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Bio-efficiency of Zinc Nanoparticles Synthesized using *Stereospermum chelonoides* (L.F.) DC

Sangeetha Manickam[1], Anandaraj Balaiah[2] and Rajan Susyambu*[3]

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A B S T R A C T

The metal nanoparticles obtained from medicinal plants showed its bio-efficiency as antibacterial, antifungal, anticancer activities. It is biosynthesized using eco-friendly and non-toxic method that fascinates the researchers around the world as an important biodeficient component. In this study pathogens were isolated from urine samples collected from UTI cases and identified as Escherichia fergusonii and Klebsiella pneumoniae, and all the organisms were resistant to multiple drugs. *Stereospermum chelonoides* leaves and flowers were collected from Thyagaraja Swamy Temple in Thiruvarur, Tamil Nadu, India and extracted using water as aqueous extract. This aqueous extract was submitted for zinc nanoparticle synthesis. Nanoparticles were characterized by UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy, Energy Dispersive X-Ray (EDX) analysis and Transmission electron microscopy (TEM) analysis. The antibacterial activity was examined by agar well diffusion method. In this study we have identified that the Zinc nanoparticles showed the potent anti-microbial activity. Similarly, nanoparticles of this plants provide good evidence for this plant extracts as an effective anti-inflammatory agent. Flavonoid based compounds could be responsible for all types of bio efficiency of this plants and its nanoparticles.

***Key words*:** *Stereospermum chelonoids*, Nanoparticles, Copper, Bio-efficiency

Nanoparticles (NPs) have received significant interest worldwide particularly by the synthesis of metals in the nanometre region. Zinc (Zn) has been used from decades owing to its excellent compatibility in humans, physical and chemical properties. Zn has been reported to be used as Zn metal or modified as oxides, silanes or sulphide. The advent of coupled quantum dots has infused intensive application especially in the field of cancer diagnostics. The synthesis of Zn NPs has been confirmed chemically but recently novel green synthesis has been given considerable importance to synthesize Zn as ZnO, ZnS or other modified precipitates. Researchers have witnessed synthesis of Zn nanoparticles, which precipitates microbial proteins, which inhibits the growth of microbial agents. It has been reported as absorbers for harmful sun ultra violet rays and hence being incorporated in sunscreen creams and lotions. The major aspiration of researchers has been to exemplar the use of Zn and modified ZnNPs as anticancer agents. The biological activity of nanoparticles depends on many factors including surface chemistry, size distribution, particle morphology, and particle reactivity in solution. Therefore, the development of nanoparticles with controlled structures that are uniform in size, morphology, and functionality is essential for various biomedical applications [1-2]. Hence in this study zinc nanoparticles were synthesized using flower and leaf mixture aqueous extracts and studies its efficiency as antibacterial, antifungal, antioxidant and anti-inflammatory agent.

Stereospermum chelonoides (L.F.) DC (Syn. *S. suaveolens*) is the holy tree of Thyagaraja Swamy Temple in Thiruvarur, Tamil Nadu, India and also Lord Patalisuvar at the Tirupatirippulyur Temple in Cuddalore District, Tamil Nadu, India. It is called as Pathiri tree. Part of this plant is one of the ingredients of classical Ayurvedic preparation Dashamularishta. This genus is belonging to the family Bignoniaceae and known for its biological activities like antimicrobial, antiprotozoal and anti-inflammatory properties. Parts like barks, flowers, roots and leaves of this plant have been used by traditional healers and rural communities for the treatment of vomiting, eructation, piles, acidity, diarrhoea, otalgia, odantalgia, rheumatalgia, malarial fever, gonorrhoea, loss of taste and other fevers [3-5]. Flowers, stem bark, leaf and root are used to treated burning sensation of urination, able to cure pitta and vatha, heart problems and cough [6-7]. In 2020, microbial diseases of respiratory, intestinal and urinary system caused high morbidity and mortality among the populations of low economic countries and affects more than 150 million people annually [8-9]. UTIs are the 5th most common type of infection globally with 15 - 20% hospital admissions [10]. One in five women developed UTI in their life time [11]. The

