Journal of Animal Research: v.12 n.06, p. 825-836. December 2022

DOI: 10.30954/2277-940X.06.2022.2

In-vitro Assessment of Antimicrobial Activity Against Isolates and Pure Culture of *Salmonella* Typhimurium by Using Green Synthesized Silver Nanoparticles

Raghupathi Ch[1*], Vijaya Kumar, A.[1], Sujatha Singh[1], Shirisha, A.[1] and Naveena, A.[2]

[1]*Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Hyderabad, INDIA*

[2]*Department of Veterinary Microbiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, INDIA*

[*]*Corresponding author: C Raghupathi; E-mail: challagurugularaghupathi@gmail.com*

Received: 22 Aug., 2022 **Revised:** 10 Oct., 2022 **Accepted:** 16 Oct., 2022

ABSTRACT

The green synthesis of metal nanoparticles has gained significant research attention in recent years. Among metallic nanoparticles, silver nanoparticles (AgNPs) exhibit a broad range of physical, chemical and biological properties due to which they are extensively applied in different fields. In this study, 1mM silver nitrate solution was combined with leaf extracts of *Ocimum sanctum* and *Azadirachta indica* under various circumstances to create green silver nanoparticles. Due to the presence of terpenoids and flavonoids, these two plant leaf extracts function as reducing agents in the synthesis of silver nanoparticles. This study discusses produced silver nanoparticles that were examined using a UV-visible spectrophotometer and the Zeta potential method. The absorption spectrum of the silver nano solution prepared by using *Ocimum sanctum* and *Azadirachta indica* leaf extracts showed a surface plasmon absorption band with maximum of 440 nm and 410 nm respectively. The zeta value of silver nanoparticles synthesized from *Ocimum sanctum* and *Azadirachta indica* leaf extract was -15.3 and -21.5 mV, size of 71.4 nm and 100.6 nm respectively. The antimicrobial activity of silver nanoparticles was compared in the current study using the agar well diffusion method on *Salmonella* Typhimurium isolates and pure cultures, as well as the anti-microbial resistance of different antibiotics on isolates and pure cultures. Final results demonstrated that silver nanoparticles exhibit antimicrobial activity on *Salmonella* Typhimurium bacteria that is nearly comparable to that of some antibiotics, and that a green synthesis protocol is an alternative to conventional physical and chemical methods that is quick, eco-friendly, and nontoxic.

HIGHLIGHTS

- Observed the antibacterial activity of silver nano particles in comparison with selected antibiotic discs on *Salmonella* Typhimurium.
- Green synthesized silver nanoparticles show promising results that can effectively replace antibiotics.

Keywords: Green synthesis, Silver nanoparticles, Uv-visible spectroscopy, Antimicrobial activity, *Salmonella* Typhimurium.

Nanotechnology is a unique field of research in contemporary science and technology, with applications in a variety of sectors such as biomedicine. Biogenic synthesis, one of the several methods currently accessible for the production of metallic nanoparticles, is becoming more popular for use in green nanotechnology (Brahmachari *et al.,* 2014). Silver nanoparticles have been prepared by using a variety of techniques, including chemical, physical, and biological ones. Those physical and chemical methods used for the synthesis of silver nanoparticles were toxic and hazardous (Awwad *et al.,* 2013). Thus, "green synthesis" refers to the employment of environmentally benign technologies for the synthesis of silver nanoparticles. Due to their exceptional physical and chemical properties, metallic nanoparticles are currently

How to cite this article: Raghupathi Ch, Vijaya Kumar, A., Singh, S., Shirisha, A. and Naveena, A. (2022). *In-vitro* Assessment of Antimicrobial Activity Against Isolates and Pure Culture of *Salmonella* Typhimurium by Using Green Synthesized Silver Nanoparticles. *J. Anim. Res.,* **12**(06): 825-836.

Source of Support: None; **Conflict of Interest:** None

of great interest such as high surface-to-volume ratio and heat transfer (Umer *et al.,* 2013). AgNPs represents a good candidate to carry out the nanostructured part of antibacterial and anticancer applications.

Azadirachta indica is a well-known immunostimulant owing to its more than 100 bioactive compounds. It also possesses anti-inflammatory, antioxidant, antibacterial, and anticancer properties (Mistry *et al.,* 2014). Tulsi leaves are mainly responsible for the gastric system, reproductive system, cardiovascular system, therapeutic potentials of the immune system, and central nervous system and are also significant in various ailments in modern science (Mondal *et al.,* 2009). Hence, Neem leaves and *Ocimum sanctum* leaves were selected as reducing agents for the present study.

These days, microorganisms might become resistant to drugs after extended exposure. This could be a result of the self-defense mechanism employed by the bacteria, which could lead to gene mutation and the development of antibiotic-inactivating enzymes (Poole *et al.,* 2002). Bacterial antibiotic resistance has grown to be a serious problem. Scientists have identified various novel strategies to meet this difficulty, one of which is the antibacterial activity of NPs. Silver is the most effective antibacterial agent among the other nanoparticles. AgNPs' shape and size determine how effective they are against bacteria (Pal *et al.,* 2007). By modifying the membrane potential, AgNPs bind to the cell membrane and change its permeability (Dakal *et al.,* 2016). In the present study, reported the green method of silver nanoparticles using extracts from tulsi and neem leaves, as well as the detection of their antibacterial activity against *Salmonella Typhimurium* isolates and pure cultures. We also examined the antibacterial activity with various antibiotics using the Kirby-Bauer disc diffusion method.

MATERIALS AND METHODS

Collection of plant leaves and preparation of leaf extract

Fresh leaves of *Ocimum sanctum* (Fig. 1) and *Azadirachta indica* (Fig. 2) were procured from various locations around the CVSc, Hyderabad and brought to the Department of Veterinary Public Health and Epidemiology laboratory. To get rid of any foreign objects like dust and etc., the leaves were rinsed in water many times before being dried in the shade. 20 g of freshly chopped *Ocimum sanctum* leaves were added to 100 ml of double-distilled water, which was then stirred at 60ºC for an hour. Whatman No. 1 filter paper was used to filter the resulting extract, and the filtrate was preserved for further use. In a similar manner, 100 ml of double-distilled water was combined with 20 g of *Azadirachta indica* leaves to create a fine powder. This powder was then added, heated for 10 minutes, cooled, and then filtered through Whatman No. 1 filter paper for further usage.

Fig. 1: Leaves of *Ocimum sanctum*

Fig. 2: Leaves of *Azadirachta indica*

Green Synthesis of Silver nanoparticles

One, two, three, four, and five ml of *Ocimum sanctum* leaf extract were added to five test tubes along with five ml of a 1 mM silver nitrate solution. The test tubes were then incubated in the dark to prevent photoactivation until the pale-yellow solution turned a rich mustard yellow color (Fig. 3).

