# Supplementary information

## Larvicidal, super hydrophobic and antibacterial properties of herbal nanoparticles from Acalypha indica for biomedical applications

S. Karthik<sup>a</sup>, R. Suriyaprabha<sup>a</sup>, M. Vinoth<sup>a</sup>, S. R. Srither<sup>a</sup>, P. Manivasakan<sup>a</sup>, V. Rajendran<sup>a</sup>,\* Suresh Valiyaveettil<sup>b</sup>

a.Centre for Nano Science and Technology, K. S. Rangasamy College of Technology, Tiruchengode-637215, Tamil Nadu, India

b.Department of Chemistry, National University of Singapore, Singapore 117543, Singapore

\*Corresponding author

Tel: 91-4288-274741-4, 274880; Fax: 91-4288-274880 (Direct), 274860

E-mail: veerajendran@gmail.com

| Sample code                 | Tensile strength |                | Tear strength  |                |   |                 |                   |   |                             |
|-----------------------------|------------------|----------------|----------------|----------------|---|-----------------|-------------------|---|-----------------------------|
|                             | Warp<br>(kg)     | Weft<br>(kg)   | Warp<br>(kg)   | Weft<br>(kg)   | Air<br>Permeability<br>(cc s <sup>-1</sup> cm <sup>-2</sup> ) | CRA<br>(W+F)°   | Thickness<br>(mm) | Bursting<br>strength<br>kg cm <sup>-2</sup> | GSM<br>g sq m <sup>-1</sup> |
| Before wash                 |                  |                |                |                |   |                 |                   |   |                             |
| CF                          | $46.1\pm0.1$     | $41.3\pm0.1$   | 21.2 ±0.1      | $17.9 \pm 0.1$ | $97.42\pm0.4$   | $98.6\pm0.2$    | $0.29\pm0.1$      | $6.31\pm0.5$                                | $120.5\pm0.5$               |
| CF-Chi                      | $47.9\pm0.1$     | $42.8\pm0.1$   | $23.9\pm0.2$   | $19.8 \pm 0.1$ | $99.97\pm0.2$   | $103.4 \pm 0.4$ | $0.33\pm0.5$      | $6.62\pm0.6$                                | $127.1\pm0.5$               |
| CF- AINp-Chi                | $48.5\pm0.4$     | $44.6 \pm 0.3$ | $27.1 \pm 0.1$ | $22.1 \pm 0.1$ | $52.36 \pm 0.2$   | $104.2\pm0.6$   | $0.34\pm0.5$      | $7.45\pm0.5$                                | $135.4 \pm 0.2$             |
| After 5 <sup>th</sup> wash  |                  |                |                |                |   |                 |                   |   |                             |
| CF                          | $33.2 \pm 0.1$   | $29.2 \pm 0.1$ | $17.3\pm0.3$   | $13.6\pm0.2$   | $98.47\pm0.2$   | $75 \pm 0.2$    | $0.27 \pm 0.1$    | $6.30\pm0.1$                                | $119.1 \pm 0.1$             |
| CF-Chi                      | $35.1 \pm 0.1$   | $32.6\pm0.2$   | $21.4\pm0.2$   | $14.1\pm0.1$   | $65.93\pm0.1$   | $92\pm0.2$      | $0.32\pm0.5$      | $6.51\pm0.1$                                | $125.5\pm0.5$               |
| CF- AINp-Chi                | $39.2\pm0.4$     | $33.2\pm0.3$   | $22.5\pm0.1$   | $15.8 \pm 0.1$ | $60.02\pm0.5$   | $95.3\pm0.2$    | $0.33\pm0.5$      | $6.18\pm0.3$                                | $128.6\pm0.5$               |
| After 10 <sup>th</sup> wash |                  |                |                |                |   |                 |                   |   |                             |
| CF                          | $26.3\pm0.3$     | $20.4\pm0.4$   | $15.4\pm0.4$   | $10.2\pm0.4$   | $99.97 \pm 0.1$   | $45.6\pm0.2$    | $0.25\pm0.1$      | $6.1\pm0.1$                                 | $118.5\pm0.5$               |
| CF-Chi                      | $29.8\pm0.1$     | $23.1 \pm 0.1$ | $16.1 \pm 0.1$ | $11.6 \pm 0.1$ | $72.44 \pm 0.1$   | $61.5 \pm 0.1$  | $0.30 \pm 0.1$    | $6.3 \pm 0.2$                               | $123.3 \pm 0.1$             |
| CF- AINp-Chi                | $30.2\pm0.2$     | $24.2 \pm 0.1$ | $18.6 \pm 0.1$ | $12.5 \pm 0.2$ | $70.12 \pm 0.1$   | $60.8\pm0.1$    | $0.31\pm0.5$      | $6.5\pm0.5$                                 | $127.2 \pm 0.2$             |