* **Rajan Susyambu**
✉ ksrajan99@gmail.com

[1-3] P.G. and Research Department of Microbiology, M. R. Government Arts College (Affiliated to Bharathidasan University, Tiruchirappalli), Mannargudi - 614 001, Thiruvarur District, Tamil Nadu, India

synthetic drug like trimethoprim, sulfamethoxazole, Fosfomycin, nitrofurantoin, cephalexin and ceftriaxone are used for the treatment of UTI infections whereas may cause adverse effects, which may damage liver and bone marrow. The irrational and discriminate use of antibiotics has led to the emergence of Multi drug resistant organisms. Inflammation was a host defence mechanism of living tissues to microbial infection, physical agents and defective immune response [12-13]. The anti-inflammatory drugs which are administered to relieve pain are found to possess various side effects which leads to the complications of liver, kidney and also to other organs [14]. This has turned the attention of the researchers to discover the anti-inflammatory principles hidden in the plants which are of non-toxic to every part of the human system [15]. Efficiency of plant extracts may improve by adding nanomaterials. Unfortunately, medicinal plants from different regions in India with anti-inflammatory properties have not been documented. Therefore, it is important to document the ethnobotanical knowledge and applications of anti-inflammatory medicinal plants [16-17]. Hence in this research, an attempt was made to study anti-inflammatory activity of nanoparticles obtained from *Stereospermum chelonoides* aqueous extracts.

MATERIALS AND METHODS

Preparation of plant material

Flower and leaves of *Stereospermum chelonoides* were collected as wild from Thyagaraja Swamy Temple in Thiruvarur, Thiruvarur District, Tamilnadu. The collected plant materials were air dried and subjected for extraction. Plant materials were collected during summer and authenticated by Dr. John Britto, Director, Rabinath Herbarium, St. Joseph's College, Tiruchirappalli (Accession Number: 3210).

Extraction of plant powder

Active components of the plant materials were extracted using water [18]. The filtrate was obtained by means of a vacuum filter pump. Filtering was repeated three times with the same plant material until the solution was clear. The filtrate was evaporated in a weighted flask, with a water bath set at 40°C. Sterile extracts obtained were stored separately in labelled, sterile capped bottles in a refrigerator at 4°C [19-20]. The filtrate was hold on at 4°C for synthesis of nanoparticles.

Synthesis of zinc oxide nanoparticles using plant extract

20 ml of the aqueous *Stereospermum chelonoides* extract was added to 80ml of 0.05 M Zinc nitrate. The solution was then transferred to a 200 mL conical flask and boiled at 70°C for 20 min with a magnetic stirrer and a heater until a brown coloured precipitate, which marked the completion of the reaction, was observed. The precipitate was collected while the product was dried in the oven at 80°C for 6 h given a powder and Zn oxide nanoparticles were examined under UV and visible spectrophotometer analysis [21].

Characterization of nanoparticles

Characterization of nanoparticles is important to understand and control nanoparticles synthesis and applications. Characterization is performed using a variety of different techniques such as scanning electron microscopy (SEM), EDX, Fourier transform infrared spectroscopy (FT-IR) and UV–Vis spectroscopy [22-24].

Bio-efficiency assay of zinc nanoparticles
Antibacterial assay

Preparation of microorganism culture

Two urinary isolates were selected from our epidemiological study of UTI infection. Urine samples were processed as per methodology [25]. On the basis of colony morphology on selective cum differential media like Mac Conkey agar and EMB agar, isolates *Escherichia fergusonii* and *Klebsiella pneumoniae* were selected and identified using biochemical tests. This organism's antibiotic susceptibility pattern was also checked with disc diffusion method [26]. Finally, identity of the test organisms was confirmed with 16srRNA sequencing and the sequences were submitted to GenBank and obtained accession number. The bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Preparation of disc with extracts

Nanoparticle (20µl) obtained were injected into a 6mm sterile disc (Hi-Media, Mumbai) and the standard antibiotics chloramphenicol used as positive control. Discs injected with water and ethanol acts as a negative control [26].

