Larvicidal and antibacterial activity of aqueous leaf extract of Peepal (*Ficus religiosa*) synthesized nanoparticles

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**A B S T R A C T**

In this study, the zinc oxide nanoparticles (ZnO NPs) and titanium dioxide nanoparticles (TiO₂ NPs) were synthesized using the aqueous leaf extract of *Ficus religiosa* (Peepal tree). The synthesized nanoparticles were tested as larvicides against the larvae of *Anopheles stephensi*. Further, the synthesized nanoparticles were tested as antibacterial agents against the *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) bacteria. The synthesized nanoparticles were characterized with UV-visible spectroscopy, X-rays powder diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX). The larvicidal mortality was observed after 24 h and 48 h by probit analysis. The antibacterial activity was evaluated using the well diffusion method. The synthesized nanoparticles were irregular shape and varied size. The larvae of *A. stephensi* were found highly susceptible against the ZnO NPs than the TiO₂ NPs and aqueous leaves extract. The highest mortality was observed in synthesized ZnO NPs against first to third instars of (LC₅₀ 50, 75, and 5 ppm) and 100% mortality in fourth instars of *A. stephensi*. The higher zone of inhibition was occurred against the *E. coli*. This report of present investigation revealed that the rapid biological synthesis of ZnO NPs and TiO₂ NPs using aqueous leaf extract of *F. religiosa* would be effective potential larvicides for mosquito control as well as antimicrobial agents with eco-friendly approach.

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1. Introduction

*F. religiosa* is commonly known as Peepal tree belonging to the family moraceae. It is also known as bodhi tree, pippala tree, peepul tree, pepal tree and ashwattha tree. It is a medicinal plant which is emerged as a good source of traditional medicine for the treatment of asthma, diabetes, diarrhea, epilepsy, gastric problems, inflammatory disorders, infectious and sexual disorders (Singh et al., 2011a). The leaves of *F. religiosa* contain tannic acid, leucine, isoleucine, methionine, tryptophan, threonine, glycine asparatic acid, serine and arginine, bark comprises of bergaptol and bergapten, the fruits consists of tyrosine and asparagines and seeds contain threonine, alanine and valine (Gupta and Singh, 2012), which are the good source of secondary metabolites used as larvicides and antimicrobial agents.
The larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf and bark of *Ficus racemosa* (Moraceae) has been assayed for their toxicity against the early fourth-instar larvae of *Culex quinquefasciatus* (Abdul Rahuman et al., 2008). The larvicidal efficacy of different extracts of *F. benghalensis* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* has been investigated (Govindarajan, 2010). Further, larvicidal efficacy of different solvent leaf extracts of *F. benghalensis* against *Cx. tritaeniorhynchus* and *An. subpictus* has been determined (Govindarajan et al., 2011). Thereafter, the larvicidal activity of Indian medicinal plants, *Commiphora berryi*, *Commiphora pentandra*, *Pelegrinimum graveolens*, *Thevetia peruviana*, *Sesamum indicum*, *Ficus microcarpa*, *Melia dubia*, *C. bonplandianus*, *F. religiosa* and *Croton lacryma* has been tested against *Ae. aegypti* mosquito (Deepa et al., 2015). The antimicrobial properties of *Ficus* extract have been reported (Mandal et al., 2000; Ao et al., 2008; Oyeleke et al., 2008; Annan and Houghton, 2008; Kuete et al., 2008; Kuete et al., 2009; Usman et al., 2009; Alimuddin et al., 2010; Lazreg Aref et al., 2010). Unfortunately, the secondary metabolites of plants have the slow reaction against mosquito (Deepa et al., 2015). The antimicrobial properties of *Sesamum indicum* vicidal activity of Indian medicinal plants, *F. religiosa* and *Croton lacryma* has been tested against *Ae. aegypti* mosquito (Deepa et al., 2015). The antimicrobial properties of *Ficus* extract have been reported (Mandal et al., 2000; Ao et al., 2008; Oyeleke et al., 2008; Annan and Houghton, 2008; Kuete et al., 2008; Kuete et al., 2009; Usman et al., 2009; Alimuddin et al., 2010; Lazreg Aref et al., 2010). Unfortunately, the secondary metabolites of plants have the slow reaction against the mosquitoes. Therefore, it is needed to develop the eco-friendly and rapid technology for the mosquito control as well as antimicrobial agent, so that people can be protected from the bacterial and vector borne diseases.

