IJPSR (2021), Volume 12, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 March 2020; received in revised form, 12 August 2020; accepted, 25 October 2020; published 01 March 2021

ANTI-BACTERIAL EFFICACY OF PHYTOGENIC SILVER AND GOLD NANOPARTICLES OF *LANTANA CAMARA*. LINN AGAINST ENTERIC PATHOGENS

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Keywords:

Lantana camara, Phytochemicals, Silver and Gold Nanoparticles, Antibacterial activity

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ABSTRACT: Antibiotic resistance could be a natural phenomenon that occurs when an antibiotic loses its ability to control or kill the bacterial growth; in other words, the bacteria become "resistant" and still continue to multiply in the presence of therapeutic levels of an antibiotic. Nano-technology is one of the foremost active areas in modern science. Nano particles exhibit a completely new or improved particle based on the specific characteristics. Plant extracts generally have excellent potential as antimicrobial compounds against microorganisms. Thus, they can be utilized in the treatment of infectious diseases caused by resistant microbes. The current study explores the phytochemical screening of aqueous extracts of *Lantana camara*, which had revealed the presence of flavonoids tannins, terpenoids, saponins, steroids, and alkaloids. Formation and stability of silver and gold nanoparticles in aqueous colloidal solution are confirmed using UV-Vis spectral, FTIR, XRD, SEM and EDX analysis. The antibacterial activity of crude, ethanol, and methanol extracts and plant-mediated silver and gold nanoparticles were done. The study concludes that the crude extracts showed the maximum antibacterial activity against all the entero pathogenic strains rather than others. Following these, the plant-mediated silver and gold nanoparticles also showed the best result of antibacterial activity at 20µl of concentration which can possess good activation when the concentration is increased. The plant-mediated nanoparticles will be cost-effective, eco-friendly, and efficient drugs for the treatment in controlling the disease particularly the diarrheal outbreak.

INTRODUCTION: Antibiotic resistance is that the ability of a microorganism to face up the consequences of an antibiotic. It is an elected sort of drug resistance that evolves naturally *via* survival through random mutation, but it could even be engineered by applying evolutionary stress on a population.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(3).1903-14

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(3).1903-14

The usage of broad-spectrum antibiotics like second and third-generation antibiotics greatly hastens the event of resistance. Most of the factors that commonly contribute towards the resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and therefore, the employment of antibiotics as livestock food additives for growth promotion [1].

Nowadays, there is a constant demand for new therapies to treat and prevent this life-threatening disease caused by resistant microorganisms. Currently the research interest has drawing its attention towards the naturally derived compounds,

which are less toxic and side effects compared to current treatments such as chemotherapy [2]. To overcome this problem, medicinal plants are often used to cure several sorts of health problems. Since ancient times, medicinal plants represent a crucial source of medically important compounds. Systemic analysis of those plants provides a spread of bioactive molecules for the occurrence of newer pharmaceutical products. Recently, there is a growing interest within the pharmacological evaluation in which the various group of plants were utilized in different traditional systems of drugs.

In previous couple of decades, most of traditionally known plants were extensively studied and reported for various medicinal properties *viz,* anti-oxidant activity, anti-bacterial activity, anti-fungal activity, anti-inflammatory activity, anti-cancer activity, anti-diabetic activity, anti-helmintic, hepato-protective activity, larvicidal activity *etc* [3]. However, this area is not much developed when compared to modern system of medicine, mainly because of the lack of scientific documentation in this field. Mostly the pharmacological activity of medicinal plants resides in their secondary metabolites, which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates, and lipids. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man [4].

Lantana camara belongs to the Verbenaceae family and is reported to be utilized in traditional medicine system for the treatment of itches, ulcers, cuts, swellings, eczema, bilious fever and cataract. The literatures have reported that different parts of the plants were utilized in the treatment of cold, infectious disease, bronchitis, chickenpox and eye injuries [5].