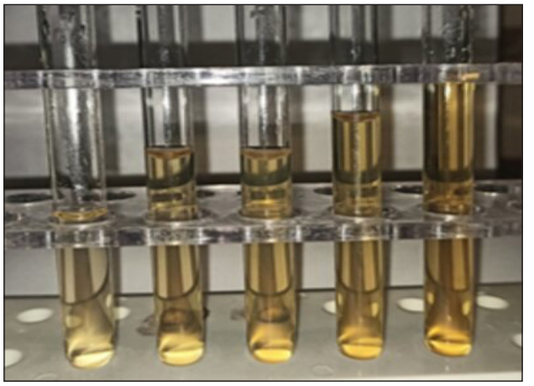

Fig. 3: After addition of *Ocimum sanctum* leaf extract to $AgNO_3$, Colour changes into dark yellow colour

Similar to this One hundred milliliter of 1 mM silver nitrate solution was prepared and every 10 ml of silver nitrate solution was transferred into 5 test tubes. Then 1, 2, 3, 4, and 5 ml of *Azadirachta indica* leaf extract were added to five test tubes and later incubated in the dark chamber to avoid photo activation till the colorless solution changed to brown color (Fig. 4).

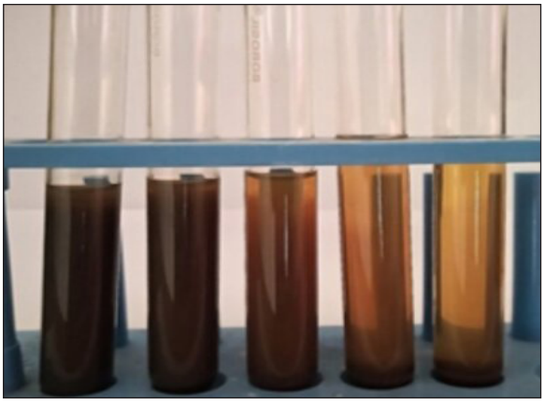

Fig. 4: After addition of *Azadirachta indica* leaf extract to $AgNO_3$, Colour changes into brown colour

Characterization of synthesized silver nanoparticles

UV-Visible spectrophotometer and Zeta Potential

The AgNPs were founded by measuring the wavelength of the reaction mixture in the Ultra-visible spectrum of the Thermo fisher spectrophotometer at a resolution of 1 nm (from 200 to 800 nm) in a 2 ml quartz cuvette with a 1 cm path length. One ml of the sample was pipette into a test tube and subsequently examined at room temperature. The prepared sample was dispersed in deionized water followed by ultra-sonication. Afterward, the solution was filtered and centrifuged for 15 min at 25ºC at 5000 rpm and the supernatant was collected. The supernatant was diluted 4 to 5 times and the particle distribution in liquid was studied in a computer-controlled particle size analyzer (ZETA sizer Nano series, Malvern instrument Nano Zs).

Isolation of *Salmonella* Typhimurium from poultry samples

For *Salmonella* Typhimurium isolation, A total of 150 poultry samples (50 each from chicken fecal, cloacal, and egg swabs) were aseptically collected from college farms. The isolation of *Salmonella* Typhimurium was carried out by using RV broth for the enrichment and BGA agar media. The presumptive *Salmonella* Typhimurium colonies were subjected to Gram staining and various biochemical tests (Table 1)

Table 1: Biochemical characterization of *Salmonella* Typhimurium

Sl. No.	Tests	*Salmonella* Typhimurium
1	Gram staining test	Negative (-ve)
2	Urease test	Negative (-ve)
3	Indole test	Negative
4	Methyl red test	Positive
5	Voges – Proskauer test	Negative
6	Simmons citrate	Positive
7	TSI	Acid butt (Y), Alkaline slant (R), H_2S (+)
8	Motility test	Motile

Pure strains

The pure strain of *Salmonella* Typhimurium was obtained from MTCC at Chandigarh, India. All the cultures were

maintained at 4ºC in nutrient broth and sub-cultured in nutrient agar at regular intervals over days.

PCR assay

Genomic DNA extraction

Genomic DNA was isolated from all the bacterial isolates using the Phenol Chloroform-Isoamyl alcohol DNA extraction method as per (Wright *et al.,* 2017) with some modifications.

Oligonucleotide primers for detection of genus *Salmonella*

The primers used from the *fli*C gene for the detection of *Salmonella* Typhimurium were custom synthesized by Xcelris labs limited are given in Table 2. Polymerase chain reaction (PCR) was carried out on extracted DNA targeting invasion A gene (*fli*C) gene which is genus-specific as per the method described by Oliveira *et al.* (2002) with little modification.

Table 2: Specific primers used for identification of *Salmonella* spp.

Primer	Target gene	Length	Primer sequence	Amplification (bp)	Reference
Fli 15	*fli*C	22	CGG TGT TGC CCAGGT TGG TAA T	620	Oliveira *et al.* (2002)
Typ 04	*fli*C	16	ACT GGT AAA GAT GGC T		

Standardization of the PCR protocol for *Salmonella* Typhimurium

PCR amplification of the *fli*C gene fragment was set up to 25 µl reactions. The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay and by varying the annealing temperature and cycling conditions.

Reaction mixture

The components of the reaction mixture were finally optimized as given in Table 3. In this study, the template preparation was done by the phenol-chloroform method. PCR assay was performed in an Eppendorf gradient Thermal cycler with a heated lid. The cycling conditions used were presented in Table 4. PCR products were stored at -20ºC until further use.

Table 3: PCR reaction mixture for *Salmonella* Typhimurium

Sl. No.	Name of the Reagent	Quantity (µl) for *Salmonella* spp
1	10X Taq polymerase buffer	2.5
2	dNTP mix	1.0
3	Primer-F	2.0
4	Primer-R	2.0
5	Taq DNA polymerase	0.3
6	Molecular grade water	q.s
7	Purified DNA /Bacterial lysate	5.0

Table 4: Amplification conditions used for *Salmonella* Typhimurium primers

Sl. No.	Step	*fli*C (*Salmonella* Typhimurium)
1	Initial denaturation	94ºC /5min
2	Final denaturation	94ºC/1min
3	Annealing	45.1ºC/30sec
4	Initial extension	72ºC/38 sec
5	Final extension	72ºC/7min
6	Hold	4ºC

Antibiotic sensitivity test

The disc diffusion assay using Muller-Hinton (MH) agar and following CLSI guidelines was used to determine the isolates' antimicrobial susceptibility. Five colonies of the isolate were injected into MH broth, and tubes were incubated at 37ºC for 2–8 hours until the turbidity reached 0.5 on the Mac Farland scale. Following turbidity correction, a sterile swab was inserted, pushed against the tube well to remove any excess liquid, and then seeded for *Salmonella* Typhimurium on the surface of a petri dish with MH agar, rotating at least twice. Using sterile forceps, four discs (Table 5) were impregnated with antimicrobials, 50 µl of each of the AgNPs from *Ocimum sanctum* and *Azadirachta indica*, $AgNO_3$ solution, and Pure leaf extracts and Distilled water were placed at equal distances from each other on the surface of the inoculated

agar plate. Subsequently, the plate was inverted and incubated at 37ºC for 24 h. Disc readings were taken after incubation, and a ruler was used to estimate the diameter of the inhibition halos. The interpretation was determined in accordance with the disc manufacturer's stated zone size interpretation chart.