Recently, the use of metal nanoparticles is a great attention in this regards. The plants synthesized ZnO NPs and TiO2 NPs are the good, rapid and eco-friendly sources for mosquito control and antibacterial agents also. The synthesis and characterization of ZnO NPs (Singh et al., 2011b; Awwad et al., 2014; Shekhawat et al., 2014; Gnanasangeetha and Thambavani, 2014; Raju Kooluru and Sharada, 2014; Suganya et al., 2015; Noorjahan et al., 2015; Varghese and George, 2015; Manokari and Shekhawat, 2015; Manokari et al., 2016a, 2016b; Fatimah et al., 2016; Sharmila Devi and Dhinesh, 2016; Pinto and Nazareth, 2016) and TiO2 NPs (Sundararajan and Gowri, 2011; Rajakumar et al., 2012; Ganapathi Rao et al., 2015; Khadar et al., 2015; Valli and Geetha, 2015; Chatterjee et al., 2016; Mythreyi et al., 2016; Dobrucka, 2017; Patidar and Jain, 2017) using the plant extract has been reported. The antimicrobial activity of synthesized ZnO NPs using plants has been reported (Jeeva Lakshmi et al., 2012; Aswathi Sreenivasan et al., 2012; Divya et al., 2013; Mishra and Sharma, 2015; Raj et al., 2015; Narendra and Sivaraj, 2016; Salih and Smail, 2016; Jeba Jane Ratney and Begila David, 2016; Paul et al., 2016).

The larvicidal activity of plant synthesized TiO2 NPs has been assessed (Rajakumar et al., 2015; Suman et al., 2015; Gandhi et al., 2016). The antimicrobial activity of synthesized TiO2 NPs using plants has been studied (Mauya et al., 2012; Malarkodi et al., 2013; Santhoshkumar et al., 2014; Murphin Kumar et al., 2014; Hariharan et al., 2017). Till now no review is available on the larvicidal and antibacterial activities of ZnO NPs and TiO2 NPs synthesized using aqueous leaf extract of Peepal (*F. religiosa*). In the present investigation, the ZnO NPs and TiO2 NPs were synthesized using the aqueous leaf extract of *F. religiosa* and access their larvicidal and antibacterial properties. This ZnO NPs and TiO2 NPs technology could be a rapid, green and eco-friendly approach for mosquito control and used as antimicrobial also.

2. Materials and methods

2.1. Collection and leaf extract preparation

The fresh and green leaves of *F. religiosa* were collected from the nearest area of ICMR-National Institute of Malaria Research, India. The leaves were rinsed with tap water and then with distilled water to remove dust and other particles. The rinsed leaves were then air dried for 1–2 h. After then, approximately 20 g of leaves were cut into fine pieces and put into a 250 ml conical flask which containing 100 ml of distilled water. Boil the flask for 1 h at 50 °C on a magnetic stirrer. After 1 h, cooled the extract and filtered through the whatman-1 filter paper and store the leaves filtrate for the experiment.

2.2. Synthesis of ZnO and TiO2 NPs

The ZnO NPs were biosynthesized by co-precipitation method described by Singh et al. (2011a) with some modification. 20 ml of leaf extract was heated at 60 °C for 10 min on a magnetic stirrer. After then, 50 ml of 0.1 M of Zinc nitrate solution and 50 ml of 0.2 M sodium hydroxide solution were added drop wise under stirring. The mixture was continued stirred for 1 h on magnetic stirrer which resulting cream colored precipitate of zinc hydroxide formed. Then, the precipitate was collected by centrifugation at 4000 rpm for 15 min and washed with deionized water and ethanol. The ZnO NPs were collected after dried in hot air oven for 48 h at 45 °C.

The TiO2 NPs were biosynthesized by the following method described by Suman et al. (2015) with some modification. The aqueous solution of TiO(OH)2 (5 mmol/L) was prepared and used for synthesis of TiO2 NPs. 20 ml leaf extract of *F. religiosa* was boiled at 50 °C. Then 80 ml of aqueous solution of 5 mmol/L TiO(OH)2 were added in the leaf extract and boil for 4 h with continuous stirring. After then reduction mixture was centrifuged at 4000 rpm for 15 min and resulting pellet was collected. The TiO2 NPs were dried in hot air oven for 48 h at 45 °C.