Antibacterial susceptibility assay

Escherichia fergusonii (MK598698) and *Klebsiella pneumoniae* (MK598697) were the significant urinary isolates isolated from UTI cases and subjected for antimicrobial assay. Inoculum of the isolates were obtained by inoculating a loop full of organisms into Nutrient broth (Hi-Media, Mumbai) and incubated at 30±0.1°C for 24 h. Mueller Hinton Agar (Hi-Media, Mumbai) was used for antibacterial activity. Spread 0.1ml of respective cultures of bacteria (10^5 bacteria per ml). Discs injected with extracts were placed on the solid agar medium by pressing slightly [26]. After Petri dishes obtained were placed at 4°C for 2 h, plates inoculated with bacteria were incubated at 35 ± 0.1°C for 24 hours. At the end of the period, inhibition zones formed on the medium were evaluated as millimetres. These studies were performed in triplicate. Sterilized distilled water and other solvents used in preparation of extracts were used as negative control. Chloramphenicol was used as a standard antibiotic (i.e., positive control)

Anti-inflammatory activity
Inhibition of egg albumin denaturation

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*. [27]. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of extracts (100, 200, 300, 400 and 500 µg/ mL respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2°C) in an incubator for 15 min and then heated at 70'c for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100-500µg/ mL) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1)$$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Assay of membrane stabilizing activity

Anti-inflammatory activity evaluated by Membrane stabilizing activity as described by Divya Singh *et al*. [28]. One

ml of phosphate buffer, 2 ml of hypotonic saline, 0.5 ml of Plant extract of various concentrations (100, 200, 300, 400 and 500 µg/ml) and 0.5 ml of 10% w/v human red blood cells. All the assay mixtures were incubated at 37°C for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was separated and the haemoglobin content was estimated by a spectrophotometer at 560nm. Diclofenac sodium (100 to 500 µg/ml) was used as reference drug. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

% of Stabilization = (Control haemolysis – Test haemolysis / Control haemolysis × 100)

In vitro anti-lipoxygenase activity

Anti-LOX assay was studied using linoleic acid as substrate and lipoxidase as enzyme purchased from Sigma, USA [29] The plant extract sample (100, 200, 300, 400 and 500µg/mL) was dissolved in 0.25 mL of 2 M borate buffer pH 9.0 and added 0.25 mL of soybean lipoxidase enzyme solution (final concentration of 20,000 U/mL). This mixture was incubated for 5 min at 25°C. After which, 1.0 mL of linoleic acid solution (0.6 mM) was added, mixed well, and absorbance was measured at 234 nm. Diclofenac sodium was used as reference standard. The percent inhibition was calculated from the following equation:

% inhibition = (Absorbance of control – Absorbance of test sample/ Absorbance of control) × 100

RESULTS AND DISCUSSION

Synthesis of zinc oxide nanoparticles using plant extract

Components in SCMPAE reduced metal ion precursors from metal salts are reduced and as a result a colour change occurs in the reaction mixture. This is the first qualitative indication that nanoparticles are being formed. The intensity of colour is directly proportional to nanoparticles production. The aqueous Zinc oxide when exposed to extract was reduced in solution, there by leading to the formation of Zinc oxide hydrosol. During the visual observation, 0.05 M Zinc nitrate and extract stirred magnetically showed the yellow mixture precipitate after 1hr. The appearance of yellowish-brown (Plate 1, Fig 1) colour is clear indication for the development of water-soluble monodispersed Zinc oxide nanoparticles.

Characterization of ZnONPs

It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. P. Figure 2 showed the UV-Vis. spectra recorded from the reaction medium after 1 hours. In the UV–Vis spectra of the reaction mixture of zinc nitrate solution with Plant extract the peak was observed at 380nm indicating the presence of ZnO nanoparticles which is synthesized by plant extract. FT-IR spectrum of zinc nanoparticles was scanned to identify the probable biomolecules responsible for efficient stabilization and capping of the ZnO nanoparticles synthesized by plant extract. The peaks observed (Plate 1, Fig 3, Table 1) for phytochemicals capped ZNO nanoparticles formed through reduction by Zinc oxide nanoparticles at 3444.19cm^{-1} indicates phenol and alcohol group, 1634.35cm^{-1} indicates 1° amines group and 692.99cm^{-1} indicates alkynes group suggested the presence of flavonoids and phenols adsorbed on the surface of ZnO nanoparticles. The results of FT-IR analysis evidenced the presence of phenol, alcohol, alkenes and aliphatic amines compounds.