Nanoparticle is a group of materials with inimitable features and extensive applications in diverse fields. Especially the metal nanoparticles, that containing silver and gold have recognized an importance in chemistry, physics and biology with their unique properties. The benefit of synthesizing silver and gold nanoparticles and their affinity for binding many biological molecules makes them attractive candidates for study [6].

Medicinal and preservative properties of silver and gold had been known for over 2000 years in the field of medicine especially Siddha, Ayurveda *etc.* Since the 19th century, metal-based compounds are widely utilized in bactericidal applications, in burns and in wound therapy, *etc*. Over the recent decades, it has been revealed that the biomolecules in plant extract such as protein, phenol, and flavonoids play a significant role in the reduction of metals ions and capping the biosynthesized nanoparticles [7]. The aim of this study was to judge the *in-vitro* antibacterial activity of various extracts of *L. camara* leaves using the five bacterial strains (Microbial Type Culture Collection - MTCC) of etheric pathogens and compared their activity with silver and gold nanoparticles synthesized from the identical extract.

MATERIALS AND METHODS:

Collection of Plant Materials: The medicinal plant sample was collected during November from the region of Periyar University, Salem, and taxonomically identified. The leaves of *Lantana camara* L. (Verbenaceae) were selected for testing the nanoparticle synthesis and its antibacterial studies.

Preparation of Plant Extracts: The leaves of the *Lantana camara* plant were cleaned under running tap water and shade-dried with occasional shifting for two weeks at room temperature. Dried leaves were crushed and powdered with a mechanical grinder, passed through a sieve, and stored in an airtight container for further use. From these, 10 g of powder was dissolved in 100 ml of deionized water and heated at 50 °C for 15 min and was filtered through the Whatman No. 1 filter paper. The filtrate was used for further studies.

Preliminary Phytochemical Screening: A preliminary phytochemical investigation was carried out for the leaf extracts with the standard protocol l8 for the detection of the alkaloids, flavonoids, phenolic compounds, tannins, glycosides, carbohydrates, saponins and terpenoids.

Chemicals and Reagents: Silver nitrate ($AgNO_3$) and chloroauric acid ($HAuCl_4$) from Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA) were used. All other chemicals and reagents were obtained commercially of analytical grade.

Preparation of Solvent Extracts: In this study, 2.5 grams of leaf powder of *L. camara* was mixed with 25 ml of Ethanol and Methanol solvent, homogenized, and incubated for 24 h at a rotary shaker. Then the extracts were filtered through a What man filter paper (No.1) and used for further studies.

Synthesis and Purification of Silver and Gold Nanoparticles: For the biosynthesis of silver and gold nanoparticles, 10 mL of 1mM aqueous $AgNO_3$ and $HAuCl_4$ solutions were separately added to 10 mL of aqueous leaf extract and incubated in the dark condition at a rotary shaker.

The resulting colloidal solutions of silver and gold were then analyzed for the visual changes and screened using an ultraviolet-visible spectrophotometer at 200-700 nm. To remove excess silver and gold ions, the silver and gold colloids were centrifuged at 10,000 rpm for 10 min washed at least three times with deionized water. Dried powders of the silver and gold nanoparticles were obtained by freeze-drying.

Characterization of Bio-synthesized Nano-particles:

Ultraviolet-Visible Spectroscopy: The suspensions of silver and gold nanoparticles were diluted by adding 2.9 mL of deionized water to 0.1 ml of the sample. Bio reduction of $AgNO_3$ and $HAuCl_4$ was observed visually as a function of time as a reference at 3 h intervals from zero hours up to 24 h. Absorption spectra of the nanoparticles were verified spectro-photometrically (Perkin-Elmer, Boston, MA) at 200 to 700 nm and operated at a resolution [9].

Fourier Transform Infrared Spectroscopy (FT-IR): Fourier transforms infrared spectroscopy was used to examine organic and inorganic chemicals, which is a powerful tool used for elucidating the structure and functional group in the sample using the FT-IR (Model: EXI).