2.3. Characterization of ZnO and TiO2 NPs

Optical properties of synthesized ZnO and TiO2 NPs were confirmed by UV–visible double beam spectroscopy (HALO DB-20) in 300–500 nm wavelength range. The XRD pattern of synthesized ZnO and TiO2 NPs were carried out using X-ray diffractometer (Bruker X-ray diffractometer D-8 Advance) Cu-Kα radiations (λ = 0.15406 nm) in 2θ range from 20° to 80°. The average size and shape of synthesized ZnO and TiO2 NPs were obtained by transmission electron microscopy (Tecnai G2). The morphology
of synthesized ZnO and TiO2 NPs were examined by scanning electron microscope (model no. Zeiss EVO MA 10). The synthesized ZnO and TiO2 NPs were analyzed for elemental analysis by energy dispersive X-ray spectroscopy (Oxford Inca Energy 250).

2.4. Rearing of larvae

The larvae of An. stephensi were reared in deionized water containing glucose and yeast powder. The colony of An. stephensi was maintained in the laboratory at 27 °C with relative humidity of (75 ± 5%) and 14 h of photoperiod using the standard method with some modifications (Geberg et al., 1994).

2.5. Bioassay, data management and statistical analysis

ZnO NPs and TiO2 NPs, synthesized using aqueous leaves extract of F. religiosa were tested for their killing activities against the An. stephensi larvae (I-IV instar). The bioassay was assessed using the standard method (World Health Organization, 2005). An. stephensi larvae were placed in a container in micro-free deionized water. After that, ZnO and TiO2 NPs with different test concentrations in 100 mL deionized water were prepared in 250 mL beakers. Bioassays were conducted separately at five different concentrations using serial dilution method, of synthesized ZnO and TiO2 NPs (25, 50, 100, 150 and 250 ppm). To test the larvicidal activity of ZnO and TiO2 NPs, 20 larvae were separately exposed to 100 mL of test concentration. Similarly, the control (without ZnO and TiO2 NPs) was run to test the natural mortality. The experiments were replicated thrice to validate the results. Thereafter, we examined their mortality after 24 h and 48 h. The data on the efficacy was subjected to probit analysis (Finney, 1971). The control mortality was corrected by Abbott’s formula (Abbott, 1925).

2.6. Antibacterial activity of ZnO and TiO2 NPs

The antibacterial activity of synthesized ZnO and TiO2 NPs was evaluated against E. coli and S. aureus. The antibacterial activity was determined using the well diffusion method. The wells were prepared on plates with Muller-Hinton agar (MHA) medium. Then, the plates were seeded with different bacterial strains using sterile swab. Four wells were prepared using gel puncture in each plate. Each well was loaded with 50 μL of different concentration of ZnO and TiO2 NPs (50, 150, 250 and 500 ppm). Then, the plates were incubated at 35 °C for 24 h and zone of inhibition was observed.

3. Results and discussion

3.1. Proposed mechanism of synthesis of ZnO and TiO2 NPs through F. religiosa

Based on the experimental work that has been done, there are series of chemical reaction that takes place. The complete hydrolysis of zinc nitrate and dihydroxy(oxo)titanium with the aid of F. religiosa aqueous leaves extract solution should result in the formation of ZnO and TiO2 nanoparticles. The richly available carbohydrates, tannin, alkaloids, steroids, terpenoids, saponin, reducing sugar and flavonoids in the plant extract acted as stabilizing and capping agents, respectively. Hence, the proposed principle of formation of ZnO and TiO2 NPs involves the ionization of zinc nitrate and hydroxylation of dihydroxy(oxo)titanium in an aqueous

Fig. 1. UV-visible spectrum of aqueous leaves extract synthesized (a) ZnO NPs and (b) TiO2 NPs.
medium to give Zn\(^{2+}\) which was reduced by phytochemical principle present in the aqueous extract of *F. religiosa*, to generate ZnO, which further aggregates to ZnO and TiO\(_2\) NPs as shown in Eq. 1 and Eq. 2.

\[
\text{TiO(OH)}_2 + \text{Ficus religiosa leaves extract} \xrightarrow{\Delta} \text{TiO}_2 + \text{H}_2\text{O}
\]

\[\text{Zn(OH)}_2\text{-aqueous leaves extract} \xrightarrow{\text{Stirring}} \text{Zn(OH)}_2 + 2\text{NO}_3^- + \text{H}_2\text{O}\]

3.2 UV-visible analysis

The formation of ZnO and TiO\(_2\) NPs during the synthesis can be observed visually. Fig. 1a is the UV–vis absorption spectrum of ZnO NPs dispersed in deionized water and the figure shows the absorption peak at 358 nm. Shah et al. (2015) stated that the UV absorption spectrum for synthesized ZnO NPs was recorded at 330 nm. Similar results were observed by Yedurkar et al. (2016). Fig. 1b shows the UV-vis absorption spectrum of TiO\(_2\) NPs with an absorption peak at 450 nm. Valli and Geetha (2015) observed the UV absorption spectrum for synthesized TiO\(_2\) NPs at 447.3 nm.

