cell cycle and cell migration, low doses (below $IC_{50}$) are selected.
156 However, for the cell death assay, higher doses close to $IC_{50}$ and above have been selected for a
157 better understanding of the extract's effect.

158

159

160 **2.4. Bioinformatics prediction**

161 Online available bioinformatics target prediction tools, Targetscan 8.0
162 (https://www.targetscan.org/mmu_80/) and miRDB (https://mirdb.org/) were used to predict the
163 target of miR-181a-5p and to find the binding region of miR-181a-5p in the target gene.

164 **2.5. Isolation of RNA and microRNA and reverse transcription quantitative real-time**
165 **polymerase chain reaction (RT-qPCR)**

166 MCF-7 and MDA-MB-231 cells were seeded in 60mm cell culture dishes at a seeding density of
167 $0.4 \times 10^6$ cells in each dish. At 60% -70% confluency, cells were treated with either 0.8% (v/v)
168 ethanol or different doses of *Lantana camara* extract (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL,
169 120 µg/mL, 180 µg/mL) for 24 hours. Total RNA was extracted using total RNA extraction reagent
170 (Takara, Japan) following the manufacturer's protocol, which is explained in detail in our previous
171 publication (Pal et al., 2024). For the isolation of microRNA, an additional overnight precipitation
172 step was added using 3M sodium acetate (SRL, India) and 100% molecular grade ethanol (Merck,
173 Germany) at -20°C temperature. The RNA sample was then treated with RNase-free DNase I
174 enzyme (Thermo Fisher Scientific, USA) according to the manufacturer's instructions to eliminate
175 any residual chromosomal DNA. The samples were subsequently quantified using a microplate
176 spectrophotometer (BioTek Epoch Microplate Spectrophotometer, Agilent Technologies Inc.,
177 USA). Total RNA was reverse transcribed into cDNA using Primescript 1$^{st}$ strand cDNA synthesis
178 kit (Takara, Japan) in accordance with the manufacturer's instructions.

179 **2.6. Transfection of MCF-7 and MDA-MB-231 cell lines**

180 MCF-7 and MDA-MB-231 cells were seeded in 35mm dishes at a density of $0.2 \times 10^6$ cells in each
181 dish. At a confluency of 60% - 70%, cells were transfected with either pcDNA3.1+ or pcmiR181a
182 using the calcium phosphate transfection method. Briefly, in a tube, 75 µL tris-EDTA buffer (pH
183 8) was taken. To this, 20 µL 2M $CaCl_2$ (SRL, India) and 15 µg purified plasmid DNA were added.
184 To this mixture, 110 µL 2X HBS was added dropwise under gentle vortex conditions. The mixture
185 was allowed to stand at room temperature for 20 minutes. In the meantime, the media of the culture
186 dishes was replaced with antibiotic-free media with 10% FBS. After that, the transfection mixture
187 was added dropwise to the culture dishes. After 6 hours, the media was replaced with fresh
188 complete DMEM media, and cells were harvested after 48 hours post-transfection for experiments.

189 **2.7. Cell cycle phase distribution assay**

190 MCF-7 and MDA-MB-231 cells were seeded in 35 mm cell culture dishes ($0.2 \times 10^6$ cells/dish)
191 and incubated overnight. Cells were then transfected with either pcDNA 3.1+ empty vector or
192 pcmiR181a. After 24 hours post transfection cells were treated with 20 µg/mL, 40 µg/mL, 80
193 µg/mL, 120 µg/mL doses of the leaf extract for MCF-7 cells and 40 µg/mL, 60 µg/mL, 120 µg/mL,
194 180 µg/mL doses of the leaf extract for MDA-MB-231 cells along with vehicle treated and
195 untreated control cells. Following 24 hours of treatment, cells were harvested from the culture
196 dishes using 1X Trypsin-EDTA and collected along with the floating cells derived from spent
197 media. Cell pellets were washed with ice-cold 1X phosphate buffer saline (PBS). Then the cells
198 were fixed with 70% ethanol and incubated at 4°C overnight. The next day, cells were pelleted
199 down and washed with ice-cold 1X PBS, and were resuspended in 500 µL propidium iodide (PI)
200 solution and incubated at 37°C for 20 minutes in the dark. Thereafter, the PI solution was removed,
201 and cells were resuspended in 500 µL 1X PBS and analyzed using a flow cytometer (BD
202 Biosciences, USA). Cell cycle distribution (Sub-G1, G1/G0, S, and G2/M) was determined, and
203 the data were represented as a bar graph.

204 **2.8. Detection of Cell death by flow cytometry**

205 Double staining using the Annexin V-FITC/PI Apoptosis detection kit by BD Biosciences was
206 performed following the manufacturer's instructions to differentiate between apoptotic and non-
207 apoptotic cell death. MCF-7 and MDA-MB-231 cells were seeded in 35 mm cell culture dishes
208 ($0.2 \times 10^6$ cells/dish) and incubated overnight, and then were transfected with either pcDNA 3.1+
209 empty vector or pcmiR181a. After 24 hours post-transfection, cells were exposed to 40 µg/mL, 80
210 µg/mL, and 120 µg/mL doses of the leaf extract for MCF-7 cells and 60 µg/mL, 120 µg/mL, and
211 180 µg/mL doses for MDA-MB-231 cells, along with vehicle-treated and untreated cells, for 24
212 hours. Following treatment, cells were harvested along with the floating cells derived from spent
213 media. Cells were stained following the protocol described in our previous publication (Pal et al.,
214 2024). Briefly, the cell pellet was washed with chilled 1X PBS, followed by resuspending the cells
215 in binding buffer. Thereafter, cells were stained with PI and Annexin V-FITC simultaneously for
216 20 minutes in the dark and were analyzed within an hour by a flow cytometer to obtain the scatter
217 plot with viable cells (bottom left quadrant), early apoptotic cells (bottom right quadrant), late

218 apoptotic cells (upper right quadrant), and non-apoptotic cells (upper left quadrant). Data was
219 represented graphically as Bar plots.

220 **2.9. Wound healing assay**

221 MCF-7 and MDA-MB-231 cells ($0.2 \times 10^6$ cells/dish) were seeded in 35mm dishes and allowed
222 to grow until a uniform monolayer of cells was formed, and then were transfected with either
223 pcDNA 3.1+ empty vector or pcmiR181a. After 24 hours post-transfection, a wound was created
224 in each dish using a sterile P10 microtip, and the spent media was removed from the culture dish.
225 The monolayer of cells was washed twice with 1X PBS, and complete media was added to the
226 cells, followed by the addition of cycloheximide (0.5µg/mL). Immediately after the addition of
227 cycloheximide, the wounded cells were treated with 5 µg/mL, 10 µg/mL, 20 µg/mL doses of the
228 leaf extract for MCF-7 cells and 40 µg/mL, 60 µg/mL, 80 µg/mL doses of the leaf extract for
229 MDA-MB-231 cells, along with vehicle-treated and untreated control cells. The cells were imaged
230 at 0 hours and 24 hours post-treatment using an inverted microscope (Evos XL Core, Invitrogen,
231 United States) with a camera. The images were analyzed, and wound closure was calculated by
232 ImageJ (ImageJ software, NIH, USA) using the following formula:

233 $$\text{Wound closure \%} = \frac{\text{Area of wound at time 0 h - Area of wound at time 't' h}}{\text{Area of wound at time 0 h}} \times 100\ \%$$

234

235 where t = specific time intervals after 0 h

236 **2.10. Cloning of 3′ UTR of *CDKN3* in pEGFPC1 vector**

237 A part of the *CDKN3* 3´UTR region was digested with HindIII and KpnI and run on 1% agarose
238 gels. The digested inserts were gel-purified, quantified, and ligated to the double-digested pEGFP-
239 C1 vector downstream *egfp* reporter gene and transformed using $CaCl_2$ (100mM). All positive
240 clones were selected from LB-agar media against kanamycin (final concentration 100 µg/mL).
241 Now the construct is recognized as pEGFPCDKN3. The empty vectors (pcDNA3.1+, pEGFPC1)
242 and pCmiR181a were the kind gift from Dr. Sumita Sengupta (nee Bandyopadhyay), University
243 of Calcutta. SnapGene (www.snapgene.com) was used for the preparation of the vector map with
244 the construct.

245

246 **2.11. Site-directed mutagenesis of the miR-181a-5p seed region on the 3′ UTR of *CDKN3***

247 Three bases at the seed region of miR-181a-5p were altered on the 3´ UTR of the *CDKN3* mRNA
248 using primers spanning the region (Table S1). Single primer amplification technique was used to
249 amplify the pEGFPCDKN3 using GXL high fidelity polymerase (Takara, Japan) using the
250 manufacturer's protocol. After amplification, the PCR products were reannealed using a
251 denaturation and slow cooling technique. After that, the product was purified using a DNA
252 purification kit (Invitrogen, USA). 2µg of purified product was digested with DpnI restriction
253 enzyme following the manufacturer's protocol. The digested product was run on agarose gel, and
254 the desired band was cut from the gel and purified using a gel purification kit. The purified product
255 was used for transforming E. coli XL-1 Blue competent cells, and positive colonies were selected
256 from LB-agar media against kanamycin (final concentration 100 µg/mL). It was further confirmed
257 by sequencing and was named as pECDKN3SDM.