The least quantity of the dried powder sample was ground with 100 mg of FT-IR grade potassium bromide and pressed into a pellet [10]. The pressed sample was kept into the sample holder, and the infrared spectra were recorded in the wavelength of 4000 - 400 cm^{-1} with resolution of 4 cm^{-1}. The obtained spectra of both nanoparticles were interpreted by comparing functional peaks with the existing peaks.

X-ray Diffraction (XRD) Analysis: X-ray diffraction is done to analyze the crystalline phases present in a material. X-ray diffraction (XRD) analysis was conducted using monochromatic Cu kα radiation (λ = 1.5406 Å) operated at 60 kV and 60 mA at a 2θ angle pattern on Bruker's X-ray Diffraction D8 instrument. The scanning was done from the region of 5-100 ºC. The images obtained were compared to the Joint Committee on Powder Diffraction Standards (JCPDS) library to study the crystalline structure [11].

SEM-EDAX Analysis: Scanning Electron Microscopy (SEM) was carried out using the SDB (small dual-beam) FEI Quanta 3D FEG with the EDAX Genesis EDX system with the resolution of 1.2 nm to study the structure and composition of the nanoparticles. Operational features of the microscope used in the experiment were 5,000 - 150,000 X magnification and 1-30 kV voltage.

EDX confirms the presence of composition and distribution of the nanoparticles through the spectrum and elemental mapping using an energy dispersive x-ray analyzer incorporated with a scanning electron microscope (SEM) system. The micro-probe analysis of nanoparticle clusters was conducted with EDAX spectrometer, USA. The acquisition time ranged from 60 to 100s, and the accelerating voltage was 20 kV [12].

Antibacterial Assessment:

Test Organisms: The major enteric pathogens such as *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, and *Vibrio paraheamolyticus* were used as the test organisms for the antibacterial activity that belongs to MTCC strains received from the IMTECH, Chandigarh.

Well Diffusion Assay Method: The antibacterial activity of the crude extract, ethanol, and methanol solvent extracts along with both the nanoparticles (Silver and Gold) of a leaf extract at the concentration of 1 mg/ml was determined using the agar well diffusion assay method respectively. Test organisms were swabbed on Muller Hinton agar plates, respectively, using L-rods. To these, the wells of 6 mm diameter were prepared with the help of a sterilized stainless-steel cork borer.

The different concentrations of the samples (20, 40, 60, 80, and 100 µl) were added to each well, and the plates were incubated at 37 °C for 24 h. The zone of inhibition was measured in millimeters, and results were recorded [13].

RESULTS:

Phytochemical Analysis: Table 1 represents the various phytochemicals present in the leaf extract of *Lantana camara*. The phytochemical studies of water extract had more positive results for alkaloids, flavonoids, phenols, tannins, carbohydrates, and terpenoids, *etc*.

The color change in the extract represents the positive of phytochemicals in the extract, and no change in color indicates negative.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *LANTANA CAMARA*

S. no.	Phytoconstituents	Leaves of *L. camara*
1	Alkaloids (Mayer's reagent)	+
2	Flavonoids (Shinoda test)	+
	Alkaline reagent	+
3	Phenolic compounds (Lead acetate test)	+
	$FeCl_3$ test	+
4	Tannins (Lead acetate test)	+
	$FeCl_3$ test	+
5	Glycosides	+
6	Carbohydrates (Fehling test -reducing sugar)	+
7	Saponins (Frothing test)	+
8	Terpenoids (Salkowski's test)	+

Ultraviolet-Visible Spectroscopy: The leaf extract of *L. camara* was mixed with an aqueous solution of silver nitrate and gold chloride. Silver nanoparticles have shown changes from yellowish to brown colour in an aqueous solution due to the excitation of surface plasma vibrations in silver ions. After the addition of the extract with gold chloride, the colour of the solution changed from yellow to reddish pink due to the reduction of ions which had indicated the formation of silver and gold nanoparticles exhibiting a color change. It is generally recognized that UV-Vis spectroscopy absorption spectra of silver and gold nanoparticles formed in the reaction media have exhibited interesting optical properties directly associated with localized surface plasmon resonance which is highly dependent on the morphology of the nanoparticles. Reduction of Ag^+ ions and Au^+ during exposure absorbance peak at 400-450 and 500-550 nm, respectively. Broadening of peak indicated nanoparticles are at the polydispersed condition.