3.3 XRD analysis

The structure of ZnO and TiO\(_2\) NPs were determined in this study using a powder diffraction system with Cu-K\(\alpha\) x-ray tube (\(\lambda = 1.541836\) Å) was used. Fig. 2 depicts the XRD pattern of synthesized ZnO NPs scanned at 2\(\theta\) range from 0 to 80 degree. Diffraction peaks at 31.66°, 34.34°, 36.15°, 47.45°, 56.46°, 62.72°, 67.86°, 68.97°, 76.79° can be assigned to (110), (002), (101), (102), (110), (103), (112), (201) and (202) plane. The strong and narrow peak denotes that the product has well crystalline nature of particles. Narendhran and Sivakumar, (2016) recorded the X-ray diffraction of ZnO NPs synthesized using the *L. aculeate*. The peaks at 2\(\theta\) values of 31.79°, 34.42°, 36.26°, 47.59°, 62.80°, 65.84°, 67.96°, 68.30°, 72.12° and 76.53° correspondence to the crystal planes of (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) of ZnO NPs. Similar results were reported by Vanathi et al. (2014) in which particles were synthesized using *E. crassipes* leaf extract. Fig. 3 shows the XRD pattern of synthesized TiO\(_2\) NPs scanned at 2\(\theta\) range from 0 to 80 degree. Diffraction peaks at 25.28°, 36.91°, 53.85°, 55.03°, 62.6°, 68.70° and 75.1° can be assigned to (110), (101), (211), (220), (204), (112) and (215) plane. Similar results were reported by Khadar et al. (2015).

3.4 TEM analysis

The shape and size of synthesized ZnO and TiO\(_2\) NPs were obtained using the TEM. Fig. 4a shows the TEM images of synthesized ZnO NPs, which depict the irregular shape and varied size nanoparticles. Fig. 4b depicts the TEM images of TiO\(_2\) NPs, which were spherical in shape and size from 70.29 to 84.93 nm with calculated size of 77.61 nm and polydisperse. Zahir et al. (2014) recorded the TEM images of the synthesized Ag NPs and TiO\(_2\) NPs spherical, quite polydisperse and individual particles showed an average size of 12.82 ± 2.50 and 83.22 ± 1.50 nm, respectively. Similar results were obtained by Rajakumar et al. (2012) and previous work (Table 1). 3.5 SEM-EDX analysis

The size and distribution of synthesized ZnO and TiO\(_2\) NPs were also confirmed by SEM shown in Fig. 5a-b. From the result it is evident that the morphology of ZnO NPs was irregular and TiO\(_2\) NPs was spherical in shape and polydisperse in nature, which is very similar to previous studies (Zahir et al., 2014; Rajakumar et al., 2012). Fig. 6 shows the EDX analysis of ZnO NPs 72.57% zinc and 27.42% of oxygen which confirm the elemental composition of ZnO NPs. Narendhran and Sivaraj (2016) showed the EDX analysis of ZnO nanoparticles.
Fig. 3. XRD pattern of synthesized TiO$_2$ NPs.

Fig. 4. TEM images of synthesized (a) ZnO NPs and (b) TiO$_2$ NPs.
37.22% of zinc and 62.78% of oxygen which confirms the elemental composition of ZnO nanoparticles. The strong signals from the zinc atoms in the nanoparticles recorded and other signals from C and O atoms were observed using EDX analysis in Parthenium-mediated ZnO nanoparticles (Rajiv et al., 2013). The EDX analysis display the optical absorption peaks of ZnO nanoparticles and these absorption peaks were due to the surface plasmon resonance of ZnO nanoparticles (Ankanna and Savithramma, 2011). Fig. 7 depicts the EDX analysis of TiO$_2$ NPs 71.99% titanium and 28.01% of oxygen which confirm the elemental composition of TiO$_2$ NPs. Santhoshkumar et al. (2014) showed the energy dispersive X-ray analysis study (EDX) which proves that the particles are crystalline in nature and indeed metallic TiO$_2$ NPs. The similar results were reported in the previous studies by (Suman et al., 2015).