Table 5: Antibiotics used in the antibiotic resistance/susceptible test

Sl. No.	Antibiotics	Abbreviations	Concentration (µg/unit)
1	Chloramphenicol	C	30
2	Tetracycline	TE	30
3	Ciprofloxacin	CIP	5
4	Ampicillin	AMP	10

RESULTS AND DISCUSSION

Green synthesis and characterization of silver nanoparticles

UV- Visible Spectroscopy

The AgNPs were characterized by UV-visible spectroscopy, for the characterization of AgNPs. *Ocimum sanctum* leaf extract was used to generate a deep mustard yellow silver nano solution, and the absorption spectrum revealed a surface plasmon absorption band with a maximum of 440 nm (Fig. 5).

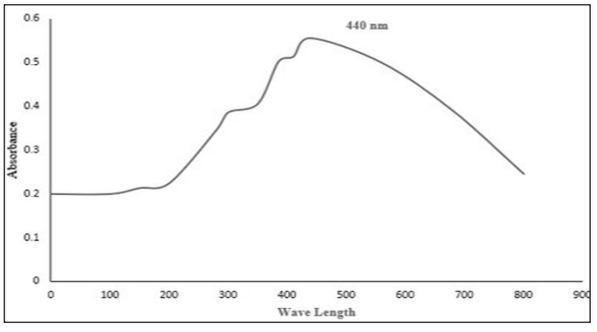

Fig. 5: AgNPs prepared with *Ocimum sanctum* showing UV-Visible absorption band at 440 nm, sample (5:5)

Brahmachari *et al.* (2014) observed the color changes from transparent to pale yellow, yellow, reddish, and finally, wine red color for the silver nanoparticles at different time intervals, Jain and Mehta (2017) observed pale yellow to dark yellow, Borah *et al.* (2013) observed pale yellow to reddish brown color, Rout *et al.* (2012) observed dark yellowish to brown color, Singhal *et al.* (2011) observed transparent to dark yellow color and Rao *et al.* (2013) observed colorless to reddish yellow color for the silver nanoparticles prepared by using *Ocimum sanctum* leaf extract and The UV absorption band of silver nanoparticles range from 400 nm to 450 nm (Ramteke *et al.,* 2013). The peaks obtained in this study for nanoparticles were within the range, clearly indicating the formation of AgNPs in both the nano solutions). Sood and Chopra (2018) reported UV-Vis spectral peak at 420 nm prepared by using *Ocimum sanctum* leaf extract, which was less than to the peak (440 nm) observed in the present study. Brahmachari *et al.* (2014) and also Rout *et al.* (2012) observed UV-Vis spectral peak at 430 nm and Borah *et al*. (2013) observed UV-Visible spectra peak at 431 nm for the AgNPs prepared by using *Ocimum sanctum*, whichwase lesser than the present study. Rao *et al.* (2013) observed UV- visible spectral peak at 406 nm, whereas Singhal *et al.* (2011) observed peak at 413 nm was very low than the present study. The absorption spectrum of the brown silver nano solution prepared by using *azadirachta indica* leaf extract showed an absorption band at 410 nm, (Fig 6) that indicated the presence of silver nanoparticles. Roy *et al.* (2017) observed the dark brown color for silver nano solution prepared with *Azadirachta indica* leaf extract, whereas Priyadarshini *et al.* (2019) observed dark brown with *Azadirachta indica* extract. Ahmed *et al.* (2016) reported yellowish to reddish brown color, Rather *et al.* (2017) observed reddish yellow to brown, Shankar *et al.* (2004) observed Yellowish to brown and Chinnasamy *et al.* (2021) observed transparent to brown colour for silver nanoparticles prepared by using *Azadirachta indica* leaf extract. The appearance of the final colors confirms the reduction of silver nitrate into silver nanoparticles due to the excitation of free electrons in the reaction mixture (Kalimuthu *et al.,* 2008) and Roy *et al.* (2017) reported UV-Vis spectral peak at 430 nm for the silver nanoparticles prepared with *Azadirachta indica* leaf extract, which was slightly higher thanthe spectral peak at 410 nm in the present study. Shankar *et al.* (2004) observed UV-Vis spectral peak at 450 nm for the AgNPs prepared by using *Azadirachta indica* leaf extract, which was higher than the spectral peak in the present study. Ahmed *et al.*

(2016) reported UV-Vis spectral peak of 436 - 446 nm range for the AgNPs prepared by using *Azadirachta indica* leaf extract, which was higher than the peak observed in the present study (410 nm), whereas Rather *et al.* (2017) reported the UV absorption peaks of silver nanoparticles prepared by using *Azadirachta indica* at 420 nm were slightly similar to the present study (410 nm). Chinnasamy *et al.* (2021) observed a peak at 400 nm which was slightly less than the present study (410nm). A broad peak at a higher wavelength indicates an increase in particle size, whereas a narrow line at a shorter wavelength represents a smaller particle size (Prathna *et al.,* 2011).

