

996 Antibiofilm and Anticancer Activities of Unripe and Ripe *Azadirachta indica*
 997 (neem) Seed Extracts

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999 **Submission ID** -bba31bf8-4a90-4c1a-81fb-35a8e398c33e

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1001 **Supplementary Section:**

1002 **Table S1.** OD values at 570 nm wavelength for the determination of MBIC of unripe and ripe
 1003 neem seed extracts against *S. aureus* and *V. cholerae*. OD_{control} for *S.aureus* was 1.241 and,
 1004 that for *V.cholerae* was 1.132.

| Doses (µg/mL) | OD ₅₇₀ | | | |
|------------------|--------------------------|-------------------|------------------------|-------------------|
| | Unripe neem seed extract | | Ripe neem seed extract | |
| | <i>S. aureus</i> | <i>V. cholera</i> | <i>S. aureus</i> | <i>V. cholere</i> |
| 50 | 0.795 | 0.803 | 0.645 | 0.656 |
| 75 | 0.732 | 0.758 | 0.595 | 0.598 |
| 100 | 0.583 | 0.701 | 0.521 | 0.532 |
| 200 | 0.482 | 0.589 | 0.459 | 0.407 |
| 300 | 0.397 | 0.521 | 0.323 | 0.271 |
| 400 | 0.285 | 0.441 | 0.173 | 0.215 |
| 500 | 0.198 | 0.362 | 0.087 | 0.136 |

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1006 **Table S2.** OD values at 570 nm wavelength for the determination of MBEC of unripe and
 1007 ripe neem seed extracts against *S. aureus* and *V. cholerae*. OD_{control} for *S.aureus* was 1.214
 1008 and, that for *V.cholerae* was 1.125.

| Doses (µg/mL) | OD ₅₇₀ | | | |
|------------------|--------------------------|-------------------|------------------------|-------------------|
| | Unripe neem seed extract | | Ripe neem seed extract | |
| | <i>S. aureus</i> | <i>V. cholera</i> | <i>S. aureus</i> | <i>V. cholere</i> |
| 50 | 0.966 | 0.912 | 0.869 | 0.753 |
| 150 | 0.882 | 0.855 | 0.783 | 0.687 |
| 300 | 0.844 | 0.753 | 0.598 | 0.641 |
| 500 | 0.587 | 0.697 | 0.403 | 0.472 |

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|------|-------|-------|-------|-------|
| 700 | 0.379 | 0.517 | 0.269 | 0.271 |
| 1000 | 0.318 | 0.203 | 0.085 | 0.101 |

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1011 **Table S3.** Fluorescence intensities of Ui, Ri, Uii, Rii, Uiii and Riii in respect to their control
 1012 system from Fig. 6, were measured as Relative Fluorescent Unit (RFU) using imageJ
 1013 software, and fold changes from their respective control system have been tabulated.

| | Bacteria | Fluorecence intensity change from their corresponding control (fold change) |
|------|--------------------|--|
| Ci | <i>S. aureus</i> | |
| | <i>V. cholerae</i> | |
| Ui | <i>S. aureus</i> | 1.08 |
| | <i>V. cholerae</i> | 1.03 |
| Ri | <i>S. aureus</i> | 1.16 |
| | <i>V. cholerae</i> | 1.07 |
| Cii | <i>S. aureus</i> | |
| | <i>V. cholerae</i> | |
| Uii | <i>S. aureus</i> | 1.35 |
| | <i>V. cholerae</i> | 1.08 |
| Rii | <i>S. aureus</i> | 1.47 |
| | <i>V. cholerae</i> | 1.31 |
| Ciii | <i>S. aureus</i> | |
| | <i>V. cholerae</i> | |
| Uiii | <i>S. aureus</i> | 2.06 |
| | <i>V. cholerae</i> | 1.59 |
| Riii | <i>S. aureus</i> | 3.14 |
| | <i>V. cholerae</i> | 2.56 |