### 3.6 Larvicidal activity of ZnO and TiO$_2$ NPs

Larvicidal activity of *F. religiosa* leaf extract, synthesized ZnO and TiO$_2$ NPs were evaluated against the larvae (I-IV) of *An. stephensi* at different concentrations (25, 50, 100, 150 and 250 ppm). The larvae of *An. stephensi* were found highly susceptible to the ZnO NPs. The fourth instar larvae have shown the 100% mortality after 24 h of exposure. Whereas, the first instar (LC$_{50}$ 50 ppm), second instar (LC$_{50}$ 75 ppm) and third instar (LC$_{50}$ 5 ppm) larvae were observed with their probit equations and 95% confidence limit, R$^2$, chi-square and p value after 24 h (Table 2). No mortality was observed in control group. The anti-parasitic activities have been assessed to determine the efficacies of synthesized zinc oxide nanoparticles (ZnO NPs) prepared by wet chemical method using zinc nitrate and sodium hydroxide as precursors and soluble starch as stabilizing agent against the larvae of cattle tick *Rhipicephalus* (Boophilus) microplus, Canestrini (Acari: Ixodidae); head louse *Pediculus humanus capitis*, De Geer (Phthiraptera: Pediculidae); larvae of malaria vector, *An. subpictus*, Grassi; and filariasis vector, *Cx. quinquefasciatus*, Say (Diptera: Culicidae) (Kirthi et al., 2011). The maximum efficacy was observed in zinc oxide nanoparticles.

| Plant species                         | Plant part used | Synthesized NPs | Size (nm) | References                      |
|--------------------------------------|-----------------|-----------------|-----------|---------------------------------|
| *Azadirachta indica, Emblica Officinalis* | leaf            | ZnO             | 51, 16    | Gnanasangeetha and Thambavani, 2014 |
| *Pyrus pyrifolia*                    | leaf            | ZnO             | 45        | Parthiban and Sundaramurthy, 2015 |
| *Ixora coccinea*                     | leaf            | ZnO             | 145.1     | Yadurkar et al., 2016           |
| *Passiflora caerulea*                 | Leaf            | ZnO             | 200       | Santoshkumar et al., 2017       |
| *Bauhinia tomentosa*                  | Leaf            | ZnO             | 22–94     | Sharmila et al., 2018           |
| *Pandanus odorifer*                   | Leaf            | ZnO             | 90        | Hussain et al., 2019            |
| *Allium sativum*                     | Skin            | ZnO             | 7.77      | Modi and Fulekar, 2020          |
| *Psidium guajava*                    | Leaf            | TiO$_2$         | 32.58     | Santhoshkumar et al., 2014      |
| *Cynodon dactylon*                    | Leaf            | TiO$_2$         | 13–34     | Hararan et al., 2017            |
| *Glycosmis cochinchinensis*           | Leaf            | TiO$_2$         | 45        | Rosi and Kalyanasundaram, 2018  |
| *Cassia fistula*                     | Leaf            | TiO$_2$         | 200       | Swathi et al., 2019             |