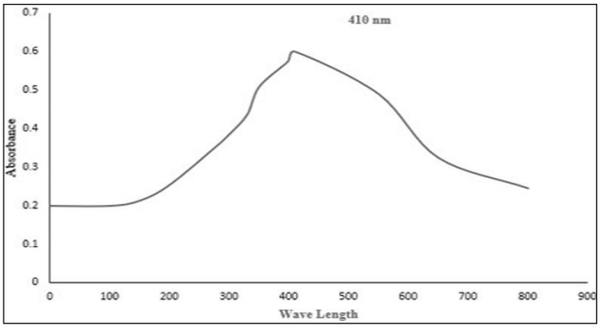

Fig. 6: AgNPs prepared with *Azadirachta indica* showing UV-Visible absorption band at 410 nm, sample (10:3)

Zeta potential and Particle size distribution by intensity

Zeta potential measures the potential stability of the nanoparticles in the colloidal suspension. The zeta potential values of silver nanoparticles synthesized from *Ocimum sanctum* and *Azadirachta indica* leaf extract were -15.3 mV and -21.5 mV respectively. Rao *et al.* (2013) used zeta potential to know the stability of AgNPs synthesized by using *Ocimum sanctum* leaf extract and observed zeta potential value of -55.0 mV, which was a lesser value than the value (-15.3 mV) obtained in the present study. Pandey *et al.* (2021) reported that zeta potential value of -26.20mV for AgNPs prepared from *Ocimum Tenuflorum* leaf extract, which was slightly lesser than the value obtained in the present study and they also observed -56.84 mV for the pure plant leaf extract, which was lesser value than the present study (-15.3 mV). Mankad *et al.* (2020) reported that zeta potential for green synthesized (*Azadirachta indica* leaf extract) silver nanoparticles ranged from -19.6 to -22.8 mV, which was similar value to the value obtained in the present study, whereas Rather *et al*. (2017) reported higher potential value of (+34.6mV) AgNPs. The negative charge provided by the active molecules of tulsi extract might have stabilized the size of the AgNPs (Pandey *et al.,* 2021). Rao *et al.* (2013) concluded that the high negative value confirms the repulsion among the particles and thereby increases the stability of the formulation.

Zeta potential of around ± 30 mV produced the complete stability of a colloidal solution (Saeb *et al.,* 2014), and the values obtained for the AgNPs prepared by using both *Ocimum sanctum* and *Azadirachta indica* leaf extract are within this range. Among the five ratios of *Ocimum sanctum* leaf extract and silver nitrate solution, the ratio of 5:5 has given the highest peak, good size, and stability, so these ratios were selected for further use, whereas among the five ratios of *Azadirachta indica* leaf extract and silver nitrate solution, the ratio of 3:10 ml has given highest peak, good size, and stability. The DLS measures size of the silver nanoparticles was 71.4 nm (Fig. 7) and 100.6 nm (Fig. 8) for AgNPs with *Ocimum sanctum* and *Azadirachta indica* respectively. These zeta potential values indicate the good stability of silver nanoparticles synthesized.

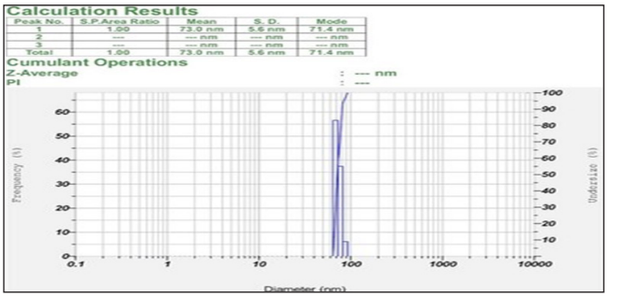

Fig. 7: Particle size distribution by intensity of AgNPs prepared with *Ocimum sanctum* leaf extract

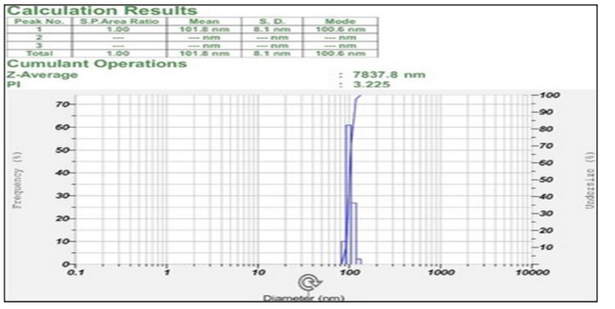

Fig. 8: Particle size distribution by intensity of AgNPs prepared with *Azadirachta indica* leaf extract

Isolation and characterization of *Salmonella* Typhimurium by the cultural method

Out of 150 samples, 50 each of poultry fecal swabs, cloacal swabs, and eggshells. 50 (33.33%) samples were positive for *Salmonella* Typhimurium by the cultural method. The isolation of *Salmonella* Typhimurium was carried out by using Rappaport Vassiliadis broth for enrichment and brilliant green agar. Colony morphology showed characteristics, like bright pink or red colonies surrounded by a red halo in the medium (Fig. 9).

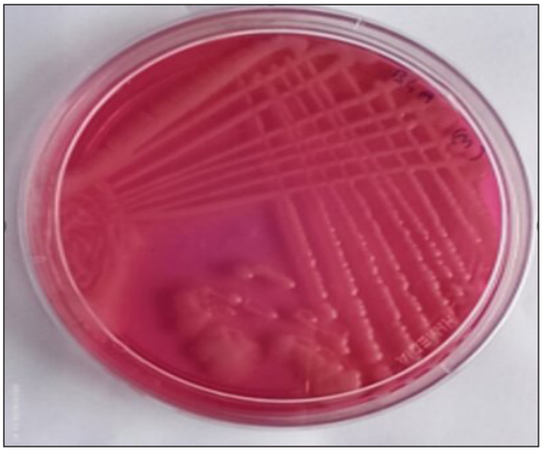

Fig. 9: Plate showing the growth of *Salmonella* Typhimurium bright pink colonies on BGA agar

Gram staining and biochemical tests (Fig. 10) were performed on the suspected colonies to confirm the results.

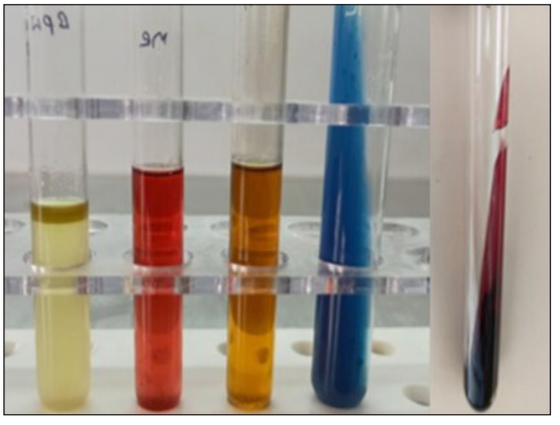

Fig. 10: Results of Biochemical characterization (IMViC: - + - +) and TSI test for *Salmonella* Typhimurium

Standardization of PCR assay to detect *Salmonella* Typhimurium

The amplification of PCR product was standardized for the identification of *Salmonella* Typhimurium by utilizing the primers of *fliC* gene. PCR reaction conditions for template *Salmonella* Typhimurium involved the variation of annealing temperature 42ºC - 62ºC, the concentration of primers (5-15 pmol), $MgCl_2$ (1.5-3 mM), template volume (2-8 µl), and the thermal cycling conditions. Results were obtained using 5 µl of bacterial lysate or 20 ng of diluted DNA as a template, 2.5 µl of 10X Taq polymerase assay buffer with 1.5 mM $MgCl_2$ were used in the reaction mixture. 1 µl of 25 mM of each dNTP, 1 µl (4 pmol/µl) of each primer, and 0.9 U/µl of Taq DNA polymerase in a final reaction volume made up to 25 µl with molecular grade water. Initial denaturation at 94ºC, 5 min, followed by 30 cycles each of denaturation at 94ºC, 60 sec, annealing at 45.1ºC, 30 sec, and extension at 72ºC, 38 sec with a final extension period of 7 min at 72ºC was found to be optimum for obtaining the desired PCR amplicon of 620 bp). Gel electrophoresis of the PCR revealed the specific amplification of a 620 bp fragment with the help of a transilluminator (Fig. 11).