Fourier Transform Infrared Spectroscopy: The bio-reduction was based on three molecules carbohydrates, glycosides, and flavonoids. The larger amount of flavonoids present in the aqueous leaves extract may play a major role in the ion reduction reaction. FTIR spectrum was used to analyze the functional group present in the *L. camara* leaves extract at the same time; the nanoparticles synthesized from the extracts is also confirmed by changes that occurred in the FTIR spectrum. The FTIR spectrums of silver nanoparticles synthesized from *L. camara* leaves extract was represented in **Fig. 1**. The functional group present in silver nanoparticles detected using FTIR had absorption peaks located at the O-H stretch, H-bonds at 3473.80, showing a high concentration of alcohols and phenols. The N-H stretchat 3261.42 indicates the presence of the 1° and 2 °C amines with amides. The C-C- stretch presence at 2061.63 and 1641.42 shows high concentrations of alkynes along with 1° amines at N-H bend at 1631.78. The C-C stretch presence at 1402.25 shows high concentrations of aromatics. The C-N stretch presence at 1083.99 shows high concentration of aliphatic amines. The =C-H bend presence at 989.48 shows high concentrations of alkenes. The –C≡C–H: C–H bend and C-Br stretch presence at 661.58 show high concentrations of alkynes and alkyl halides.

The FTIR spectrums of gold nanoparticles synthesized from *L. camara* leaf extract are represented in **Fig. 2**. The functional group present in gold nanoparticles detected using FTIR had absorption peaks located at the O-H stretch free

hydroxyl group, and H- bonded presence at 3471.87 shows high concentrations of alcohols and phenols. The N–H bend at 1631.78 shows high concen-trations of Primary amines. The C-C stretch (in-ring) presence at 1400.32 shows high concen-trations of aromatics. The C-O and C-N stretch at 1083.99 & 1033.85 shows high concentrations of alcohols, carboxylic acids, esters, ethers, and aliphatic amines. The =C-H bend presence at 989.48 shows high concentrations of alkenes. The C–Br stretch at 570.93 shows the concentrations of alkyl halides.