**Fig. 5.** SEM images of synthesized (a) ZnO NPs and (b) TiO$_2$ NPs.
against the *R. microplus*, *P. humanus capitis*, and the larvae of *An. subpictus*, *Cx. quinquefasciatus* with LC(50) values of 29.14, 11.80, 11.14, and 12.39 mg/L; r (2) = 0.805, 0.876, 0.894, and 0.904, respectively. The synthesized ZnO NPs showed the LC (50) and r (2) values against the *R. microplus* (13.41 mg/L; 0.982), *P. humanus capitis* (11.80 mg/L; 0.966), and the larvae of *An. subpictus* (3.19; 0.945 mg/L), against *Cx. quinquefasciatus* (4.87 mg/L; 0.970), respectively.The TiO2 NPs were found effective against the larvae of *An. stephensi*. The mortality was recorded after 48 h of exposure. The first instar (LC50 15 ppm), second instar (LC50 50 ppm), third instar (LC50 25 ppm) and fourth instar (LC50 25 ppm) larvae were observed with their probit equations and 95% confidential limit, R², chi-square and p value after 24 h (Table 2). The larvicidal activity of titanium dioxide nanoparticles (TiO2 NPs) synthesized from the root aqueous extract of *M. citrifolia* against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* has been assessed (Suman et al., 2015). The biosynthesized TiO2 NPS showed maximum activity against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* when compared to the aqueous extract of *M. citrifolia*. Similarly, the antiparasitic activity of TiO2 NPs against the larvae of *R. microplus*, *H. anatolicum anatolicum* and *H. bispinosa*, fourth instar larvae of *An. subpictus*, and *Cx. quinquefasciatus* has been assessed by (Rajakumar et al., 2015). The maximum efficacy was observed in synthesized TiO2 NPs against the larvae of *R. microplus*, *H. anatolicum anatolicum*, *H. bispinosa*, *An. subpictus*, and *Cx. quinquefasciatus* with LC value of 28.56, 33.17, 23.81, 5.84, and 4.34 mg/L, respectively. Recently, the larvicidal and the pediculicidal activity of synthesized titanium dioxide nanoparticles (TiO2 NPs) using the leaf aqueous extract of *V. negundo* against the fourth instar larvae of the malaria vector, *An. subpictus* Grassi and filariasis vector, *Cx. quinquefasciatus* Say and the head louse, *P. humanus capitis* De Geer has been carried out by (Gandhi et al., 2016). The maximum activity has been observed in the synthesized TiO2 NPs against *An. subpictus*, *Cx. quinquefasciatus* and lice, (LC50 = 7.52, 7.23 and 24.32 mg/L; $\chi^2 = 0.161, 2.678$ and $4.495$; $r^2 = 0.663, 0.742$ and 0.924), respectively. The larvicidal activity of synthesized ZnO and TiO2 has been reported by other researchers (Table 3) The larvae of *An. stephensi* have also shown the mortality against the aqueous leaves extract of *F. religiosa* and mortality was recorded after 48 h. The first instar (LC50 250 ppm), second instar (LC50 250 ppm), third instar (LC50 200 ppm) and fourth instar (LC50 200 ppm) larvae were observed with their probit equations and 95% confidential limit, R², chi-square and p value after...
The larvicidal efficacy of different extracts of *F. benghalensis* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* has been investigated (Govindarajan, 2010). The lethal concentration (LC50) values of *F. benghalensis* against early second, third and fourth larvae of *Cx. quinquefasciatus*, *Ae. Aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm respectively. Further, the larvicidal efficacy of different solvent leaf extracts of *F. benghalensis* against *Cx. tritaeniorhynchus* and *An. subpictus* has been determined (Govindarajan et al., 2011). The LC50 and LC90 values of *F. benghalensis* against early third instar of *Cx. tritaeniorhynchus* and *An. subpictus* were 100.88, 159.76 ppm and 56.66, 85.84 ppm, respectively. Thereafter, the Larvicidal activity of Indian medicinal plants, *C. berryi*, *C. pentandra*, *P. graveolens*, *T. peruviana*, *S.indicum*, *F. microcarpa*, *M. dubia*, *C. bonplandianus*, *F. religiosa* and *C. lacryma* has been tested against *Ae. aegypti* mosquito. They found the highest LC50 values of methanol extracts of *F. microcarpa* against *Ae. aegypti* larvae were 91.63 ppm followed by, LC50 values of *C. lacryma*, *P. graveolens*, *C. berryi*, and *M. dubia* extracts against *Ae. aegypti* larvae were 92.77, 95.65, 96.52 and 100.12 ppm, respectively.3.7Antibacterial activityThe antibacterial assay for biologically synthesized ZnO and TiO2 NPs against the pathogens is shown in Fig. 8. Well diffusion method was used to provide the evidence for and validate the antibacterial activity of ZnO and TiO2 NPs against *E. coli* (Gram negative) and *S. aureus*, (Gram positive) bacteria. The antibacterial activity of the ZnO and TiO2 NPs was indicated by the formation of the zone. The diameter of the inhibition zone was measured in millimetre. The maximum zone of inhibition was observed against ZnO NPs in *E. coli* (8, 10, 12 and 14 mm) and *S. aureus* (6, 8, 10 and 12) (Table 4 and Fig. 8). Several research confirming antimicrobial activity of ZnO NPs against the food related bacteria *B. subtilis*,
Table 2
Efficacy of *F. religiosa* aqueous leaf extract, Syntheiszed ZnO NPs and TiO$_2$ NPs against the *An. stephensi* larvae with their probit equation, LC$_{50}$ with 95% CI, $\chi^2$, $p$ and $R^2$ values.

| Instar | Concentrations (ppm) | % mortality | Probit equation | LC$_{50}$ (±CI) | $\chi^2$ | $p$  | $R^2$ |
|--------|----------------------|-------------|----------------|-----------------|--------|------|------|
| Extract I | 25 | 0 | $y = 0.2203x + 0.6641$ | 250 ± 1.22 | 1.95 | 0.744 | 0.903 |
| | 50 | 10 | | | | | |
| | 100 | 30 | | | | | |
| | 150 | 40 | | | | | |
| | 250 | 50 | | | | | |
| II | 25 | 0 | $y = 0.2203x + 0.6641$ | 250 ± 1.22 | 1.95 | 0.744 | 0.903 |
| | 50 | 10 | | | | | |
| | 100 | 30 | | | | | |
| | 150 | 40 | | | | | |
| | 250 | 50 | | | | | |
| III | 25 | 30 | $y = 0.1016x + 28.32$ | 200 ± 1.18 | 2.27 | 0.686 | 0.825 |
| | 50 | 30 | | | | | |
| | 100 | 40 | | | | | |
| | 150 | 50 | | | | | |
| | 250 | 50 | | | | | |
| IV | 25 | 0 | $y = 0.3125x - 15.938$ | 200 ± 1.14 | 1.54 | 0.820 | 0.919 |
| | 50 | 20 | | | | | |
| | 100 | 30 | | | | | |
| | 150 | 50 | | | | | |
| | 250 | 50 | | | | | |