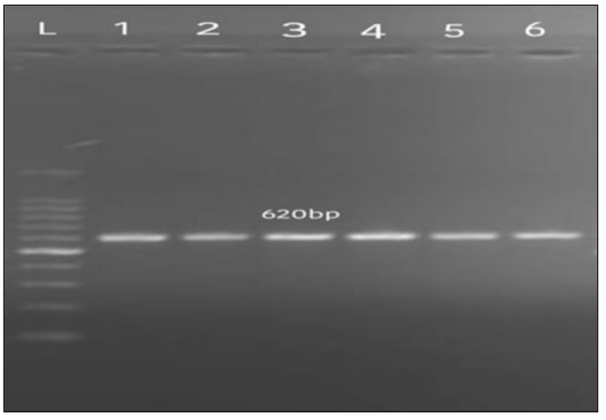

Fig. 11: PCR result of for *Salmonella* Typhimurium at bp (620); Lane L: 100 bp DNA ladder Lane 1,2,3,4,5,6,7: showing positive results for *Salmonella* Typhimurium

Incidence of *Salmonella* Typhimurium in Poultry Samples

Out of 150 total poultry samples collected from different sources, 50 (33.33 %) poultry samples were positive by

traditional cultural methods, whereas 55 (36.66%) samples were positive by PCR assay for *Salmonella* Typhimurium. The efficiency of the cultural method compared to PCR assay was 90.90 percent. Islam *et al.* (2014) reported an incidence of 47.92% of *Salmonella* Typhimurium in the poultry samples collected from different poultry farms, which was higher than the incidence (33.3%) observed in the present study, whereas Ejeh *et al.* (2017) and Hakkani *et al*. (2016) reported very low incidence (11.4%) and (25%) of *Salmonella* Typhimurium compared to the present study (33.33%). Anumolu and Lakkineni (2012) reported a very low incidence (15.38%) of *Salmonella* Typhimurium compared to the present study (36.66%). The incidence of 86% and 92% of *Salmonella* spp in the poultry samples by cultural and PCR assay reported by Ramya *et al.* (2012) was much higher than the incidence (33.33%) and (36.66%) observed in the present study. Out of 50 poultry, fecal samples 19 (38 %) and 21 (42 %) were positive for *Salmonella* Typhimurium by cultural and PCR assay respectively, with the efficiency of (90.47%) cultural method compared to PCR assay. Ramya *et al.* (2012) reported an incidence of 88% of *Salmonella spp* in poultry fecal samples by cultural method, which was higher than the incidence (38%) observed in the present study. The incidence of *Salmonella* Typhimurium from poultry fecal samples (42%) by PCR assay in the present study was very lower than the incidence (92%) reported by Ramya *et al.* (2012). Out of 50 poultry, cloacal samples 15 (30 %) and 16 (32%) were positive for *Salmonella* Typhimurium by cultural and PCR assay respectively, with the efficiency of the cultural method compared to PCR assay was 93.75 percent. Ramya *et al.* (2012) reported an incidence of 84% of *Salmonella* spp in the poultry cloacal swab samples by cultural method, which was higher than the incidence (30%) observed in the present study, whereas lower incidence (6.9%) reported by Amini *et al.* (2010) and Eyigor *et al.* (2002) in the poultry cloacal swab samples. The incidence of *Salmonella* Typhimurium in the poultry cloacal swabs (32%) by PCR assay in the present study is very lower than the incidence (92%) reported by Ramya *et al.* (2012). In the present study the incidence of *Salmonella* Typhimurium was higher in the poultry faecal samples (38%) compared to the incidence in the poultry cloacal swab samples (30%), which was similar to the findings reported by Ramya *et al*. (2012). Out of 50 poultry egg swabs 16 (32%) and 18 (36 %) were positive for *Salmonella* Typhimurium by cultural and PCR assay respectively, with the efficiency of cultural method compared to PCR assay was 88.88 percent. Haritha (2019) reported an incidence of 30% and 34% by cultural and PCR respectively of *Salmonella spp* in the poultry egg swabs, which was almost similar to the incidence (30 %) and (36%) observed in the present study.

Antimicrobial Activity Against *Salmonella* Typhimurium

The antimicrobial activity was reported as the mean zone of inhibition. The antimicrobial activity of leaf extract of Tulsi, Neem leaf extract, Pure $AgNO_3$ solution, and AgNPs prepared with Tulsi and Neem leaf extract on *Salmonella* Typhimurium was described in the (Table 6, Fig. 12, Fig 13). Distilled water has been taken as control which showed no mean of ZOI against *Salmonella* Typhimurium. Similarly, the mean of ZOI for antibiotic discs like Chloramphenicol, Ciprofloxacin, Tetracycline and Ampicillin was reported against pure culture and isolates of *Salmonella* Typhimurium from poultry samples (Fig. 14 and 15).