258 **2.12. GFP Reporter assay**

259 MCF-7 cells were chosen for doing reporter assay. Cells were seeded in 60mm dishes in triplicate
260 at a density of $0.4\times10^6$ cells per dish, a day before the experiment. The next day, the cells were
261 transiently transfected by pcDNA3.1+, pEGFPC1, pcmiR181a, pEGFPCDKN3, pECDKN3SDM
262 in antibiotic-free DMEM media (DMEM, 10%FBS), followed by changing the transfection
263 medium after 6 hours of incubation. Cells were harvested after 48 hours using 1X trypsin-EDTA
264 solution. Expression of GFP was observed through Western blot analysis. GAPDH was used as a
265 loading control.

266 **2.13. Western Blot analysis**

267 MCF-7 cells were seeded in 60mm dishes at a seeding density of $0.3\times10^6$ cells/dish and were
268 grown till 60% - 70% confluency. The next day, the cells were transiently transfected by
269 pcDNA3.1+, pEGFPC1, pcmiR181a, pEGFPCDKN3, pECDKN3SDM in antibiotic-free DMEM
270 media (DMEM, 10%FBS), followed by changing the transfection medium after 6 hours of
271 incubation. Cells were harvested after 48 hours using 1X trypsin-EDTA solution and were washed
272 twice with 1X PBS. Thereafter, these cells were lysed with RIPA cell lysis buffer (Himedia, India)
273 on ice, followed by protein estimation by the Bradford protein estimation method. 60µg of protein
274 was run on a 12% SDS page gel at a constant 150 volts for 1.5 hours, followed by transfer on a

275 PVDF membrane at 160mA for 1 hour. After the transfer was complete, the membrane was washed
276 with 1X TBST and was blocked with 5% BSA in TBST for 1hour at room temperature under
277 gentle rocking conditions. After blocking was complete membrane was again washed with
278 1XTBST for 5 minutes, and then incubated with primary antibody against GFP (ABclonal, AE078,
279 1:5000 dilution) and GAPDH (Affinity Biosciences, AF7021, 1:3000 dilution) overnight at 4ºC.
280 The next day, the membrane was again washed with 1XTBST thrice and was incubated with
281 horseradish peroxidase conjugated secondary antibody to goat anti-rabbit IgG (1:5000, Cell
282 Signaling Technology) for 1hour at room temperature. After that membrane was again washed
283 with 1XTBST thrice and developed with enhanced chemiluminescence, scanned, and recorded.

284 **2.14. Statistical analysis**

285 All graphs were generated using GraphPad Prism 9. Error bars indicate mean ± SEM of at least
286 three independent experiments. A parametric paired t-test was used for analysis of statistical
287 significance. *P*-values <0.05 were considered to be statistically significant, while *P*>0.05 were
288 considered non-significant (ns).

289 **3. Results**

290 **3.1. *Lantana camara* leaf extract upregulated the expression of hsa-miR-181a-5p in MCF-7**
291 **and MDA-MB-231 cell lines**

292 To observe the effect of *Lantana camara* treatment on the expression of miR-181a-5p in MCF-7
293 and MDA-MB-231 cells, miRNA was isolated and cDNA prepared after 24 hours of leaf extract
294 treatment, and quantitative real-time PCR was done. The PCR results were represented as Bar plots
295 (Figure 1). It is observed that both MCF-7 and MDA-MB-231 cells have a low expression of miR-
296 181a-5p in the vehicle control condition, whereas treatment with *Lantana camara* increased the
297 expression of miR-181a-5p in both cell lines significantly (Figure 1), with the increase in *Lantana*
298 *camara* dose. For MCF-7 (Figure 1A) cells at 20 µg/mL dose, there is a non-significant increase,
299 but at higher doses, the expression of miR-181a has increased significantly. Similarly, for MDA-
300 MB-231 cells (Figure 1B), a significant increase in miR-181a expression was observed at 120
301 µg/mL and 180 µg/mL doses. From these results, it can be concluded that *Lantana camara* leaf
302 extract could alter the expression of miR-181a in both cells, indicating a potential role of miR-
303 181a in breast cancer.

304 **3.2. *Lantana camara* leaf extract treatment and ectopic expression of miR-181a-5p altered**
305 **the mRNA level expression of miR-181a-5p target mRNAs: *BCL-2*, *MCL-1*, and *CDKN3* in**
306 **MCF-7 and MDA-MB-231 cell lines**

307 It is known that miRNA can bind to different target mRNAs and control their function by various
308 mechanisms. To further investigate the effect of leaf extract on the hsa-miR-181a-5p targets, a few
309 genes involved in cancer cell survival and proliferation were chosen, and changes in their mRNA
310 level expression upon 24h *L. camara* treatment were observed through real-time quantitative PCR
311 (Figures 2-4). Data revealed that the mRNA level expression of miR-181a-5p targets- *BCL-2*,
312 *MCL-1*, and *CDKN3* decreased with the leaf extract treatment in both cell lines. Furthermore, to
313 investigate the effect of ectopic expression of miR-181a on the mRNA level expression of these
314 genes, both MCF-7 and MDA-MB-231 cells were transfected with pcDNA3.1+ and pcmiR181a
315 plasmids, and the mRNA level expression of target genes was observed. Results revealed that the
316 expression of *BCL-2*, *MCL-1*, and *CDKN3* mRNAs (Figures 2-4) was significantly reduced in
317 miR-181a overexpressing MCF-7 and MDA-MB-231 cells when compared to control cells.
318 Therefore, these results also indicate the involvement of miR181a in the regulation of these
319 mRNAs. As the above-mentioned genes are critical players in the proliferation, survival, and
320 migration of cells, the effects of overexpression of miR-181a-5p in the regulation of these vital
321 parameters were also explored.

322 **3.3. Ectopic expression of miR-181a-5p increased sub-G1 population in both MCF-7 and**
323 **MDA-MB-231 cells in the presence of *Lantana camara* leaf extract**

324 Cell cycle analysis provides insight into cell growth, proliferation, and response to various stimuli
325 and treatments. Therefore, observation of the distribution of cells through different cell cycle
326 phases helps to understand the effect of overexpression of miR-181a-5p in the presence and
327 absence of the leaf extract. In our previous publication, it has already been established that *Lantana*
328 *camara* extract was able to induce G0/G1 phase arrest in MDA-MB-231 cells. Here, we intend to
329 show whether the overexpression of miR-181a-5p could further enhance the effects of the extract.
330 Figures 5 and 6 show the representative images of the MCF-7 and MDA-MB-231 cells in different
331 phases of the cell cycle (sub-G1, G0/G1, S-phase, and G2/M). Data has been analyzed and plotted
332 as Bar plots (Figure 5C, 5D, and 6C, 6D). Results of the experiment showed that in both MCF-7
333 and MDA-MB-231 cell lines, there is an increase in the sub-G1 population in miR-181a

334 overexpressed cells compared to control cells (MCF-7_empty vector and MDA_empty vector)
335 (Tables S2 and S3). This sub-G1 phase population increase was more for MCF-7_miR181a than
336 MDA_miR181a cells (Tables S2 and S3), and the presence of *Lantana camara* extract further
337 increased the sub-G1 accumulation in both cell lines. This increase in the Sub-G1 population is
338 indicative of DNA fragmentation and cellular death. For the MCF-7 cell line increase in the sub-
339 G1 population was dose dependent, followed by a subsequent decrease in G0/G1, S-phase, and
340 G2/M phase of the cell cycle (Table S2). For the MDA-MB-231 cell line, only at the 180 µg/mL
341 dose, there is a significant increase in sub-G1 population, indicating the highest DNA
342 fragmentation at this condition, when miR-181a was transiently expressed compared to the control
343 of the MDA_pcmiR181a group, with non-significant changes in other phases of the cell cycle.
344 However, the increase in the sub-G1 population was higher in all the conditions in MDA_miR181a
345 cells than in the MDA_empty vector cells (Table S3). An increase in the sub-G1 population in
346 both cell lines gives a clear hint of the cell death in both cell lines.

347 **3.4. Ectopic expression of miR-181a-5p induced cell death in MCF-7 and MDA-MB-231 cells**
348 **in the presence and absence of *Lantana camara* leaf extract**

349 An increase in the sub-G1 population is indicative of DNA breakage and hints towards cell death.
350 To further confirm the cell death in both MCF-7 and MDA-MB-231 cells and to observe the mode
351 of cell death, Annexin V-FITC/PI double staining was performed. Figures 7 and 8 represent the
352 results of the experiment summarized in Table S4 and S5. For MCF-7 cells, it was observed that
353 MCF-7 cells with transient overexpression of miR-181a-5p showed a significant increase in cell
354 death in untreated and vehicle-treated conditions when compared to MCF-7 cells transfected with
355 an empty vector. In untreated control condition for MCF-7_pcmiR181a cells % death cell was
356 observed to be 5.01 ± 0.54% (early apoptotic), 19.23 ± 3.35 % (late apoptotic), 13.93 ± 0.99 (non-
357 apoptotic), whereas for MCF-7_Empty vector, it was found to be 2.28 ± 0.40 % (early apoptotic),
358 6.33 ± 2.53 % (late apoptotic), 7.81 ± 1.67 % (non- apoptotic) cell death which was significantly
359 low compared to MCF-7_pcmiR181a. The death cell population increased further with the
360 treatment of *L. camara* extract in the MCF-7_pcmiR181a cells when compared to MCF-7_empty
361 vector. However, the increase in cell death was not dose-dependent. In MCF-7_empty vector cells,
362 there was a dose-dependent increase in cell death. Further comparison between MCF-7_empty

363 vector and MCF-7_pcmiR181a (Table S4) revealed a significant increase in cell death at 120
364 µg/mL dose in the late apoptotic cell death quadrant (20.83 ± 1.41 % to 28.88 ± 2.43 %).

