**Appendix**

**Investigation of the loading and loading efficiency of microcapsules**

Absorbance at 243 nm

A portion of 0.05 g of Microcapsules + 1000 µL of 70% Ethanol

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test sample | Absorbance at 243nm | Concentration (µL/mL) | Loading of microcapsules (µL/g) | Loading efficiency (%) |
| 1 | 4.653 | 120.885 | 2820.64 | 78.5 |
| 2 | 4.678 | 125.70 | 2933 | 81.64 |
| 3 | 4.710 | 131.846 | 3076.41 | 85.63 |

Total dry weight of lime oil microcapsules =1.67 g

Total loading of lime oil = 1.67 g × 2820.64 µL/g = 4710.469 µL

Loading efficiency = 4710.469 µL × 100% = 78.5 %

6000 µL

**Folin Ciocalteu Assay**

**Table 2.1.** UV-Visible absorbance at 750 nm for pyrogallol concentration series

|  |  |
| --- | --- |
| Pyrogallol concentration (ppm) | Absorbance at 750 nm |
| 50 | 0.055 |
| 100 | 0.117 |
| 200 | 0.239 |
| 400 | 0.463 |
| 600 | 0.693 |
| 800 | 0.855 |
| 1000 | 1.068 |

**Figure.** Pyrogallol standard curve

**Table 2.2.** Antioxidant capacity (AOC) of unencapsulated lime oil and lime oil microcapsules

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test sample | AOC | | | |
| Trail 1 | Trail 2 | Trail 3 | Average |
| Unencapsulated lime oil | 916 | 893 | 939 | 916 |
| Crushed microcapsules | 1280 | 1348 | 1382 | 1336 |

**Brine shrimp lethality assay to test the cytotoxic activity of lime oil microcapsules**

**Table 3.** Percentage mortality of brine shrimp nauplii against time for different lime oil samples

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hour | Negative control (solvent) | | | | Intact microcapsules | | | | Crushed microcapsules | | | | Unencapsulated lime oil | | | |
|  | 1 | 2 | 3 | A | 1 | 2 | 3 | A | 1 | 2 | 3 | A | 1 | 2 | 3 | A |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | 10 | 8 | 8 | 9 | 8 | 8 | 9 | 8 | 8 |
| 3 | 10 | 10 | 9 | 10 | 9 | 9 | 10 | 9 | 8 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 4 | 9 | 9 | 8 | 9 | 9 | 8 | 9 | 9 | 7 | 6 | 7 | 7 | 6 | 5 | 6 | 6 |
| 5 | 9 | 8 | 9 | 9 | 8 | 8 | 9 | 8 | 6 | 5 | 7 | 6 | 5 | 4 | 4 | 4 |
| 6 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 | 5 | 3 | 4 | 4 | 3 | 2 | 2 | 2 |
| 7 | 8 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| 8 | 7 | 7 | 7 | 7 | 6 | 6 | 7 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

A – Average

**Antibacterial activity of lime oil microcapsules**

Diameter of the inhibition zone (mm) =

d1

d2

**Table 4.** Diameter of the inhibition zones for bacterial species with different lime oil samples

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Bacterial species | Diameter of the inhibition zones (mm) | | | | | | | | | | | | | |
| Positive control  (Gentamicin) | | | | | Unencapsulated lime oil | | | | | 0.25 g/mL crushed  Microcapsules | | | |
| Trial no: | 1 | 2 | 3 | | A | 1 | 2 | 3 | | A | 1 | 2 | 3 | A |
| *Escherichia coli* | 23.5 | 24 | 23 | 24 | | 7 | 7 | 8 | 8 | | 8 | 8 | 8 | 8 |
| *Bacillus cereus* | 24 | 22 | 23.5 | 23 | | 9 | 7 | 8 | 8 | | 8 | 9 | 8 | 8 |
| *Salmonella typhimurium* | 24 | 24 | 24 | 24 | | 7 | 7 | 8 | 7 | | 7 | 7 | 7 | 7 |
| *Staphylococcus aureus* | 25 | 24 | 24 | 24 | | 8 | 9 | 9 | 8 | | 7 | 7.5 | 7.5 | 7 |

A - Average

**Investigation of Anti-bacterial activity of microcapsule incorporated cotton fabric**

**Table 5.** Antibacterial assay results according to prepared cotton fabric

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Bacterial species | Diameter of the inhibition zones (mm) | | | | | | | | | | | |
| Positive control (Gentamicin)  25 µL/mL | | | | Lime oil incorporated cotton fabric | | | | | | | |
| Fabric with intact microcapsules | | | | Fabric subjected to mechanical crushing | | | |
| Trail no: | 1 | 2 | 3 | A | 1 | 2 | 3 | A | 1 | 2 | 3 | A |
| *E. coli* | 16 | 15.5 | 16 | 16 | 7 | 7 | 7 | 7 | 10 | 10.5 | 10 | 10 |
| *B. cereus* | 19.5 | 19 | 20.5 | 20 | 8 | 7 | 8 | 8 | 14 | 13 | 15 | 14 |
| *S. typhimurium* | 16 | 15 | 15.5 | 16 | 7 | 7 | 7.5 | 7 | 10 | 10 | 9 | 10 |
| *S. aureus* | 21 | 20 | 22 | 21 | 7 | 7 | 7 | 7 | 11 | 11.5 | 10 | 11 |

A - Average