**Table (i):** Physical properties of un-coated and nanoparticles-chitosan coated cotton fabrics.

#### i) Antimicrobial assessment of AINPs nanoparticles and nanoparticles coated fabrics

The antibacterial activities of CF-Chi and CF-AINPs-Chi fabrics are shown in Fig. 1. As expected, a higher area for the zone of inhibition observed for CF-AINp-Chi fabrics confers more inhibitory action (31.58 $\pm$ 0.06 and 25.24 $\pm$ 0.34) than CF-Chi fabrics (Table ii). However, in case of *S. aureus*, the difference in the magnitude of zone of inhibition is slightly higher (34 $\pm$ 0.08 and 26.81 $\pm$ 0.07). In case of CF fabrics there is no evidence for the formation of inhibition (Fig. i). In the control experiments, methanol solvent and aqueous extraction of neem nanoparticles showed a zone of inhibition of 20 and 27 mm, respectively, against *S. aureus* bacterium. Similarly, inhibition zone areas of 14 and 20 mm were found for methanol and aqueous extraction of neem nanoparticles, respectively, against the growth of *E. coli*<sup>1</sup>. A close observation of the results indicates that CF-AINP-Chi fabric is efficient against both *E. coli* and *S. aureus*. The obtained higher antimicrobial action of AINPs sample is due to the presence of phytochemical compounds present in the neem leaves, which include several alkaloids, tannins, saponins and steroids<sup>2</sup>. The above bacterial susceptibility investigations using plant extract and herbal nanoparticles confer the exotic medicinal properties and therapeutic uses of AINPs.

|--|

|                | Zone of inhibition (mm)  |                             |              |  |  |  |
|----------------|--------------------------|-----------------------------|--------------|--|--|--|
| Test organisms | CF-Chi coated<br>fabrics | CF- AINp-Chi coated fabrics | CF (Control) |  |  |  |
| E. coli        | $25.45 \pm 0.34$         | $31.58 \pm 0.06$            | -            |  |  |  |
| S. aureus      | $26.81 \pm 0.07$         | $34.01 \pm 0.08$            | -            |  |  |  |

#### ii) Bacterial reduction test

The herbal nanocomposite coated and un-coated fabrics were cut into round shape as per the standard size (10 mm radius), as stated by American Association of Textile Chemists and Colorists (AATCC). The samples were covered with sterile AATCC bacteriostasis broth (10<sup>-4</sup> CFU/ml) for the inoculation of loopful test organisms. Initial cell concentration was calculated using viable cell count method. The test samples were incubated at 37 °C for 18 h; final number of cells in control and the test samples were calculated using a viable cell count method. After incubation, the number of colonies in each dilution plates was counted. The percentage in bacterial reduction percentage (%) was calculated as  $(A - B)/A \times 100$  where A is the initial number of cells and B the final number of cells.

#### iii) Percentage reduction test (AATCC 100) and washing durability for coated fabrics

The quantitative assessment of the antibacterial activity for the CF and CF-AINp-Chi fabrics after percentage reduction test evaluation is shown in Table iii. The percentage reduction test of organisms treated with CF-AINp-Chi fabrics before and after washing is 99 and 93 %, respectively, while the same for CF-Chi fabrics were found to be 77 and 70 %, respectively. The percentage reduction test did not show significant bactericidal activity for CF fabric. After 5<sup>th</sup> and 10<sup>th</sup> washings, the antibacterial activity was reduced owing to the removal of herbal nanoparticles from the fabric surface. The wash durability of CF-AINp-Chi fabrics after 10<sup>th</sup> wash is found to be high which confirms the enhanced functional properties of herbal nanoparticles coated fabrics.

|                       | Bacterial reduction percentage (%) |               |                             |               |  |  |  |  |
|-----------------------|------------------------------------|---------------|-----------------------------|---------------|--|--|--|--|
| No.of<br>washes       | CF-Chi coate                       | ed fabrics    | CF- AINp-Chi coated fabrics |               |  |  |  |  |
|                       | E. coli                            | S. aureus     | E. coli                     | S. aureus     |  |  |  |  |
| 0 wash                | $81 \pm 0.84$                      | $77 \pm 0.26$ | $94\pm0.76$                 | $99 \pm 0.82$ |  |  |  |  |
| 5 <sup>th</sup> wash  | $69\pm0.92$                        | $59 \pm 0.16$ | $80 \pm 0.74$               | 81 ± 0.79     |  |  |  |  |
| 10 <sup>th</sup> wash | $41 \pm 0.12$                      | $35 \pm 0.01$ | $47\pm0.06$                 | $45 \pm 0.12$ |  |  |  |  |

Table (iii): Bacterial reduction percentage of Azadirachta indica chitosan coated fabrics.