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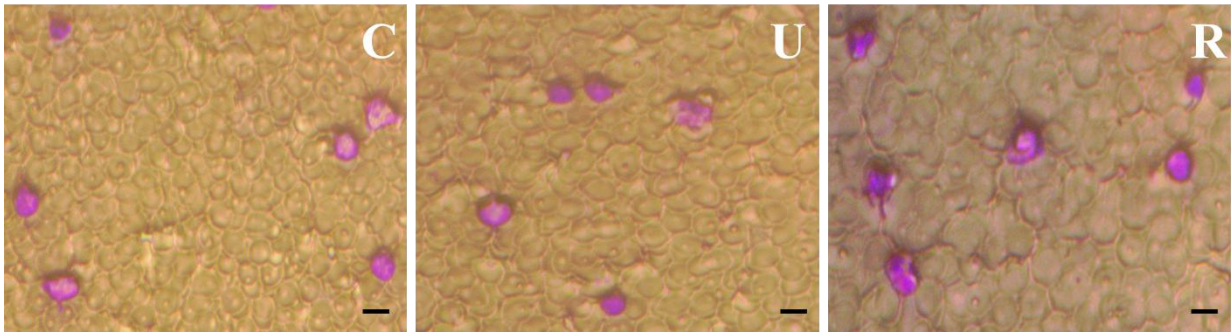
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1026 **Table S4.** Fold change of live/dead bacterial numbers comparing with their respective control
1027 system from Fig. 7, have been tabulated. ImagJ software was used to measure this fold
1028 changes.

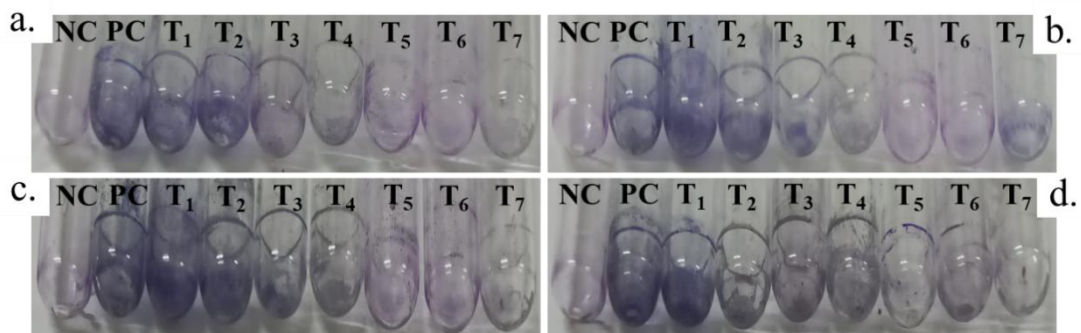
| | | Live bacterial cell number | Reduction of live bacterial cell number (fold change) | | Dead bacterial cell number | Increment of dead bacterial cell number (fold change) |
|--------------------|----|-----------------------------------|--|-----|-----------------------------------|--|
| <i>S. aureus</i> | Ci | 953 | | Cii | 98 | |
| | Ui | 582 | 1.63 | Uii | 468 | 4.97 |
| | Ri | 130 | 7.33 | Rii | 822 | 8.38 |
| <i>V. cholerae</i> | Ci | 788 | | Cii | 144 | |
| | Ui | 502 | 1.56 | Uii | 388 | 2.69 |
| | Ri | 170 | 4.63 | Rii | 898 | 6.23 |



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1030 **Fig. S1.** Leishman-stained films of human blood lymphocytes in the cytotoxicity study.
 1031 Lymphocytes showing no detectable morphological change after the treatment with unripe
 1032 (U) and ripe (R) neem seed extracts at their highest dose (20 mg) applied. Lymphocytes
 1033 treated without any seed extracts were used as positive control (C). Scale bar: 10 μ m.

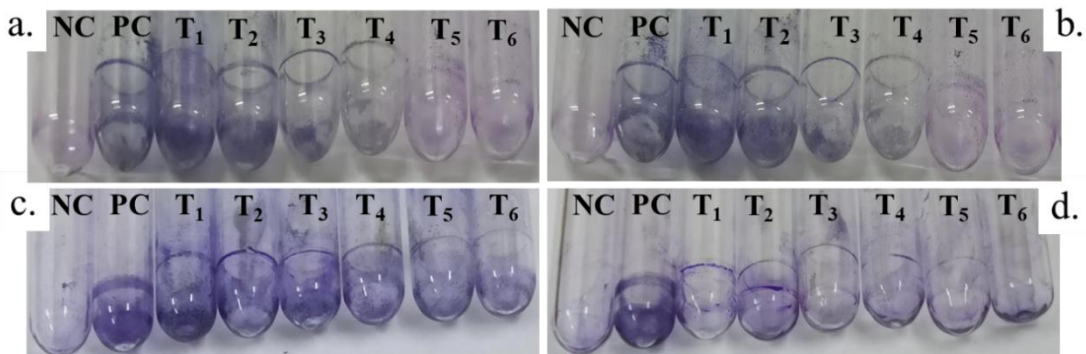
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1036 **Fig. S2.** Crystal violet biofilm assay in borosilicate tubes for the estimation of MBIC of
 1037 unripe (a & b) and ripe (c & d) neem seed extracts against *S. aureus* (a & c) and *V. cholerae*
 1038 (b & d). NC: Negative control containing only LB broth; PC: Positive control containing LB
 1039 broth with the respective bacteria; T₁-T₇: LB broth containing different concentrations of
 1040 unripe and ripe neem seed extracts, respectively for each bacteria used in this study.