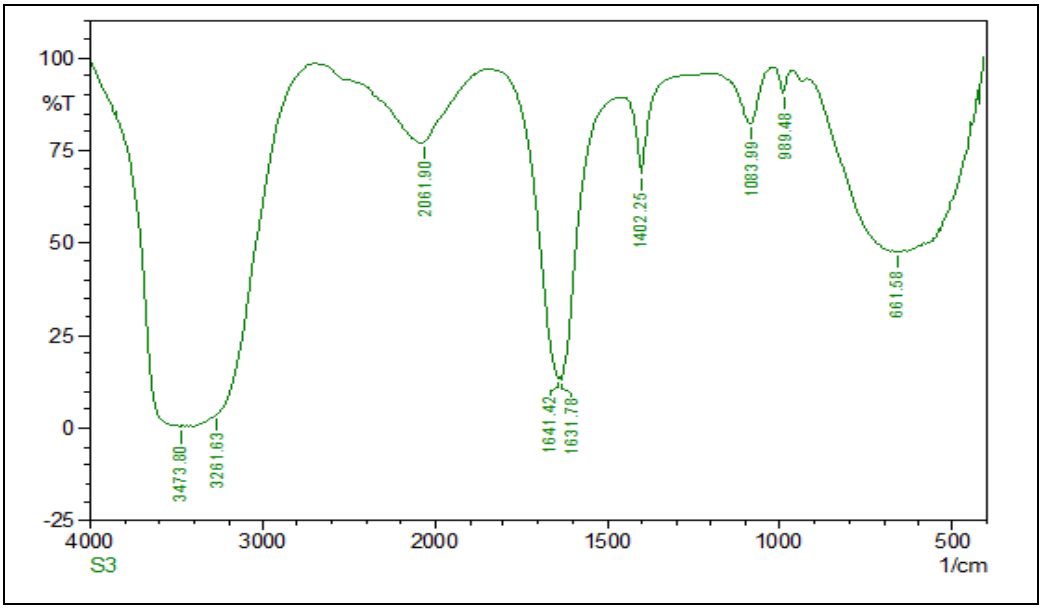

FIG. 1: FTIR SPECTRA OF AGNPS OF *LANTANA CAMARA* LEAF EXTRACT

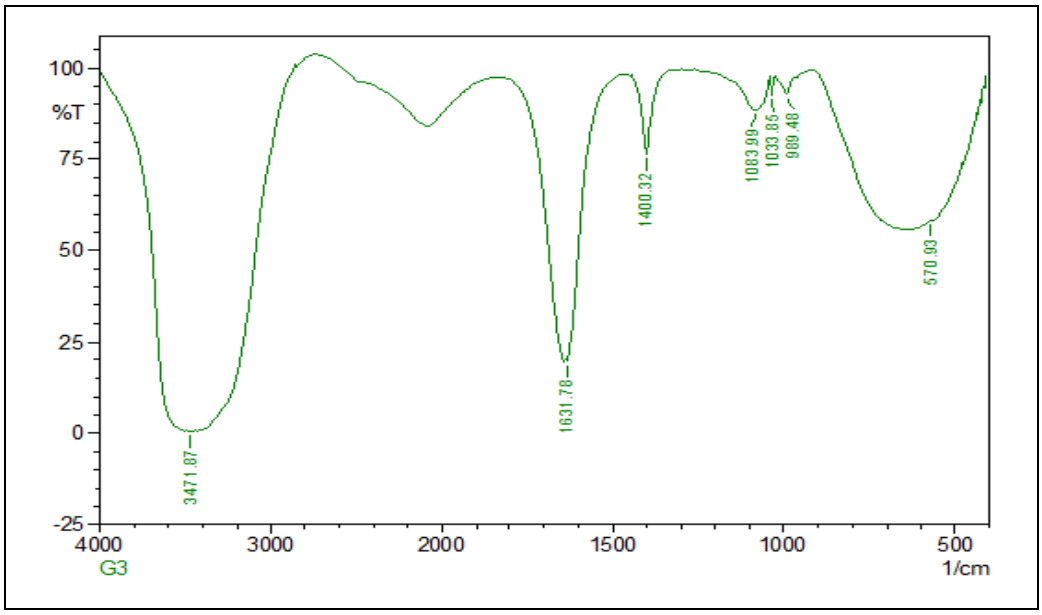

FIG. 2: FTIR SPECTRA OF AUNPS OF *LANTANA CAMARA* LEAF EXTRACT

X-Ray Diffraction (XRD) Analysis: The XRD analysis of silver nanoparticles of *L. camara* leaf extract showed 2θ values or Bragg reflections at 27-29°, 38°, 40.5°,44-46°, 49°,64° and 77°that were indexed at (111), (200), (220) and (311) are the major lattice planes **Fig. 3**. The gold nanoparticles of the same extract indicated the peaks at the angle starting from 28°, 36°, 38°, 40°,44°, 64° and 77° that indexed at (110), (102), (111), (220) and (311) planes have been depicted on **Fig. 4.**These peaks followed the Bragg's rule and clearly matched up to the crystalline structure of silver and gold. This study indicated the pure crystalline nature of the biosynthesized nano-particles, respectively.