365 For the MDA-MB-231 cell line (Figure 8A, 8B), it was observed that transient expression of miR-
366 181a-5p alone was able to induce apoptotic cell death (early and late apoptotic) in untreated control
367 and vehicle control conditions when compared to MDA_empty vector cells (Table S5).
368 Interestingly, the introduction of *L. camara* extract had shifted the death cell population
369 significantly towards non-apoptotic cell death, indicating that the combined effect of miR-181a-
370 5p and *Lantana camara* plant extract could also trigger other types of cell death pathways besides
371 apoptosis. In conclusion, miR-181a-5p could induce both apoptotic and non-apoptotic cell death
372 significantly in both MCF-7 and MDA-MB-231 cell lines in the presence and absence of the leaf
373 ethanolic extract.

374 **3.5. Ectopic expression of miR-181a-5p inhibited cell migration in MCF-7 and MDA-MB-**
375 **231 cells in the presence of *Lantana camara* leaf extract**

376 One of the characteristics of cancer is its ability to migrate to distant parts of the body from the
377 original tumor site. Breast tumor, specifically the triple-negative subtype, is known for its
378 migratory ability. Breast cancer can often migrate to the bones, lungs, kidneys, and brain
379 (Ibragimova et al., 2023). MDA-MB-231, being a triple-negative breast cancer cell, has good
380 migratory potential. MCF-7 cells are luminal A-type breast cancer cells and do not have good
381 migration ability, but can still migrate to nearby bones and lymph nodes. Hence, studying the
382 migration of cancer cells is of great importance for the development of effective therapy. To study
383 the effect of miR-181a-5p on MCF-7 and MDA-MB-231 cells, a scratch assay was performed after
384 transiently overexpressing miR-181a-5p in both cell lines. The results of the experiment have been
385 summarized in Figures 9 and 10. For the MCF-7 cell line (Figure 9), it has been observed that the
386 migration of the cells has reduced significantly in both MCF-7_Empty vector and MCF-
387 7_miR181a conditions with the increase in the *Lantana camara* dose, and the effect was maximum
388 at 20 µg/mL dose. Comparing MCF-7_Empty vector and MCF-7_miR181a conditions, it was also
389 observed that miR181a could not reduce the migration of the cells alone, but the introduction of
390 the leaf extract reduced migration significantly (Figure 9C). In MDA-MB-231 cells, similar effects
391 were observed with maximum inhibition of migration at 80 µg/mL dose (Figure 10B, 10C). From
392 the above findings, it can be concluded that overexpression of miR-181a alone could not inhibit

393 the migration of the breast cancer cells efficiently, but in the presence of the *L. camara* leaf extract,
394 miR-181a reduced the migration potential of breast cancer cells significantly.

395 **3.6. miR-181a-5p targets the 3′ untranslated region of the *CDKN3* mRNA**

396 *In silico* target scan (Target scan Human version 8) revealed the binding site of hsa-miR-181a to
397 the 3′ UTR of *CDKN3* mRNA. To confirm the interaction between hsa-miR-181a and the *CDKN3*
398 gene, a reporter assay was performed. MCF-7 cells were transiently transfected with
399 pEGFPC1+pcDNA3.1+, pEGFPCDKN3+pcDNA3.1+, pEGFPCDKN3+pcmiR181a,
400 pECDKN3SDM + pcmiR181a (Figure 11). After 48h, cells were harvested, and a whole cell lysate
401 was prepared to check the protein level expression of the GFP reporter gene in all the conditions.
402 It was observed that under pEGFPC1+pcDNA3.1+ and pEGFPCDKN3+pcDNA3.1+ conditions,
403 the expression of GFP was quite high, which was significantly reduced under
404 pEGFPCDKN3+pcmiR181a conditions (Figure 11D, 11E). The expression of GFP was partially
405 regained when the mutated *CDKN3* construct was present, i.e, pECDKN3SDM + pcmiR181a
406 condition. Hence, it can be concluded from the findings that miR-181a-5p can specifically bind to
407 *CDKN3* 3' UTR and result in the translational repression of *CDKN3*. Figure 11C, Bar plots
408 representing the relative mRNA expression of *CDKN3* in MCF-7 and MDA-MB-231 cells.

409 **4. Discussion**

410 Breast cancer is one of the predominant causes of death among females globally. Among different
411 subtypes of breast cancer, Luminal A type is the most predominant (60%) and has a better
412 prognosis and overall survival (Burguin et al., 2021). Triple-negative breast cancer, on the other
413 hand, is the most invasive type of breast cancer and has a worse prognosis and lower life
414 expectancy (Burguin et al., 2021). Treatment options for breast cancer are also limited, mainly for
415 triple-negative breast cancer, due to the absence of hormone receptors. For luminal A-type cancer,
416 although there are various treatment options like surgery, hormone therapy, and chemotherapy but
417 all these come with several long-term side effects (Burguin et al., 2021). For triple-negative breast
418 cancer, chemotherapy is the only treatment option, but excessive use of chemotherapy gives rise
419 to drug resistance and severe side effects (Mahmoud et al., 2022). To overcome these challenges,
420 natural products isolated from plants can be an alternative option. Several breast cancer drugs,
421 which are derived from plants like Taxol, Paclitaxel, vincristine, and sulforaphane etc, are

422 effectively used for breast cancer treatment. Recent studies have also reported that various plant-
423 derived compounds like genistein, resveratrol, curcumin, and lycopene have exhibited their
424 cytotoxic and antimigratory effects on triple-negative breast cancer (Ke et al., 2022). In our
425 preliminary work, it has been reported that *Lantana camara* leaf ethanolic extract exhibits
426 significant cytotoxicity in MCF-7 and MDA-MB-231 cells. The extract has also induced G0/G1
427 phase cell cycle arrest in MDA-MB-231 cells, resulting in DNA condensation in the nucleus,
428 followed by cell death(Pal et al., 2024). Moreover, *L. camara* can also inhibit cell migration in the
429 aggressive MDA-MB-231 cells (Pal et al., 2024). All these findings point towards the therapeutic
430 potential of the extract. To further elucidate the detailed underlying mechanism of the extract, we
431 have checked the expression of a vital miRNA, miR-181a, after *L. camara* treatment. miRNAs are
432 small RNA molecules (17-25 nucleotides) that play a vital role in breast cancer and other cancers.
433 miRNAs have the inherent property to target different mRNA molecules and inhibit their activity
434 either by repressing translation or by sequestering into the P-body (O'Brien et al., 2018). miR-
435 181a-5p is a well-known microRNA that plays a pivotal role in various types of cancers, including
436 breast cancer (Martino et al., 2025). Not only that, recently miRNAs are emerging as an effective
437 therapeutic tool for cancer therapy. In the current study, it was found that *L. camara* upregulated
438 the expression of miR-181a in both the MCF-7 (Figure 1) and MDA-MB-231 (Figure 1) cell lines,
439 and also downregulated the expression of miR-181a target genes, including *BCL-2*, *MCL-1*, and
440 *CDKN3*. *BCL-2* and *MCL-1* (Figures 2, 3) are two vital anti-apoptotic genes that play a crucial
441 role in resisting cell death (Roberts et al., 2021). Downregulation of these genes gives a hint
442 towards cell death, whereas the *CDKN3* (Figure 4) gene is an important regulator of cell
443 proliferation and migration in cancer (C. Zhang et al., 2024). Overexpression of miR-181a in
444 MCF-7 and MDA-MB-231 cell lines also downregulated the same expression of the above genes.

445 To delineate the role of miR-181a in the survival and migration of breast cancer cells, in
446 combination with *Lantana camara* cell cycle phase distribution assay, Annexin V-FITC/PI
447 apoptosis assay, and wound healing assay, was performed. From the results of experiment 3.3, it
448 can be concluded that miR181a alone and in the presence of *L. camara* extract could induce DNA
449 fragmentation, which is evident by the increase in the Sub-G1population in both cell lines (Table
450 S2 and S3). This increase in Sub-G1 population is an indication of cell death, and it was further
451 confirmed by performing Annexin V-FITC/PI double staining. Results of the experiment (Figures
452 7, 8) supported the findings of the previous experiment 3.3.