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1043 **Fig. S3.** Crystal violet biofilm assay in borosilicate tubes for the estimation of MBEC of
 1044 unripe (a & b) and ripe (c & d) neem seed extracts against *S. aureus* (a & c) and *V. cholerae*
 1045 (b & d). NC: Negative control containing only LB broth; PC: Positive control containing LB

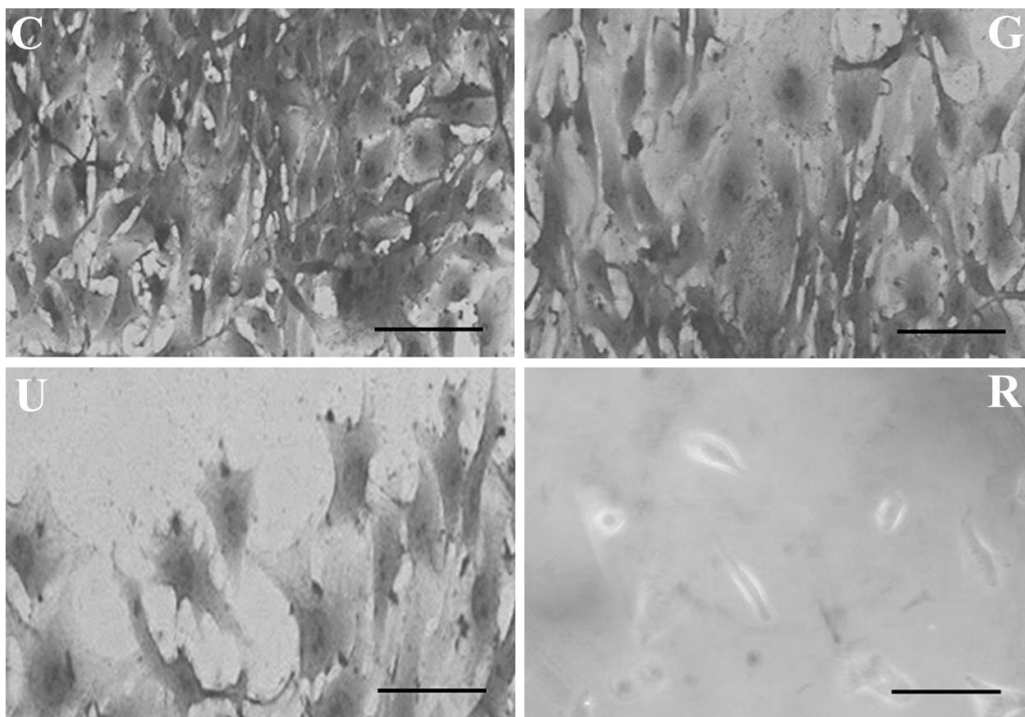
1046 broth with the respective bacteria; T₁-T₇: Bacterial preformed biofilm treated with different
1047 concentrations of unripe and ripe neem seed extracts, respectively for each bacteria used in
1048 this study for biofilm eradication.

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1054 **Fig. S4.** Phase-contrast microscopic studies of MDA-MB-231 breast cancer cells upon
1055 treatment with neem seed extracts. Morphological changes of MDA-MB-231 breast cancer
1056 cells were recorded as treated with control drug gemcitabine (G), unripe (U) and ripe (R)
1057 neem seed extracts for 24 h at their IC₅₀ doses. Cancer cells without addition of drug or seed
1058 extracts were considered as positive control (C). Cells were cultured upto 70-80%
1059 confluence before addition of the drug and/or neem seed extracts. Scale bar: 50 μ m.

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