Table 1 FT-IR analysis of ZnONPs synthesiszed from SCMPAE

Frequency (cm^{-1})	Bond	Functional group
3444.19	O-H stretch, H-bonded	Alcohols, phenols
1634.35	N-H bend	1° amines
692.99	-C≡C-H: C-H bend	Alkynes

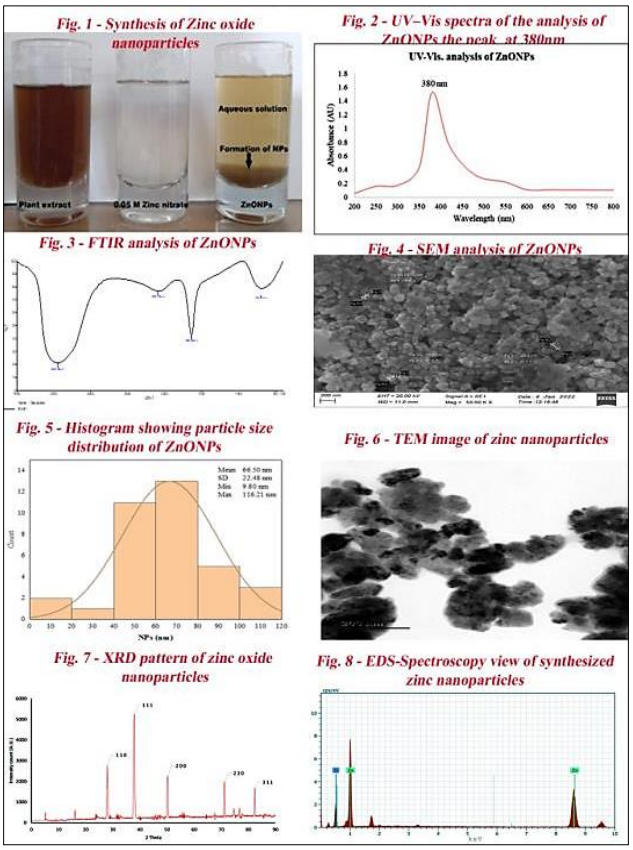

Plate 1 Biosynthesis and characterization of zinc nanoparticles using *Stereospermum chelonoides*

SEM analysis was carried out to understand the topology and the size of the ZnONPs, which showed the synthesis of higher density polydisperse spherical ZnONPs of various sizes that ranged from 10.00 to 84.34 nm respectively as well cubic and crystalline nature of the nanoparticles. Most of the nanoparticles gathered and only a little of them were dispersed, when observed under SEM (Plate 1, Fig 4). To find out the particle size of the nanoparticles the dynamic light scattering measurement was performed. Laser diffraction had shown that particle size was found in the range of 1 to 120 nm (Plate 1, Fig 5). The histograms plotted on the obtained data to study the particle size distribution reveals that the size of the nanoparticles ranged from 9.80 to 116.21 nm and the average particle size was found to be 66.50 ± 22.48 nm.

TEM is one of the reliable tools for NPs characterization. The morphological feature of synthesized ZnO NPs was determined using TEM as shown in (Plate 1, Fig 6). Synthesized ZnO NPs that was observed under TEM showed a homogenous shape that seem to be spherical and crystalline nature of the nanoparticles. XRD is generally used for determining the chemical constituents and crystal structure of a material. The identifying the presence of ZnO NPs in the sample can be achieved by using XRD to study the diffraction peaks of the ZnO NPs. X-ray diffraction pattern of ZnO NPs from the leaves extract is shown in (Plate 1, Fig 7). The crystalline nature of ZnO NPs was further established from X-ray diffraction (XRD) analysis display the XRD pattern of nanoparticles acquired

from colloid samples. A number of Bragg reflections with 2ø values of 28.02°, 37.95°, 50.24°, 71.20° and 82.36° indicated the 110, 111, 200, 220 and 311 reflections of metallic zinc visibly representing the crystalline spherical of zinc. The line broadening of peaks is mainly due to small particle size. Indexing has been done and data are in (Table 2).