Table 6: The antimicrobial activity of antibiotics, silver nitrate solution and silver nano particles on pure cultures and isolates of *Salmonella* Typhimurium from poultry samples

Sl. No.	Antibiotic/ material	Zone of inhibition (mm)	
		On Pure Cultures	On Isolates (Mean ± SE)
1	$AgNO_3$ Solution	12	11.10±0.31
2	*Ocimum sanctum* Leaf extract	2	1.02±0.12
3	*Azadirachta indica* Leaf extract	3	2.06±0.17
4	*Ocimum sanctum* AgNP's	19	17.18±0.33
5	*Azadirachta indica* AgNP's	23	21.08±0.40
6	Chloramphenicol	26	25.04±0.24
7	Ciprofloxacin	22	21.24±0.38
8	Tetracycline	18	17.02±0.30
9	Ampicillin	17	15.04±0.24

In the current investigation, the mean ZOI against *Azadirachta indica* leaf extract for pure cultures and isolates of *Salmonella* Typhimurium from poultry samples was 3 and 2.06±0.17mm, respectively. This was

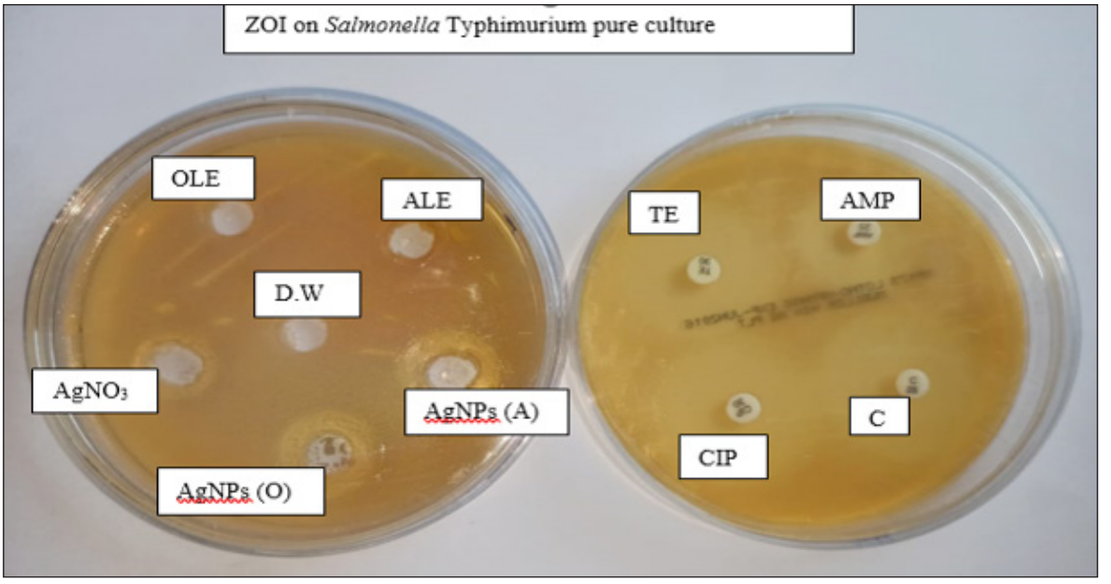

Fig. 12: ZOI on pure culture of *Salmonella* Typhimurium. $AgNO_3$: Silver nitrate solution; AgNPs (O): Silver Nanoparticles prepared with *Ocimum sanctum*; OLE: *Ocimum sanctum* leaf extract; AgNPs (A): Silver Nanoparticles prepared with *Azadirachta indica*; ALE: *Azadirachta indica* leaf extract.

Fig. 13: ZOI of Antibiotics for pure culture of *Salmonella* Typhimurium. **(A)** Ampicilin; CIP: Ciprofloxacin; **(C)** Chloramphenicol; TE: Tetracycline

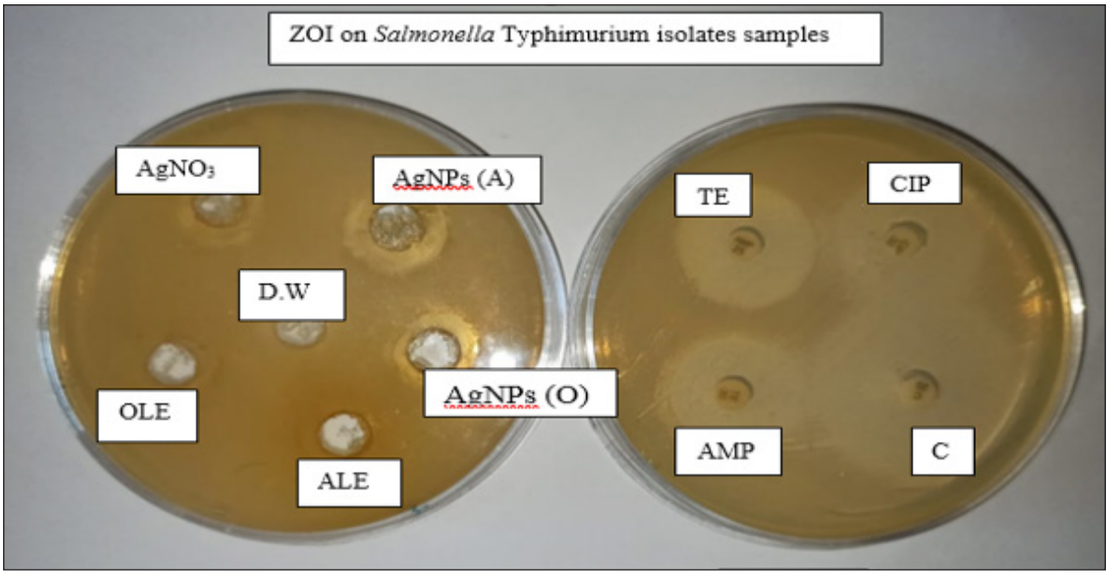

Fig. 14: Zone of Inhibition on Isolates of *Salmonella* Typhimurium

Fig. 15: Zone of Inhibition of Antibiotics for Isolates of *Salmonella* Typhimurium