| ZnO NPs I | 25 | 40 | $y = 0.2297x + 35.586$ | 50 ± 0.23 | 2.67 | 0.615 | 0.898 |
| | 50 | 50 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 80 | | | | | |
| II | 25 | 40 | $y = 0.2297x + 35.586$ | 75 ± 0.25 | 2.74 | 0.603 | 0.898 |
| | 50 | 50 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 80 | | | | | |
| III | 25 | 50 | $y = 0.1859x + 42.617$ | 5 ± 0.23 | 2.74 | 0.602 | 0.988 |
| | 50 | 50 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 90 | | | | | |
| IV | 25 | 60 | $y = 0.1234x + 49.805$ | 25 ± 0.34 | 2.710 | 0.608 | 0.938 |
| | 50 | 60 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 80 | | | | | |

| TiO$_2$ NPs I | 25 | 50 | $y = 0.1188x + 62.344$ | 15 ± 0.12 | 2.912 | 0.573 | 0.868 |
| | 50 | 70 | | | | | |
| | 100 | 80 | | | | | |
| | 150 | 80 | | | | | |
| | 250 | 80 | | | | | |
| II | 25 | 40 | $y = 0.2141x + 37.383$ | 50 ± 0.29 | 2.727 | 0.604 | 0.991 |
| | 50 | 50 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 90 | | | | | |
| III | 25 | 50 | $y = 0.1234x + 49.805$ | 25 ± 0.34 | 2.689 | 0.611 | 0.938 |
| | 50 | 60 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 80 | | | | | |
| IV | 25 | 50 | $y = 0.1234x + 49.805$ | 25 ± 0.34 | 2.710 | 0.608 | 0.938 |
| | 50 | 60 | | | | | |
| | 100 | 60 | | | | | |
| | 150 | 70 | | | | | |
| | 250 | 80 | | | | | |

** 100% mortality.

*E. coli*, *P. fluorescens*, *S. typhimurium* and *S. aureus* has been reported (Russell and Hugo, 1994; Ip et al., 2006). ZnO NPs are also known to exhibit antimicrobial activities against *L. monocytogenes*, *S. enteritidis* and *E. coli* (Russell and Hugo, 1994). The formation of hydrogen peroxide from the surface of ZnO is considered to be mainly responsible for its antimicrobial property (Rai et al., 2009). While, TiO$_2$ NPs has shown the zone of inhibition in *E. coli* (7, 9, 10, and 13) and *S. aureus* (5, 6, 8 and 10) (Table 4 and Fig. 8). The higher zone of inhibition occurred at 500 ppm concentration of synthesized ZnO and TiO$_2$ NPs. The antibacterial
activity of TiO$_2$ by pure plant extracts of *B. variegata* and *T. cordifolia* has been studied (Maurya et al., 2012). Plant extract/TiO$_2$ nanocomposites have shown various levels of antibacterial activity on different test microorganisms. The highest antibacterial potentiality expressed in terms of zone of inhibition (ZOI) in mm was exhibited by the aqueous extract of *B. variegata*/TiO$_2$ (45 mm against *E. faecalis* and 30 mm against *E. coli*) and benzene extract of *T. cordifolia*/TiO$_2$ (26 mm) nanocomposites. Similar results were reported (Kumar et al., 2014) using the biosynthesized and chemically synthesized titania nanoparticles and other researchers also (Table 5).