453 miR-181a alone and in the presence of the extract caused significant cell death in both cell lines
454 (Figures 7 and 8) in both apoptotic and non-apoptotic pathways. Non-apoptotic death was more
455 prevalent in MDA-MB-231 cells transiently expressing miR-181a under leaf extract treatment
456 conditions. Non-apoptotic population of death cells may indicate different cell death pathways like
457 necrosis, pyroptosis, necroptosis, ferroptosis, perthonatos, autophagy, etc (Zhang et al., 2025).
458 Hence, Further exploration of these may give deeper insight into the cell death mechanism
459 triggered in the MDA_pcmiR181a cells in the presence of *Lantana camara* extract. One of the
460 features of cancer cells is their ability to metastasize to distant parts of the body from the primary
461 tumor site, and it is more predominant in late-stage cancer. Although Luminal A subtype is less
462 invasive in nature; these cancer cells can migrate to the bones (Fan et al., 2023). MCF-7 is luminal
463 A type breast cancer cell line, and MDA-MB-231 is a highly invasive, late-stage triple-negative
464 breast cancer cell line (Welsh, 2013); therefore, it was important to check the effect of miR-181a
465 overexpression on cancer cell migration. Here, in this study, it has been observed that miR-181a
466 was able to inhibit the migration of both MCF-7 and MDA-MB-231 cell lines in the presence of
467 *Lantana camara* extract (Figures 9, 10). However, miR-181a could not inhibit migration alone.
468 Hence, it can be concluded that the combined effect of miR-181a and the extract could also impede
469 the migratory property of both cell lines. As already mentioned earlier, miRNA generally binds to
470 the 3′ UTR of target mRNAs and controls their function. In the literature, it is reported that miR-
471 181a can target the 3′ UTR of *BCL-2* and *MCL-1* and regulate their function (Ouyang et al., 2012).
472 However, no report is available on the binding of miR-181a-5p to *CDKN3*. To validate that miR-
473 181a can target *CDKN3* 3′ UTR, a GFP-reporter assay was done, and the result (Figure 11)
474 confirms their interaction. In this study, we have examined three crucial parameters of cancer cells:
475 cell proliferation, survival, and migration, and found that overexpression of miR-181a-5p was able
476 to significantly affect all three parameters either alone or in the presence of the leaf ethanolic
477 extract.

478 All these findings make *Lantana camara* leaf extract and miR181a potential candidates for breast
479 cancer treatment in the future. However, future studies to identify the bioactive compound(s) in
480 *Lantana camara* leaf extract and an *in vivo* study to delineate its effect may open new paths for
481 breast cancer management.

482

## 5. Conclusion

To summarize, we report that *Lantana camara* upregulated the expression of miRNA-181a-5p and downregulated some important target mRNAs of the miR-181a. Transient overexpression of miR-181a in breast cancer cells also showed the similar results. Moreover, overexpression of miR-181a was able to induce sub-G1 accumulation and cell death, and it increased in the presence of the leaf extract. miR-181a overexpression also impeded cell migration in the presence of the extract in both MCF-7 and MDA-MB-231 cells. All these findings together make *Lantana camara* and miR-181a-5p potential candidates for breast cancer management in the future.

## Authors' contributions

**Arundhaty Pal:** Conceptualisation, Formal analysis, Investigation, Methodology, Writing – original draft. **Sourav Sanyal:** Formal analysis, Investigation, Methodology, Writing – original draft. **Tapas Kumar Sengupta:** Conceptualisation, Funding acquisition, Project administration, Supervision, Writing – original draft.

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## Availability of Data and Materials

In this study, all data generated or analyzed are included in the article and supplementary section.

## Declarations Conflict of interest

The authors declare that there is no conflict of interest**.**

## Ethical Approval

Not applicable.

## Consent to Participate

All authors had consented to participate in the study.

## Consent for Publication

All authors have given consent for publication.

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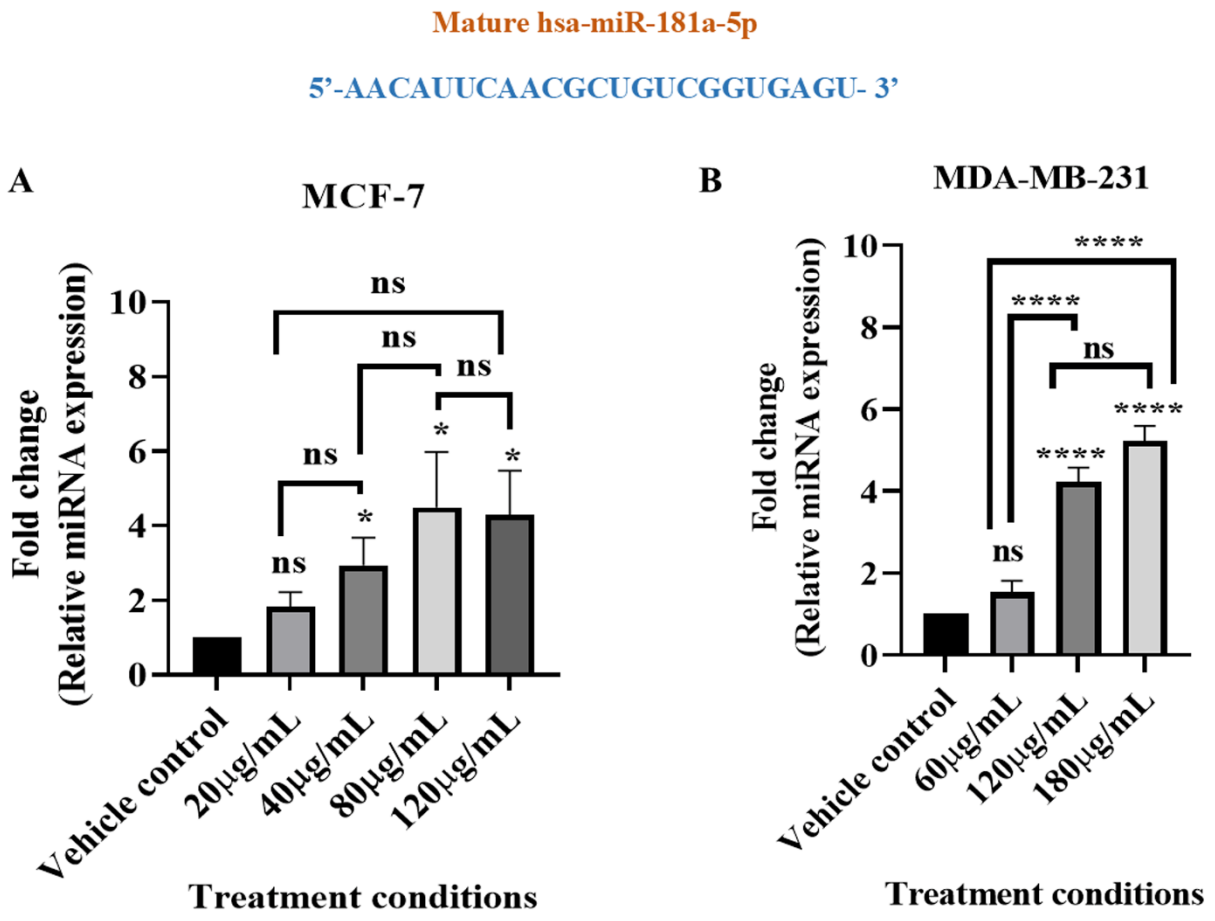
612

613

614

615 **Figure 1**

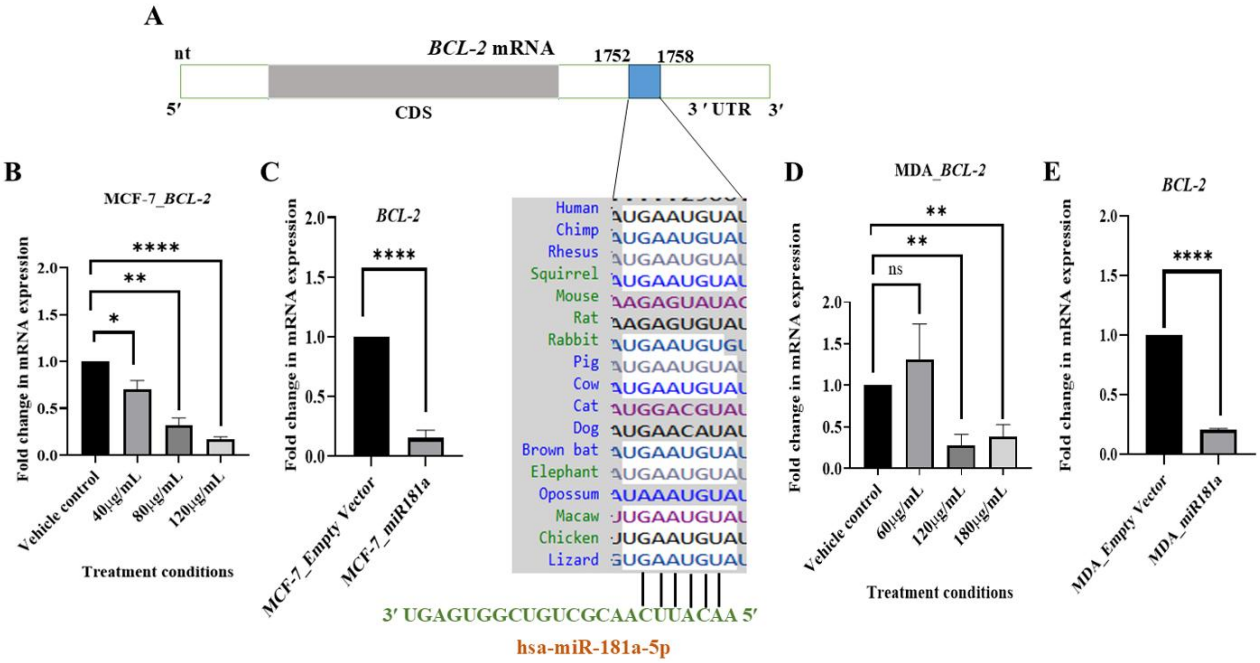



616

617 **Figure 1. *Lantana camara* upregulated the expression of miR-181a-5p in MCF-7 and MDA-**
618 **MB-231 cell lines**. (A), and (B) Bar plots showing the expression of miR-181a-5p in MCF-7 and
619 MDa-MB-231 cells. Data is plotted as mean ± SEM from three independent biological replicates.
620 Asterisks indicate statistically significant differences when compared to vehicle control
621 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ and ns signifies non-significant).