smaller than the ZOI of 10 mm reported by Jamiu and Bello (2018). Terpenoids and flavanones, two significant phytochemicals found in neem, are essential for stabilizing nanoparticles, acting as capping and reducing agents (Benerjee *et al*., 2014), and exhibiting anti-oxidant activity by neutralizing lipid free radicals (Maisuthisakul *et al*., 2007) or chelating silver ions (Balasundaram *et al*., 2014). Samantaray *et al*. (2020) reported zero ZOI with AgNPs prepared by using Tridax procumbens leaf extract against *Salmonella* Typhimurium. The mean of ZOI against pure $AgNO_3$ solution was 12 and 11.10±0.31 mm respectively for pure culture and isolates of *Salmonella* Typhimurium from poultry samples in the present study, which was slightly higher than the ZOI (10mm) reported by Singh *et al*. (2021). Jamiu and Bello (2018) reported ZOI as (12 mm) for $AgNO_3$ solution, which was similar to the mean width of the zone of inhibition in the present study. The mean of ZOI against silver nanoparticles prepared using *Azadirachta indica* leaf extract was 23 and 21.08±0.40 mm respectively for pure culture and isolates of *Salmonella* Typhimurium from poultry samples in the present study. This zone of inhibition for AgNPs was higher compared to the $AgNO_3$ solution in the present study. Loo *et al.* (2018) reported less zone of inhibition (20 mm) against *Salmonella* Typhimurium for silver nanoparticles prepared from Pu- erh tea leaf extract. The mean of ZOI against *Ocimum sanctum* leaf extract for pure cultures and isolates from poultry samples against *Salmonella* Typhimurium in the current investigation was 2 and 1.02 ± 0.12 mm, respectively. The antibacterial activity of *Ocimum sanctum* extract might be due to Eugenol (1-hydroxy-2-methoxy-4-allyl benzene) present in the leaf extract, which affects bacterial growth (Priyadarshini *et al*., 2019). The mean of ZOI of silver nanoparticles prepared using *Ocimum sanctum* leaf extract against *Salmonella* Typhimurium was 19 and 17.02±0.33 mm respectively for pure cultures and isolates from poultry samples, which was similar to the value reported by Shaik *et al.* (2018). The mean of ZOI of Chloramphenicol, Ampicillin, Tetracycline, and Ciprofloxacin was 26, 17, 18 and 22 mm respectively against *Salmonella* Typhimurium pure cultures, whereas the mean of ZOI against *Salmonella* Typhimurium isolates from poultry samples was 25.04±0.24, 15.04±0.24, 17.02±0.30 and 21.24±0.38 mm respectively. The mean of ZOI against *Salmonella* Typhimurium with antibiotics used in this study was high with Chloramphenicol (25.04±0.24 mm) followed by Ciprofloxacin (21.24±0.38 mm) and less with Tetracycline (17.02±0.30 mm) and Ampicillin (15.04±0.24 mm). Hari *et al.* (2014) observed the mean ZOI of Chloramphenicol on *Salmonella* Typhimurium, was similar to (25.04±0.24 mm) the mean width of the zone of inhibition in the present study.

CONCLUSION

The conclusion of the present study indicates that the AgNPs prepared with both *Ocimum sanctum* and *Azadirachta indica* leaf extract were almost equally efficient against both *Salmonella* Typhimurium and the AgNPs antibacterial activity is almost similar to most of the antibiotics studied in this work. According to the current study, silver nanoparticles are a promising future technology that can effectively replace antibiotics. but, for efficient utilization of silver nanoparticles, further research is needed.

ACKNOWLEDGMENTS

This work was supported financially by the P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, and also my special thanks to the National Institute of Pharmaceutical Education and Research (NIPER) institute, Hyderabad for providing good facilities.

REFERENCES

Ahmed, S., Ahmad, M., Swami, B.L. and Ikram, S. 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J. Adv. Res.*, **7(1)**: 17-28.

Anumolu, V.K. and Lakkineni, V.R. 2012. Screening of poultry samples for *Salmonella* Typhimurium by PCR assay. *Vet. World.*, **5(3)**: 169-172.

Awwad, A.M., Salem, N.M. and Abdeen, A.O. 2013. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. *Int. J. Ind. Chem.*, **4(1)**: 1-6.

Balasundram, N., Sundram, K. and Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food chem.*, **99(1)**: 191-203.

Banerjee, P., Satapathy, M., Mukhopahayay, A. and Das, P. 2014. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour. Bioprocess.*, **1**(1): 1-10.

Borah, D., Deka, P., Bhattacharjee, P., Changmai, A. and Yadav, R.N.S. 2013. *Ocimum sanctum* mediated silver nano particles showed better antimicrobial activities compared to citrate stabilized silver nano particles against multidrug resistant bacteria. *J. Pharm. Res.*, **7(6)**: 478-482.

Brahmachari, G., Sarkar, S., Ghosh, R., Barman, S., Mandal, N.C., Jash, S.K. and Roy, R. 2014. Sunlight-induced rapid and efficient biogenic synthesis of silver nanoparticles using aqueous leaf extract of *Ocimum sanctum Linn*. with enhanced antibacterial activity. *Org. Med. Chem. Lett*., **4(1)**: 1-10.

Chinnasamy, G., Chandrasekharan, S., Koh, T.W. and Bhatnagar, S. 2021. Synthesis, characterization, antibacterial and wound healing efficacy of silver nanoparticles from *Azadirachta indica*. *Front. Microbiol*., **12**: 611560.

Dakal, T.C., Kumar, A., Majumdar, R.S. and Yadav, V. 2016. Mechanistic basis of antimicrobial actions of silver nanoparticles. *Front. Microbiol*., **7**: 1831.

Ejeh, F.E., Lawan, F.A., Abdulsalam, H., Mamman, P. H. and Kwanashie, C.N. 2017. Multiple antimicrobial resistance of *Escherichia coli* and *Salmonella* species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria. *Sokoto J. Vet. Sci*., **15(3)**: 45-53.

Eyigor, A., Carli, K.T. and Unal, C.B. 2002. Implementation of real-time PCR to tetrathionate broth enrichment step of *Salmonella* detection in poultry *lett. Appl. Microbiol*., **34(1)**: 37-41.

Hakkani, M.G., Rahman, M.M., Akter, M.R., Hossain, M.K., Nazmi, A. and Rahman, M.S. 2016. Isolation and identification of microorganisms from cloacal swabs in poultry. *AAJBB* **1(3)**: 441-447.

Hari, N., Thomas, T. K. and Nair, A. J. 2014. Comparative study on the synergistic action of differentially synthesized silver nanoparticles with β-cephem antibiotics and chloramphenicol. *J. Nanosci*., 2014, ID 201482.

Islam, M.M., Islam, M.N., Sharifuzzaman, F.M., Rahman, M.A., Sharifuzzaman, J.U., Sarker, E.H. and Sharifuzzaman, M.M. 2014. Isolation and identification of *Escherichia coli* and *Salmonella* from poultry litter and feed. *Int. J. Nat. Soc. Sci.*, **1(1)**: 1-7.

Jain, S. and Mehata, M.S. 2017. Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. *Sci. Rep*., **7(1)**: 1-13.

Jamiu, A.T. and Bello, S.A. 2018. Biosynthesis of silver nanoparticles using *azadirachta indica* leaf extract and assessment of its antibacterial activity on some pathogenic enteric bacteria. *Int. J. Novel. Res. Life Sci*., **5**: 25-31.

Kalimuthu, K., Babu, R.S., Venkataraman, D., Bilal, M. and Gurunathan, S. 2008. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids Surf B Biointerfaces*., **65(1)**: 150-153.

Kumarss, A., Taghi, Z.S., Gholamreza, N., Reza, R., Javid, A. and Shahrnaz, B. A. 2010. Molecular detection of *invA* and *spv* virulence genes in *Salmonella enteritidis* isolated from human and animals in Iran. *Afr. J. Microbiol. Res*., **4**(21): 2202-2210.