Table 2 Simple peak indexing

Peak position 2θ	1000 × Sin2θ	1000 × Sin2θ/39.5	Reflection	Remarks
28.02	62.50	2	110	$1^2 + 1^2 + 0^2 = 2$
37.95	102.4	3	111	$1^2 + 1^2 + 1^2 = 3$
50.24	176.4	4	200	$2^2 + 0^2 + 0^2 = 4$
71.20	336.4	8	220	$2^2 + 2^2 + 0^2 = 8$
82.36	422.5	11	311	$3^2 + 1^2 + 1^2 = 11$

The peak at degrees and average particle size has been assessed by Debye-Scherrer formula [31].

$$D = 0.9\ \lambda/\beta\ \text{Cos}\ \theta$$

Where 'λ' is wave length of X-Ray (0.1541 nm)

'β' is FWHM (Full width at half maximum),

'θ' is the diffraction angle and 'D' is particle diameter size.

The average crystalline size according to Debye–Scherrer equation calculated was found to be 83.449nm and the details are in (Table 3).

Table 3 The grain size of ZnO nanoparticle

2θ of the intense peak (deg)	Miller indices (hkl)	θ of the intense peak (deg)	FWHM of intense peak (β) radians	Size of the particle (D) nm
28.02	110	19.01	0.2445	268.466
37.95	111	22.02	0.3311	21.183
50.24	200	25.12	0.4384	18.510
71.20	220	35.60	0.6213	25.635
82.36	311	41.18	0.7187	11.908
Average nanoparticle size				83.449

EDS were used to find elemental composition in the reaction mixture. EDS of ZnO NPs revealed the presence of pure zinc (Zn 70.29%) and was the major constituent element compared to oxide (29.71%) as shown in (Table 2, Plate 1, Fig 8). The EDX reading proved that the required phase of Zinc (Zn) was present in the ZnONPs.

Biopotential assay of zinc nanoparticles

Antimicrobial activity

Disc diffusion method was followed to assess antimicrobial activity of zinc nanoparticles synthesized from SCMPAE. Results were illustrated in (Plate 2, Fig 9-10). Results reveled that zinc nanoparticles provides good results when compared to crude extracts as well as pure zinc nitrate. ZnONPs produced 14.05±0.56 and 13.80±0.54 mm zone of inhibition against *Escherichia fergusonii* and *Klebsiella pneumoniae* respectively (Table 4).

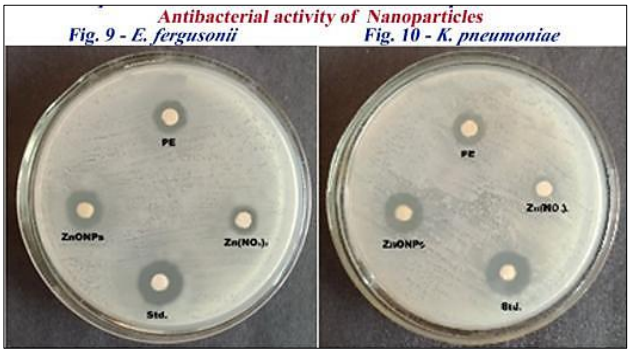

Plate 2 Antimicrobial potentials of zinc nanoparticles synthesized from *Stereospermum chelonoides* leaf and flower powder mixture

Table 4 Anti-microbial activity of ZnONPs synthesized using *Stereospermum chelonoides* extract

Microbial strains	Dose (50 µl)			Std. (30 µG)
	Zinc nitrate (0.05 M- (50 µl))	PE (50 µl)	ZnONPs (50 µl)	
Bacterial strains				
Escherichia coli	8.50±0.17	10.80±0.33	14.05±0.56	18.70±0.88
Klebsiells pneumoniae	8.35±0.16	10.55±0.31	13.80±0.54	18.65±0.88

Values expressed as Mean ± SD; Standard:
Chloramphenicol and Fluconazole; PE: Plant extract; mm: Millimeter

Anti-inflammatory activity

Three different *in-vitro* methods were used to assess anti-inflammatory activity of *Stereospermum chelonoides* leaf and flower mixture induced zinc nanoparticles (Table 5). Results revealed that all the three methods indicated the role of zinc nanoparticles as an effective anti-inflammatory agent. Effectiveness of nanoparticles are increased with increased concentration of the particles. ZnONPs produce protein damage protection up to 89.44±1.52%, RBC membrane stabilization 88.52±1.39% and Lipoxygenase inhibitory activities 86.76±1.54% at 500 µg/mL concentrations with 269.20 µg/mL, 310.45 µg/mL and 282.24 µg/mL of IC_{50}/ SC_{50} respectively.