622

623 **Figure 2**



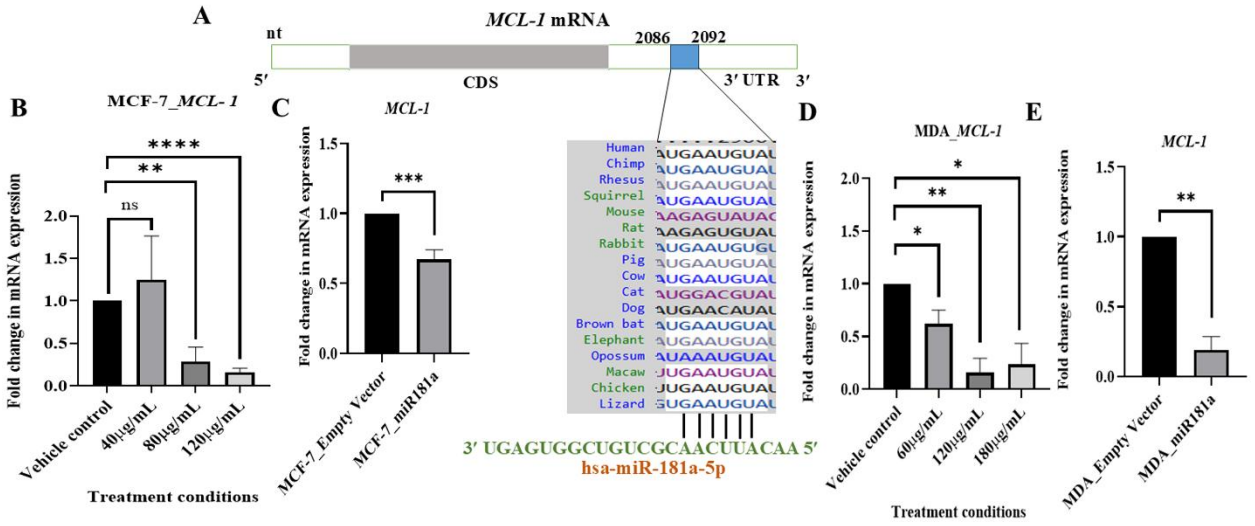

624

625 **Figure 2. mRNA level expression of *BCL-2* decreased upon *Lantana camara* treatment and**
626 **miR-181a-5p overexpression conditions in MCF-7 and MDA-MB-231 cells.** (A) Schematic
627 representation of in silico search of miR-181a-5p seed region in 3′ UTR of *BCL-2* mRNA and its
628 cross-species conservation. (B), and (D) bar plots showing the mRNA level expression of *BCL-2*
629 in MCF-7 and MDA-MB-231 under *Lantana camara* treatment conditions. (C) and (E) bar plots
630 showing the mRNA level expression of *BCL-2* in MCF-7 and MDA-MB-231 under miR-181a-5p
631 overexpression conditions. Data is plotted as mean ± SEM from three independent biological
632 replicates. Asterisks indicate statistically significant differences when compared to control
633 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ and ns signifies non-significant).

634

635

636 **Figure 3**

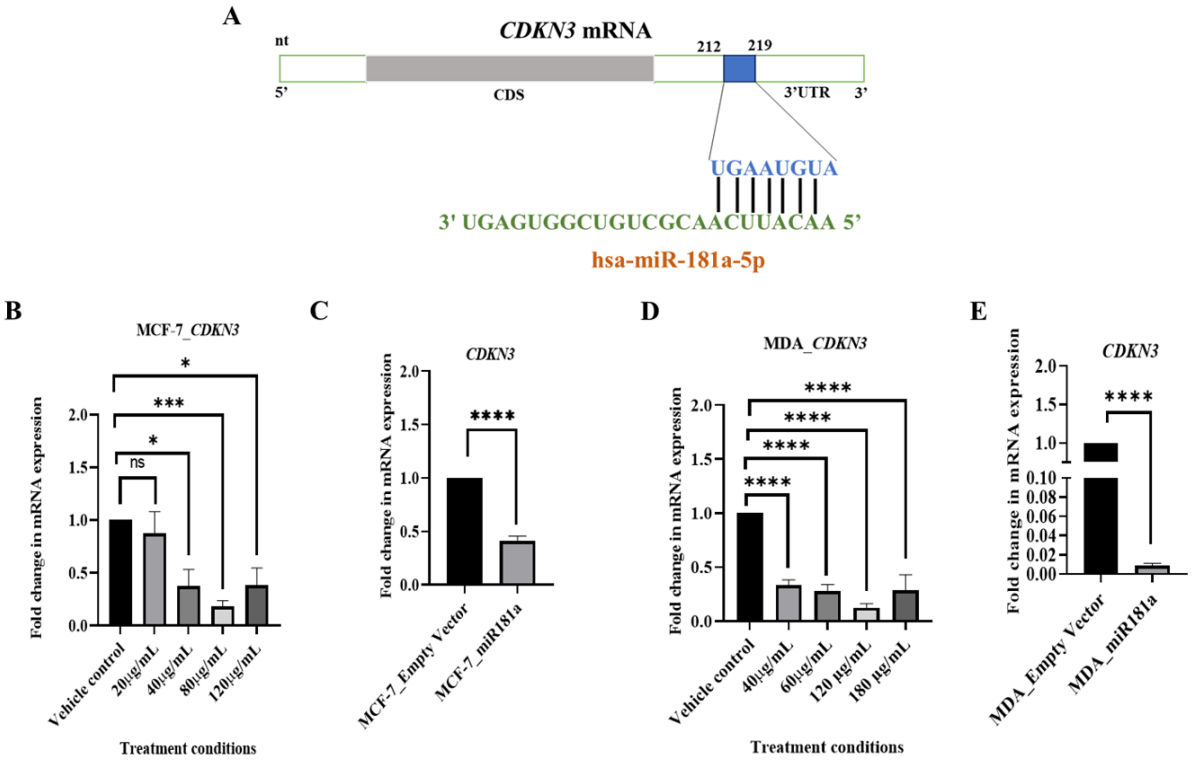



637

638 **Figure 3: mRNA level expression of *MCL-1* decreased upon *Lantana camara* treatment and**
639 **miR-181a-5p overexpression conditions in MCF-7 and MDA-MB-231 cells**. (A) Schematic
640 representation of in silico search of miR-181a-5p seed region in 3′ UTR of *MCL-1* mRNA and its
641 cross-species conservation. (B), and (D) bar plots showing the mRNA level expression of *MCL-1*
642 in MCF-7 and MDA-MB-231 under *Lantana camara* treatment conditions. (C), and (E) bar plots
643 showing the mRNA level expression of *MCL-1* in MCF-7 and MDA-MB-231 under miR-181a-5p
644 overexpression conditions. Data is plotted as mean ± SEM from three independent biological
645 replicates. Asterisks indicate statistically significant differences when compared to control
646 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ and ns signifies non-significant).