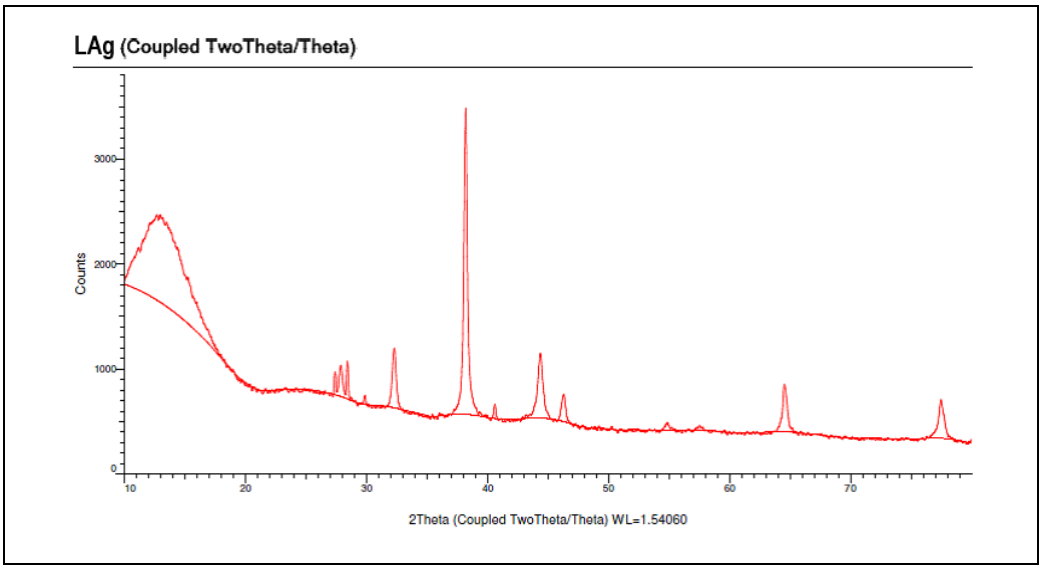

FIG. 3: XRD PATTERN OF AGNPS OF *LANTANA CAMARA* LEAVES

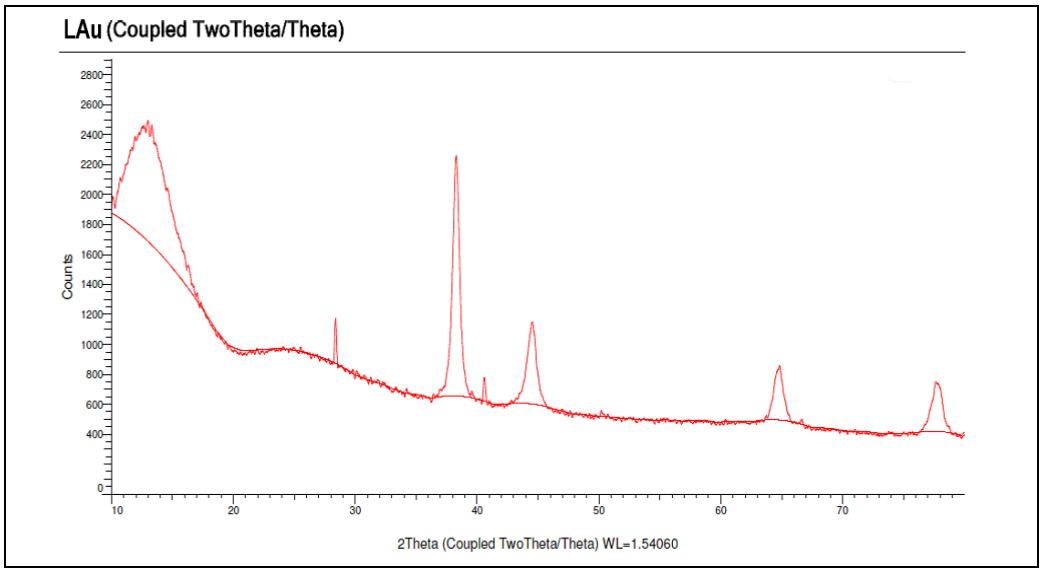

FIG. 4: XRD PATTERN OF AUNPS OF *LANTANA CAMARA* LEAVES

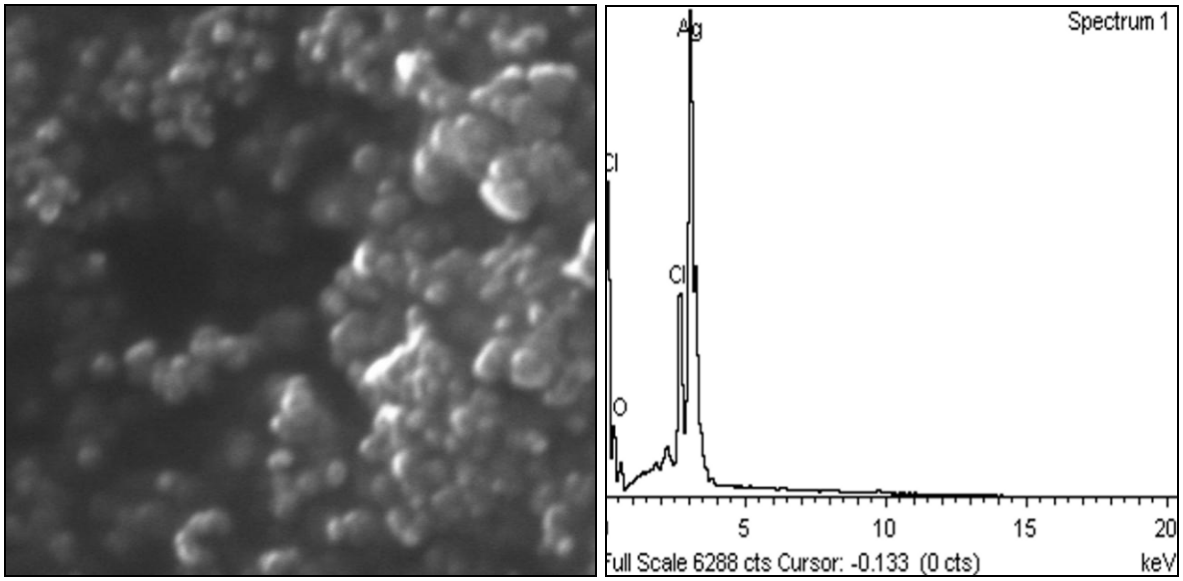

FIG. 5: SEM & EDX IMAGE OF AGNPS OF *LANTANA CAMARA* LEAVES

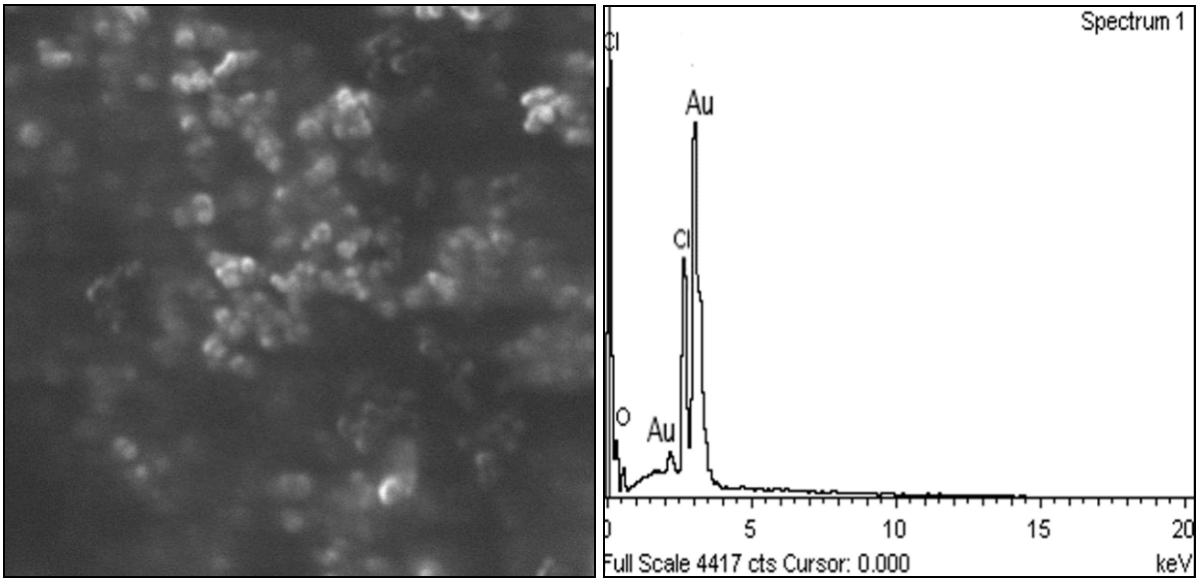

FIG. 6: SEM & EDX IMAGE OF AUNPS OF *LANTANA CAMARA* LEAVES

SEM-EDAX Analysis: The characterization study of the silver and gold nanoparticles of the leaf extract of *L. camara* on SEM showed the spherical shaped particles with a size range of 25 - 48 nm and most of them are agglomerates. The EDX reports have confirmed the presence of metallic