**Fig. i.** Antimicrobial activity of un-coated (A), CF-Chi (B) and CF-AINP-Chi (C) coated fabrics) against *E. coli* (a) and *S. aureus* (b).

## iv) Characterization of un-coated and coated fabrics

The surface morphological features and EDS analysis of un-coated fabrics are shown in Fig. ii a & b. The SEM image and EDX of the chitosan coated cotton fabrics (CF-Chi) shows cluster-type polymer structures on the fiber surface (Fig. ii c & d). The chitosan polymer is strongly adhered to the surface of the fabrics even after 5<sup>th</sup> and 10<sup>th</sup> washes (Fig. iii. a & iv. a).



Fig. ii. SEM (a, c, e) and EDX (b, d, f) images of un-coated (a), CF-Chi (c) and CF-AINp-Chi (e) coated fabric before wash. The wash durability (fastness) of CF-Chi fabric surface is further confirmed through EDS measurements Fig. iii. b & iv b. Fig. iii. c & d and Fig. iv. c & d confirms the presence, uniform distribution and washes durability of 5<sup>th</sup> and 10<sup>th</sup> washes AINPs-Chi nanocomposite particle and their elemental composition analysis on coated fabrics.



Fig. iii. SEM (a, c) and EDX (b, d) images of CF-Chi (top) and CF-AINPs-Chi (bottom) coated fabrics after 5<sup>th</sup> washings.

The above observation indicates that the CF-Chi and CF-AINPs-Chi fabrics reveal the strong adhesion on the fabrics, even after consequent washes. Nevertheless, the percentage of nanoparticles coated on the fabric decreases significantly with increase in number of washings. This is again supported by the changes in measured thickness of the CF, CF-Chi and CF-AINPs-Chi fabrics before and after wash results of herbal nanoparticles<sup>3</sup>. In many applications such as wound dressing, such antimicrobial fabrics are employed for one time use only.



# **Fig. iv.** SEM (a, c) and EDX (b, d) images of CF-Chi (top) and CF-AINPs-Chi (bottom) coated fabrics after 10<sup>th</sup> washings.

In addition, the coating thickness of nanoparticles on fabric reflects the air permeability of fabric<sup>4</sup>. Air permeability test on coated fabrics showed significant reduction (45%) in air permeability for CF-AINPs-Chi when compared to that of CF fabric and CF-Chi fabric. The observed reduction in air permeability is due to the absorption of composite materials in between the fibers of the fabric, which reduces the passage of air. Similarly, the air permeability of the CF-AINPs-Chi fabric was increased to 61% and 72% after 5<sup>th</sup> and 10<sup>th</sup> washes, respectively, while the chitosan coated fabric showed an increase up to 70% and 76%, respectively. This is attributed to the removal of the coatings from the fabric surface.

The mechanical properties of the CF, CF-Chi and CF-AINPs-Chi fabrics are determined and correlated with the functional properties of the fabrics. The longitudinal and transverse weaves are analyzed using tensile and tear strength for chitosan coated and *Azadirachta indica* chitosan coated fabrics. Before washing, the breaking weight of interstices (warp and weft yarn) values for tensile and tear strengths of CF-AINPs-Chi fabric are increased as compared to CF and CF-Chi fabrics. Generally, the overall tensile and tear strength for the fabrics specimen CF-Chi and CF-AINPs-Chi is much higher than the CF fabric (Table i). This is due to the sharing of the load by the coated nanoparticle on the fabrics which result an enhancement in tensile strength. Similarly, the CF-AINPs-Chi fabrics again compliments with higher bursting strength of CF and CF-Chi fabrics. The nanoparticles are strongly adhered to the surface of cotton fabrics which resulted in an increase in crease recovery angle of fabrics (CRA). The CRA of CF-AINPs-Chi fabric shows  $104^{\circ} \pm 0.76^{\circ}$  which is higher than the CF and CF-Chi fabric. The observed higher magnitude of crease recovery resulted from higher absorption of AINPs-Chi nanoparticles on the surface of fabrics without imparting stiffness to the fabric. As expected the CRA of the CF-AINPs-Chi fabrics is reduced after 5<sup>th</sup> and 10<sup>th</sup> washes due to the loss of coating with increase in number of washings.

### References

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