647

648 **Figure 4**

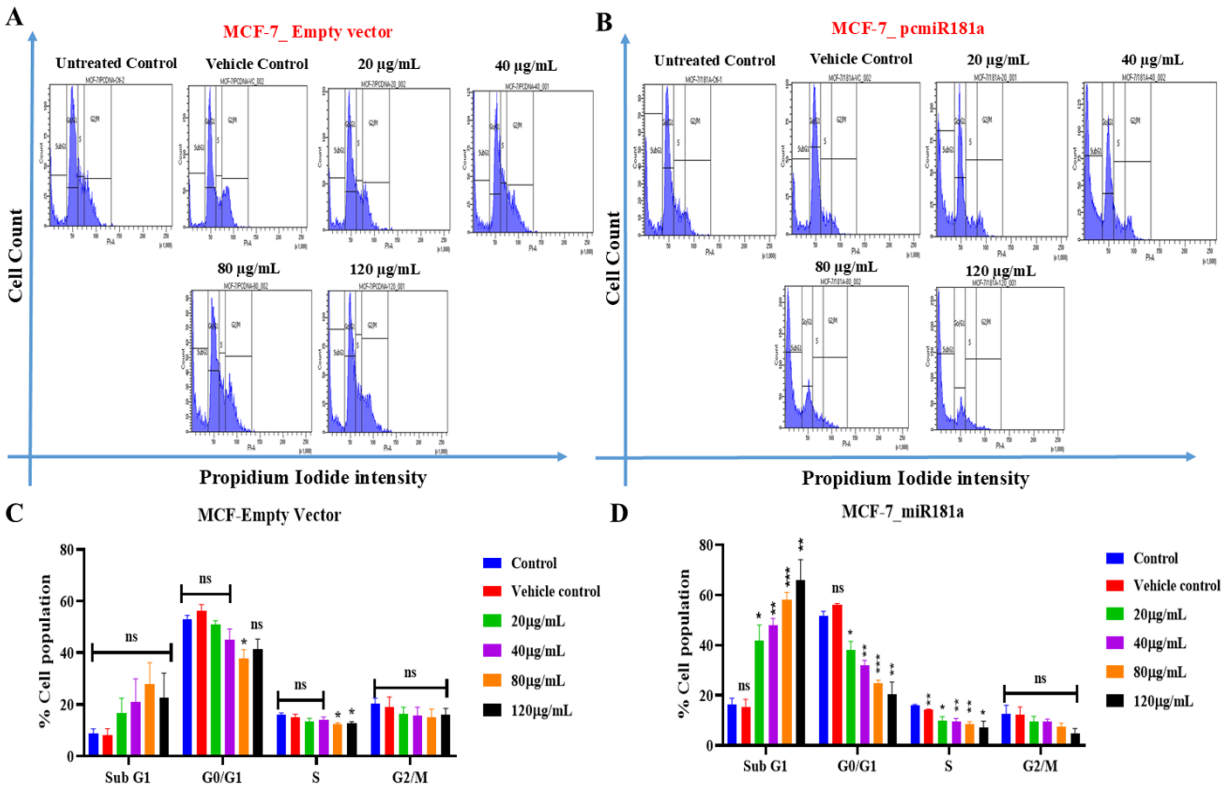



649

650 **Figure 4. mRNA level expression of *CDKN3* decreased upon *Lantana camara* treatment and**
651 **miR-181a-5p overexpression conditions in MCF-7 and MDA-MB-231 cells.** (A) Schematic
652 representation of in silico search of miR-181a-5p seed region in 3′ UTR of *CDKN3* mRNA. (B),
653 and (D) bar plots showing the mRNA level expression of *CDKN3* in MCF-7 and MDA-MB-231
654 under *Lantana camara* treatment conditions. (C), and (E) bar plots showing the mRNA level
655 expression of *CDKN3* in MCF-7 and MDA-MB-231 under miR-181a-5p overexpression
656 conditions. Data is plotted as mean ± SEM from three independent biological replicates. Asterisks
657 indicate statistically significant differences when compared to control (*$P < 0.05$; **$P < 0.01$;
658 ***$P < 0.001$; ****$P < 0.0001$ and ns signifies non-significant).

659

660 **Figure 5**

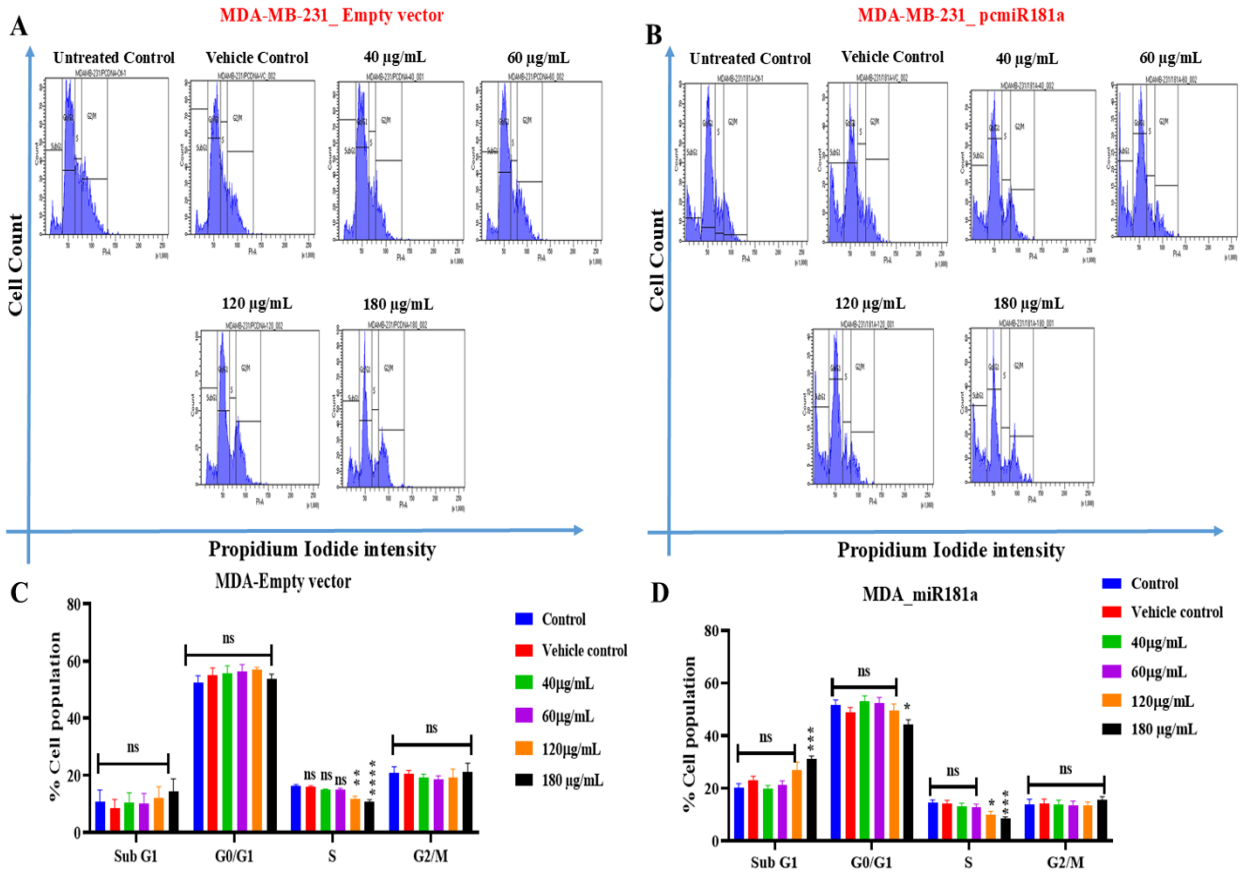



661

662 **Figure 5. Ectopic expression of miR-181a-5p in association with *Lantana camara* leaf extract**
663 **increased sub-G1 population in MCF-7 cells.** (A) and (B) are the representative images showing
664 the distribution of cells in sub-G1, G0/G1, S, and G2/M phases of the cell cycle under different
665 treatment conditions. (C) and (D) graphical representation of the data obtained from cell cycle
666 phase distribution assay. Data is plotted as mean ± SEM from three independent biological
667 replicates. Asterisks indicate statistically significant differences when compared to control
668 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and ns signifies non-significant).