### Table 3

Comparative larvicidal efficacy of synthesized ZnO and TiO$_2$ nanoparticles against different mosquito species.

| Plant species          | Common name      | Plant part used | Test NPs tested | Mosquito species                  | References                  |
|------------------------|------------------|-----------------|-----------------|-----------------------------------|-----------------------------|
| *Momordica charantia*  | Bitter guard     | Leaf            | ZnO             | *An. stephensi Cx. quinquefasciatus* | Gandhi et al., 2016        |
| *Syzgium cumini*       | Black plum       | Seed            | ZnO             | *Ae. aegypti*                     | Roopan et al., 2015         |
| *Scadoxus multijorpus* | Blood lily       | Leaf            | ZnO             | *Ae. aegypti*                     | Abdullaah Al-Dhabh and Valan Arasu, 2018 |
| *Morinda citrifolia*   | Noni             | Root            | TiO$_2$         | *An. stephensi A. aegypti, Cx. quinquefasciatus* | Suman et al., 2015          |
| *Mangifera indica*     | Mango            | Leaf            | TiO$_2$         | *An. stephensi C. quinquefasciatus* | Rajakumar et al., 2015      |
| *Vitex negundo*        | Chinese chaste tree | Leaf          | TiO$_2$             | *An. stephensi C. quinquefasciatus* | Gandhi et al., 2016        |

### Table 4

Antibacterial activity of synthesized ZnO NPs and TiO$_2$ NPs against *E. coli* and *S. aureus*.

| Species | Zone of inhibition/mm |
|---------|-----------------------|
|         | 50                    | 150                  | 250                  | 500                  |
| ZnO NPs | *E. coli* 8 ± 0.612   | 10 ± 0.654           | 12 ± 0.712           | 14 ± 0.801          |
|         | *S. aureus* 6 ± 0.563  | 8 ± 0.612            | 10 ± 0.654           | 12 ± 0.712          |
| TiO$_2$ NPs | *E. coli* 7 ± 0.552   | 9 ± 0.642           | 10 ± 0.654           | 13 ± 0.752          |
|         | *S. aureus* 5 ± 0.456  | 6 ± 0.563           | 8 ± 0.612           | 10 ± 0.654          |

![Fig. 8. Antibacterial activity of synthesized (a) ZnO NPs and (b) TiO$_2$ NPs against *E. coli* and *S. aureus*.](image)

activity of TiO$_2$ by pure plant extracts of *B. variegata* and *T. cordifolia* has been studied (Maurya et al., 2012). Plant extract/TiO$_2$ nanocomposites have shown various levels of antibacterial activity on different test microorganisms. The highest antibacterial potentiality expressed in terms of zone of inhibition (ZOI) in mm was exhibited by the aqueous extract of *B. variegata*/TiO$_2$ (45 mm against *E. faecalis* and 30 mm against *E. coli*) and benzene extract of *T. cordifolia*/TiO$_2$ (26 mm) nanocomposites. Similar results were reported (Kumar et al., 2014) using the biosynthesized and chemically synthesized titania nanoparticles and other researchers also (Table 5).

### Conclusion
The present study, synthesis of ZnO NPs and TiO$_2$ NPs from the *F. religiosa* is a green,
Table 5

| Plant species                  | Plant part used | NPs tested | Species                                      | References               |
|-------------------------------|-----------------|------------|----------------------------------------------|--------------------------|
| Catharanthus roseus           | Leaf            | ZnO        | B. thuringiensis, E. coli, S. aureus, P. aeruginosa | Bhumi and Savithramma, 2014 |
| Brassica oleracea             | Leaf            | ZnO        | E. coli, V. cholera, C. Botulinum, S. aureus, B. subtilis | Raj et al., 2015         |
| Trifolium pratense            | Flower          | ZnO        | S. aureus, P. aeruginosa, E. coli           | Dobrucka and Dugaszewska, 2016 |
| Bauhinia tomentosa            | Leaf            | ZnO        | B. thuringiensis, E. coli, S. aureus, P. aeruginosa | Sharmila et al., 2018    |
| Pandanus odorifer             | Leaf            | ZnO        | B. subtilis, E. coli                        | Hussain et al., 2019     |
| Aloe vera                     | Leaf            | ZnO        | E. coli                                     | Iwzanie Rasli et al., 2020 |
| Psidium guajava               | Leaf            | TiO₂       | E. coli, S. aureus                          | Santhoshkumar et al., 2014 |
| Cynodon dactylon              | Leaf            | TiO₂       | E. coli                                     | Hariharan et al., 2017   |
| Glycosmis                     | Leaf            | TiO₂       | S. suprathyricus, B. subtilis, E. coli, cochincheninis P. aeruginosa | Rosi and Kalyanasundaram, 2018 |
| Cassia fistula                | Leaf            | TiO₂       | E. coli, S. aureus                          | Swathi et al., 2019      |

rapid, cost-effective, non-toxic and eco-friendly approach for mosquito control as well as antimicrobial agent also.

Declaration of Competing Interest

Authors declare that there is no conflict of interest.

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