Loo, Y.Y., Rukayadi, Y., Nor-Khaizura, M.A.R., Kuan, C.H., Chieng, B.W., Nishibuchi, M. and Radu, S. 2018. *In vitro* antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. *Front. Microbiol*., **9**: 1555.

Maisuthisakul, P., Suttajit, M. and Pongsawatmanit, R. 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chem*., **100**(4): 1409-1418.

Mankad, M., Patil, G., Patel, D., Patel, P. and Patel, A. 2020. Comparative studies of sunlight mediated green synthesis of silver nanoparaticles from *Azadirachta indica* leaf extract and its antibacterial effect on *Xanthomonas oryzae pv. oryzae*. *Arab. J. Chem.* **13**(1): 2865-2872.

Mistry, K.S., Sanghvi, Z., Parmar, G. and Shah, S. 2014. The antimicrobial activity of *Azadirachta indica, Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% *chlorhexidine gluconate* on common endodontic pathogens: An in vitro study. *Eur. J. Dent*., **8(02)**: 172-177.

Mondal, S., Mirdha, B.R. and Mahapatra, S.C. 2009. The science behind sacredness of Tulsi (*Ocimum sanctum Linn*.). *Indian J. Physiol. Pharmacol.*, **53**(4): 291-306.

Oliveira, S.D., Santos, L.R., Schuch, D.M.T., Silva, A.B., Salle, C.T.P. and Canal, C.W. 2002. Detection and identification of *salmonellas* from poultry-related samples by PCR. *Vet. Microbiol*., **87**(1): 25-35.

Pal, S., Tak, Y.K. and Song, J.M. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli. Appl. Environ. Microbiol.*, **73(6)**: 1712-1720.

Pandey, V.K., Upadhyay, S.N. and Mishra, P.K. 2021. Light-induced synthesis of silver nanoparticles using *Ocimum tenuiflorum* extract: Characterisation and application. *J. Chem. Res*., **45(1-2)**: 179-186.

Poole, K. 2002. Mechanisms of bacterial biocide and antibiotic resistance. *J. Appl. Microbiol.*, **92**: 55S-64S.

Prathna, T.C., Chandrasekaran, N., Raichur, A.M. and Mukherjee, A. 2011. Biomimetic synthesis of silver nanoparticles by *Citrus limon* (lemon) aqueous extract and theoretical prediction of particle size. *Colloids Surf. B: Biointerfaces*., **82**(1): 152-159.

Priyadarshini, S., Sulava, S., Bhol, R. and Jena, S. 2019. Green synthesis of silver nanoparticles using *Azadirachta indica* and *Ocimum sanctum* leaf extract. *Curr. Sci*., **117**(1300): 1300-1307.

Ramteke, C., Chakrabarti, T., Sarangi, B.K. and Pandey, R.A. 2013. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *J. Chem.,* 2013:ID 278925.

Ramya, P., Madhavarao, T. and Rao, L.V. 2012. Study on the incidence of *Salmonella enteritidis* in poultry and meat samples by cultural and PCR methods. *Vet World*, **5**(9): 541-545.

Rao, Y.S., Kotakadi, V.S., Prasad, T.N.V.K.V., Reddy, A.V. and Gopal, D.S. 2013. Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulasi (*Ocimum sanctum*) leaf extract. *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **103**: 156-159.

Rather, M.A., Bhat, I.A., Sharma, N., Gora, A., Ganie, P.A. and Sharma, R. 2017. Synthesis and characterization of *Azadirachta indica* constructed silver nanoparticles and their immunomodulatory activity in fish. *Aquac. Res.*, **48(7)**: 3742-3754.

Routhu, H., Anumolu, V., Singh, S., Shashikumar, M. and Krishnaiah, N. 2020. Studies on the Prevalence of *Salmonella* in poultry in and around greater Hyderabad municipal corporation. *Int. J. Livest. Prod*., **10(3)**: 115-122.

Roy, P., Das, B., Mohanty, A. and Mohapatra, S. 2017. Green synthesis of silver nanoparticles using *Azadirachta indica* leaf extract and its antimicrobial study. *Appl. Nanosci*., **7(8)**: 843-850.

Saeb, A., Alshammari, A.S., Al-Brahim, H. and Al-Rubeaan, K.A. 2014. Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. *Sci. World J.,* 2014:704708.

Samantaray, U.S., Mishra,A. and Singh, Y.D. 2020. Biosynthesis and antimicrobial activities of silver nanoparticles (AgNPs) by using Leaf extracts of *Tagetes erecta* (Marigold) and *Tridax procumbens* (Tridax). *Int. J. Res. Appl. Sci. Biotechnol*., **7**(6): 209-226.

Shaik, M.R., Khan, M., Kuniyil, M., Al-Warthan, A., Alkhathlan, H.Z., Siddiqui, M.R.H. and Adil, S.F. 2018. Plant-extract-assisted green synthesis of silver nanoparticles using *Origanum vulgare* L. extract and their microbicidal activities. *Sustainability*, **10(4)**: 913.

Shankar, S.S., Rai, A., Ahmad, A. and Sastry, M. 2004. Biosynthesis of silver and gold nanoparticles from extracts of different parts of the geranium plant. *Appl. Nano Sci.*, **1**: 69-77.

Singh, C., Sharma, V., Naik, P.K., Khandelwal, V. and Singh, H. 2011. A green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiber officinale*. *Dig. J. Nanomater. Biostructures*., **6(2)**: 535-542.

Singh, R., Hano, C., Nath, G. and Sharma, B. 2021. Green biosynthesis of silver nanoparticles using leaf extract of *Carissa carandas* L. and their antioxidant and antimicrobial activity against human pathogenic bacteria. *Biomolecules*, **11**(2): 299.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A.R. and Singh, R.P. 2011. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. *J. Nanopart. Res*., **13(7)**: 2981-2988.

Sood, R. and Chopra, D.S. 2018. Optimization of reaction conditions to fabricate *Ocimum sanctum* synthesized silver nanoparticles and its application to nano-gel systems for burn wounds. *Mater. Sci. Eng. C*., **92**: 575-589.

Umer, A., Naveed, S., Ramzan, N. and Rafique, M.S. 2012. Selection of a suitable method for the synthesis of copper nanoparticles. *Nano*, **7(05)**: 1230005.

Wright, M.H., Adelskov, J. and Greene, A.C. 2017. Bacterial DNA extraction using individual enzymes and phenol/ chloroform separation. *J. Microbiol. Biol. Educ*., **18(2)**: 18-2.

Yogeswari, R., Sikha, B. and Akshya, K.O. 2012. Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulashi) and study of their antibacterial and antifungal activities. *J. Microbiol. Antimicrob*., **4(6)**: 103-109.