669

670 **Figure 6**

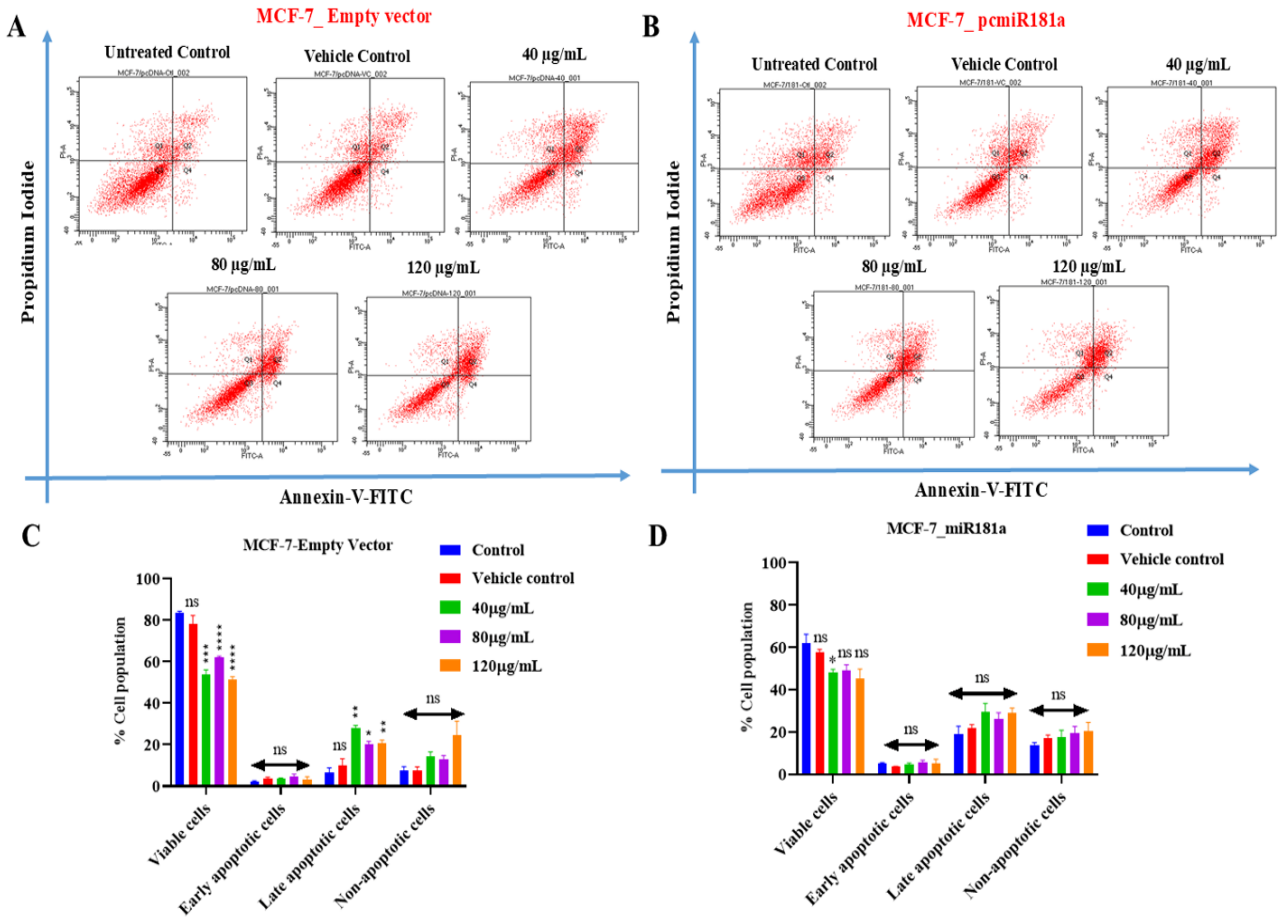



671

672 **Figure 6. Ectopic expression of miR-181a-5p in association with *Lantana camara* leaf extract**
673 **increased sub-G1 population in MDA-MB-231cells.** (A) and (B) are the representative images
674 showing distribution of cells in sub-G1, G0/G1, S and G2/M phases of cell cycle under different
675 treatment conditions. (C) and (D) graphical representation of the data obtained from cell cycle
676 phase distribution assay. Data is plotted as mean ± SEM from three independent biological
677 replicates. Asterisks indicate statistically significant differences when compared to control
678 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ and ns signifies non-significant).

679

680 **Figure 7**

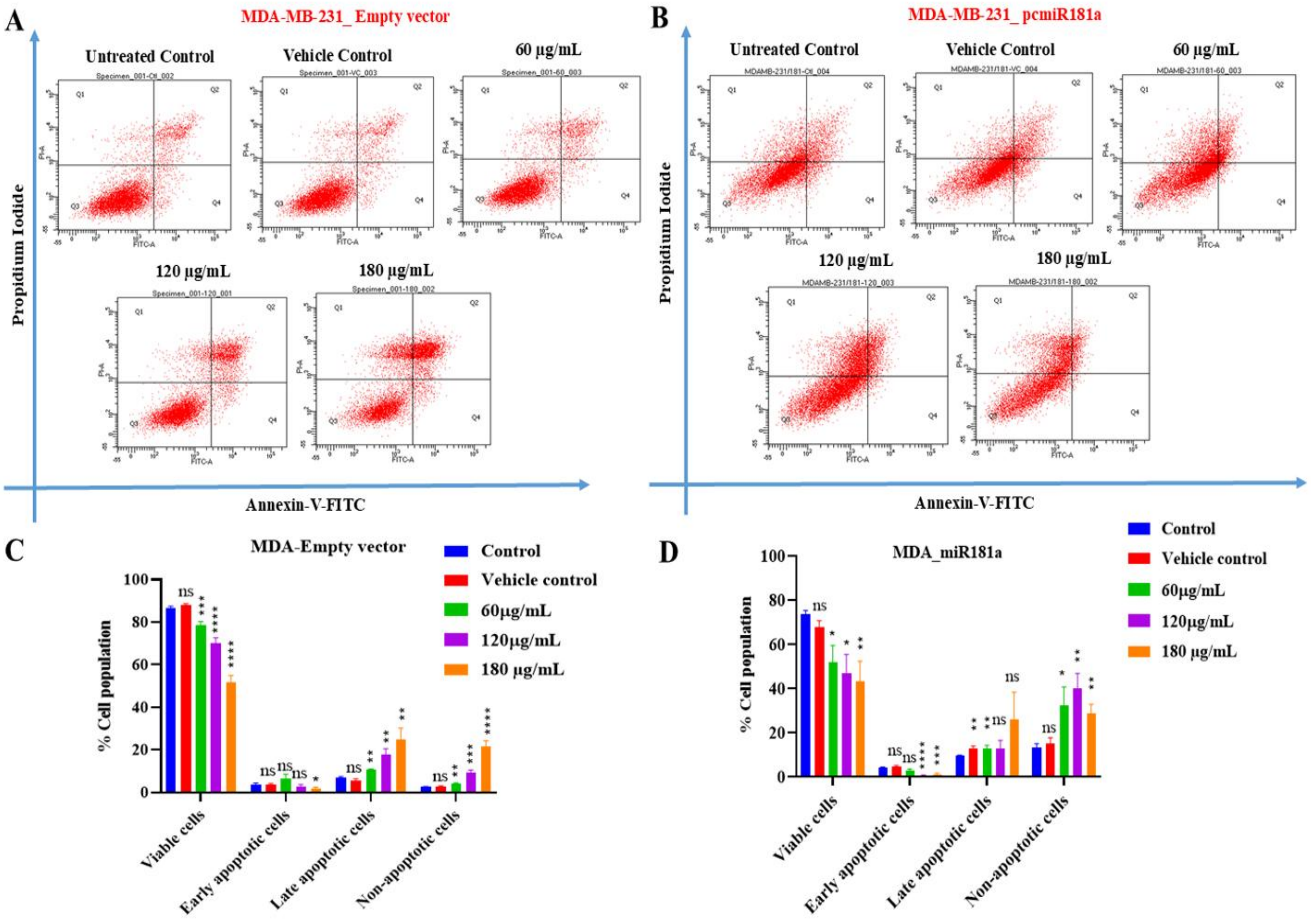



681

682 **Figure 7. Ectopic expression of miR-181a-5p in association with *Lantana camara* leaf extract**
683 **induced cell death in MCF-7 cells.** (A) and (B) are the representative scatter plots showing
684 distribution of viable (bottom left quadrant), early apoptotic (bottom right quadrant), late apoptotic
685 (upper right quadrant), and non-apoptotic (upper left quadrant) cell populations under different
686 treatment conditions. (C) and (D) graphical representation of the data obtained from cell death
687 assay. Data is plotted as mean ± SEM from three independent biological replicates. Asterisks
688 indicate statistically significant differences when compared to control (*$P < 0.05$; **$P < 0.01$ and
689 ns signifies non-significant).

690

691 **Figure 8**

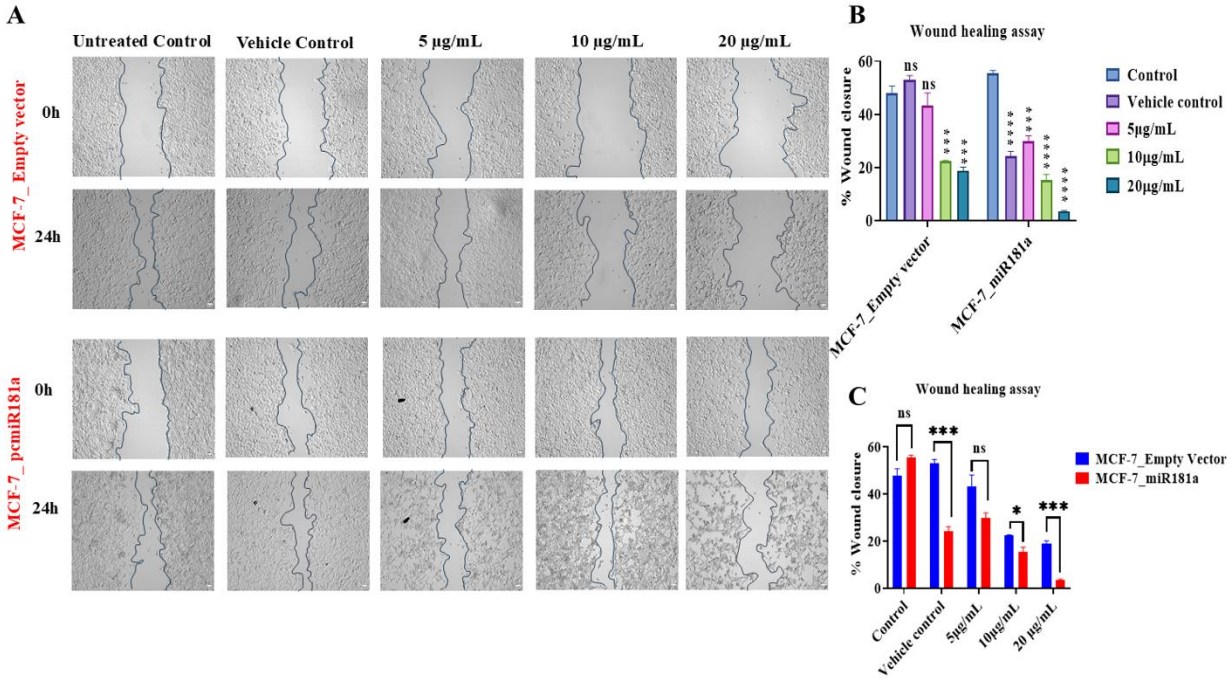



692

693 **Figure 8. Ectopic expression of miR-181a-5p in association with *Lantana camara* leaf extract**
694 **induced cell death in MDA-MB-231 cells.** (A) and (B) are the representative scatter plots
695 showing distribution of viable (bottom left quadrant), early apoptotic (bottom right quadrant), late
696 apoptotic (upper right quadrant), and non-apoptotic (upper left quadrant) cell populations under
697 different treatment conditions. (C) and (D) graphical representation of the data obtained from cell
698 death assay. Data is plotted as mean ± SEM from three independent biological replicates. Asterisks
699 indicate statistically significant differences when compared to control (*$P < 0.05$; **$P < 0.01$ and
700 ns signifies non-significant).