Concentration

The anti-inflammatory activity of Isorhamnetin also plays an important role in kidney protection. It reduces inflammatory mediators and decrease oxidative stress in kidney thereby protecting it [30]. Luteolin 7-glucoside is one of the flavonoids showing biological activities like antioxidant, anticancer, anti-inflammatory, and antiapoptotic properties [31-

32]. The antioxidant activity of luteolin and its glycosides have been evaluated and confirmed [33]. It has been observed that luteolin inhibits inflammatory responses induced by lipopolysaccharide (LPS), tumour necrosis factor-α (TNF-α), and IL-6 in a dose-dependent manner [34]. Tumour Necrosis Factor alpha (TNF α) is an inflammatory cytokine produced by macrophages during acute inflammation and is responsible for a diverse range of signalling events within cells, leading to necrosis or apoptosis [35]. Compounds detected in SCMPEE possess anti TNF α activity. The expression of iNOS in the CNS is very tightly regulated, and several intrinsic and extrinsic stimuli can induce its expression in immune cells [36].

Table 5 Anti-inflammatory power of SCMPAE induced zinc nanoparticles

Concentrations (µg/mL)	Egg albumin inhibitions activity (% Inhibition)	Membrane stabilizing activity (% Stabilisation)	Lipoxygenase inhibitory activities (% Inhibition)
100	19.37±0.23	13.28±0.37	18.95±0.37
200	35.76±0.47	28.55±0.53	33.45±0.54
300	57.37±0.73	42.58±0.79	55.38±0.79
400	75.84±1.24	67.14±1.04	70.85±0.96
500	89.44±1.52	88.52±1.39	86.76±1.54
IC_{50}/ SC_{50} (µg/mL)	269.20	310.45	282.24

Values were expressed as mean ± Standard deviation for triplicates;
IC: Inhibitions concentration; SC – Stabilization

The flavanol-type flavonoid quercetin has increased in popularity because of its ability to modulate signal transduction pathways. Direct antioxidant properties may play a role in the abrogation of both DNA damage, but potentially of more importance quercetin, can also target multiple signalling pathways associated with oncogenesis and tumour progression, which include DNA damage, inflammation and obesity. Quercetin can also upregulate proteins that abrogate free radical damage, such as p53. The concurrent targeting of quercetin's multiple bioactivities presents a potent chemo preventative strategy, but because bioavailability of quercetin is poor it will be necessary to develop quercetin analogues to maximize the full chemo preventative potential of the compound. This review will explore the structural and mechanistic properties of quercetin as they relate to its ability to act as a chemo preventative compound [37]. O-glycoside derivatives of Quercetin are of two types, they are 3-O-glycoside and other O-glycoside derivatives. O-Glycoside quercetin derivatives represents broad-spectrum biological activities [38].

The research in the field of therapeutics is of great importance for the improvement of the quality of human life and for reducing human diseases. A vast number of diseases is due to pathogenic organisms. Pathogens are microorganisms that are harmful to the human body. Bacteria, viruses, fungus, prion, protozoan, viroid, etc. are the different types of pathogens. Microbial infections are drastically increased in living beings due to multidrug-resistant microorganisms even though the human body can defend against potential pathogens. Selectively targeting bacterial proteins which are critical to essential bacterial life processes like cell wall biosynthesis, translation, DNA replication etc. with novel compounds forms the basis of antibiotics development programs. Dihydrofolate reductase (DHFR) is one such protein, which, due to its critical role in nucleotide biosynthesis, has been a central drug target [39-40].

CONCLUSION

On the basis of above results, it was concluded that *Strereospermum chelonoides* induced zinc nanoparticles possess an efficient antimicrobial and anti-inflammatory activity. Antimicrobial activity is on MDR pathogens isolated from UTI cases. Nanoparticles possess wide spectrum of activity which could be due to flavonoid based compound in extracts as well as in nanoparticles. This compound could be considered as the alternative source in the control of multi drug resistant, ESBL producing bacteria.

Conflict of interest

The authors have no conflicts of interest.

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