701

702 **Figure 9**

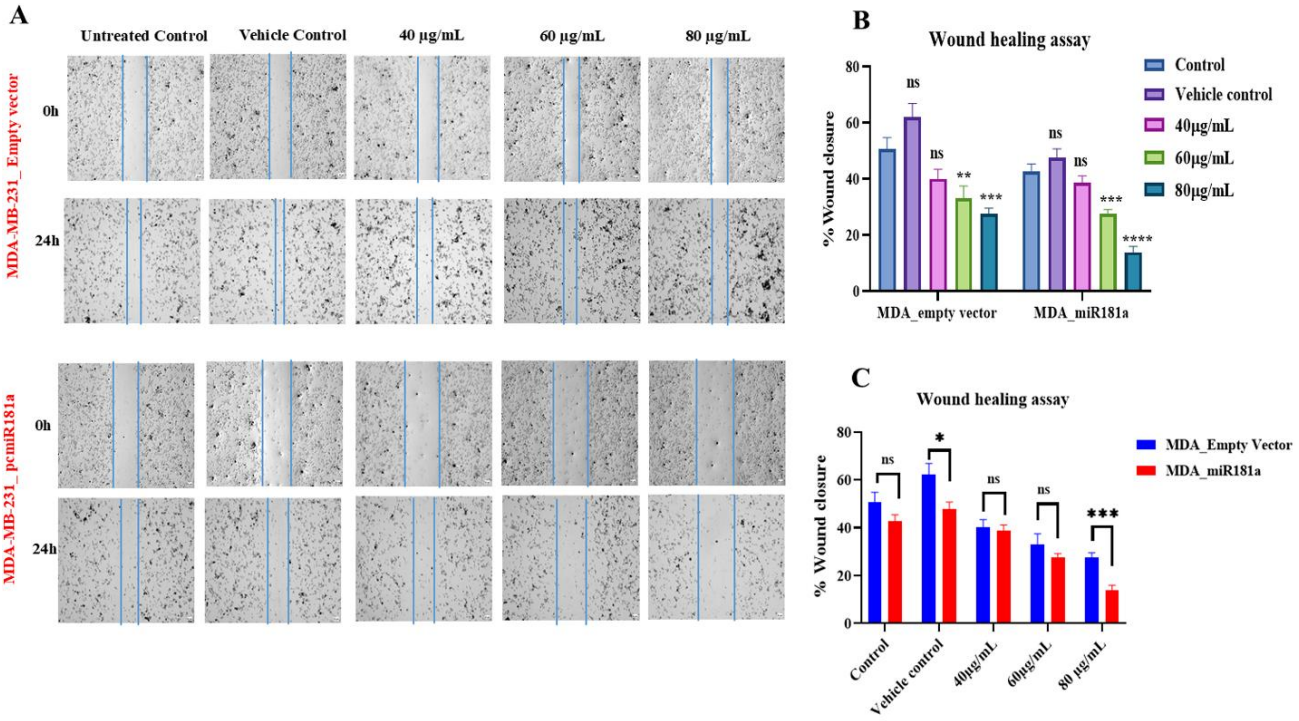



703

704 **Figure 9. Ectopic expression of miR-181a-5p in association with *Lantana camara* leaf extract**
705 **inhibited migration in MCF-7 cells.** (A) For the wound healing assay, untreated, vehicle-treated
706 and extract-treated MCF-7 cells (transfected with either empty vector (upper panel) or pcmiR181a
707 (lower panel)) were subjected to a scratch at monolayer condition and then incubated for another
708 24 hours. Wound closure was visualized using a phase contrast microscope, and images were
709 captured at 0 h and 24 h under 10X magnification. (B), (C) Bar diagram representing wound
710 closure potential of MCF-7 cells after 24h. Data is represented as means ± SEM of three
711 independent biological replicates, where * is $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and ns signifies
712 non-significant.

713

714 **Figure 10**

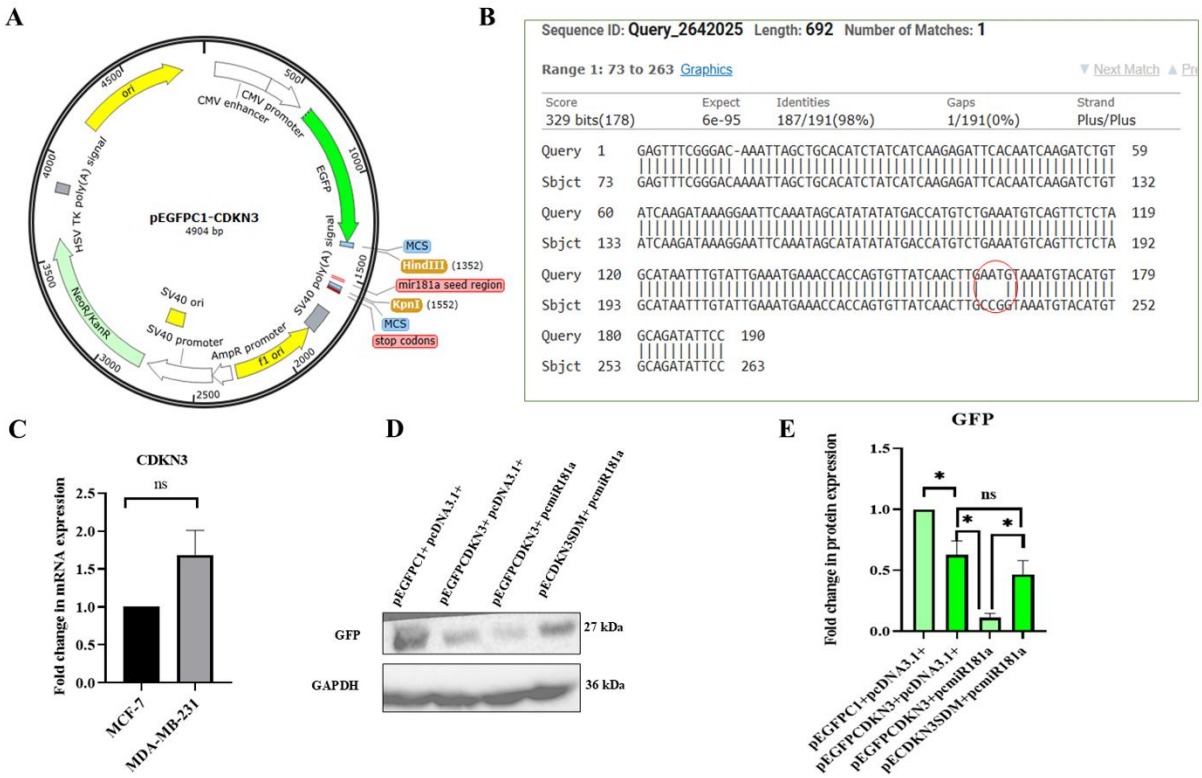



715

716 **Figure 10. Ectopic expression of miR-181a-5p in association with *Lantana camara* leaf extract**
717 **inhibited migration in MDA-MB-231 cells.** (A) For the wound healing assay, untreated, vehicle-
718 treated and extract-treated MDA-MB-231 cells (transfected with either empty vector (upper panel)
719 or pcmiR181a (lower panel)) were subjected to a scratch at monolayer condition and then
720 incubated for another 24 hours. Wound closure was visualized with a phase contrast microscope,
721 and images were captured at 0h and 24h under 10X magnification. (B), (C) Bar diagram
722 representing wound closure potential of MDA-MB-231 cells after 24h. Data is represented as
723 means ± SEM of three independent biological replicates, where * is $P \leq 0.05$, ** $P \leq 0.01$, ***
724 $P \leq 0.001$, and ns signifies non-significant.

725

726 **Figure 11**




727

728 **Figure 11: miR-181a-5p targets the 3′ UTR of the *CDKN3* mRNA.** (A) Vector map of
729 pEGFPCDKN3 with cloned 3′ UTR of *CDKN3* mRNA with miR-181a-5p seed region. (B)
730 Alignment of wild-type *CDKN3* 3′ UTR sequence with mutated 3′ UTR in the miR-181a-5p seed
731 region (red circle). (C) Relative expression of *CDKN3* mRNA in MCF-7 and MDA-MB-231. (D)
732 Representative Western blot pictures for GFP and GAPDH (loading control) (E) Bar plot showing
733 the results of Western blot. Data is plotted as mean ± SEM from three independent biological
734 replicates. Asterisks indicate statistically significant differences when compared to the control
735 (*$P < 0.05$, ns signifies non-significant).

736