silver and gold ions and their pure crystalline nature, together with the signals of O and Cl atoms on analysis of nanoparticles which has been represented on **Fig. 5** & **6**.

Antibacterial Assessment: The antibacterial activity of crude, ethanol, and methanol extracts of leaves of *L. camara* investigated against enteric pathogens were represented on **Fig. 7** and **9** respectively. The aqueous (Crude) leaf extract of *L. camara* showed the maximum zone of inhibition on *Shigella dysenteriae* followed by *E. coli*, *S. typhi*, *Vibrio cholerae,* and *Vibrio paraheamolyticus*, almost all the enteric pathogens are repressed. At the same time, the zone of inhibition of methanolic extract showed the topmost activity against Vibrio's followed by the *Shigella dysenteriae* and other pathogens when comparing to the ethanolic extract of the same plant. Probably increase in the concentration of the extracts has increased the antibacterial activity. At furthermost, the minimum concentration (20 µl) of silver nanoparticles (AgNPs) synthesized from the leaf extract revealed the highest activity against *Shigella dysenteriae* followed by *E. coli* and other enteric pathogens when comparing to gold nanoparticles (AuNPs) which has been depicted on **Fig. 10**.

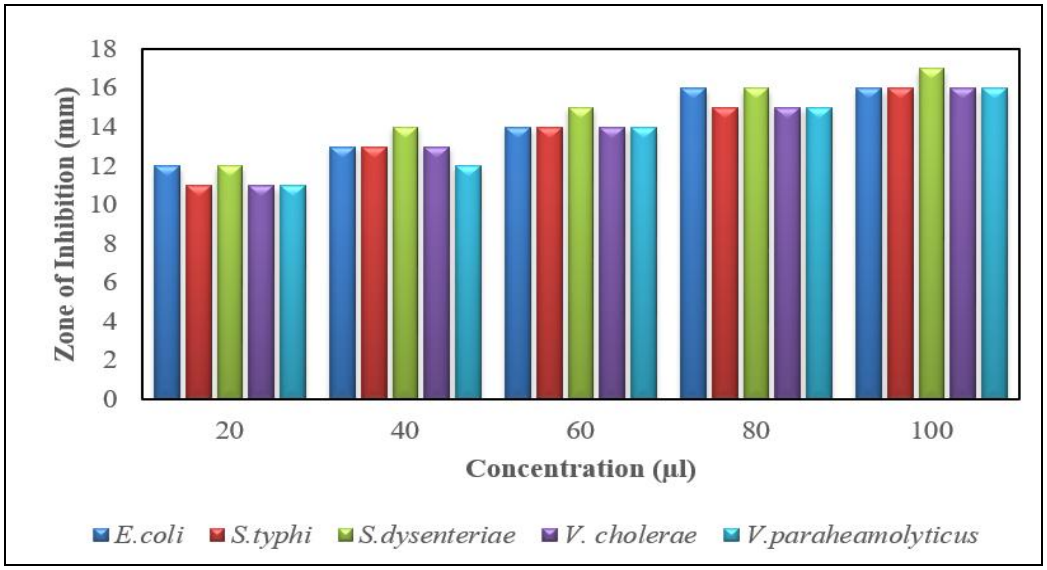

FIG. 7: ANTIBATERIAL ACTIVITY OG CRUDE EXTRACT OF LEAVES

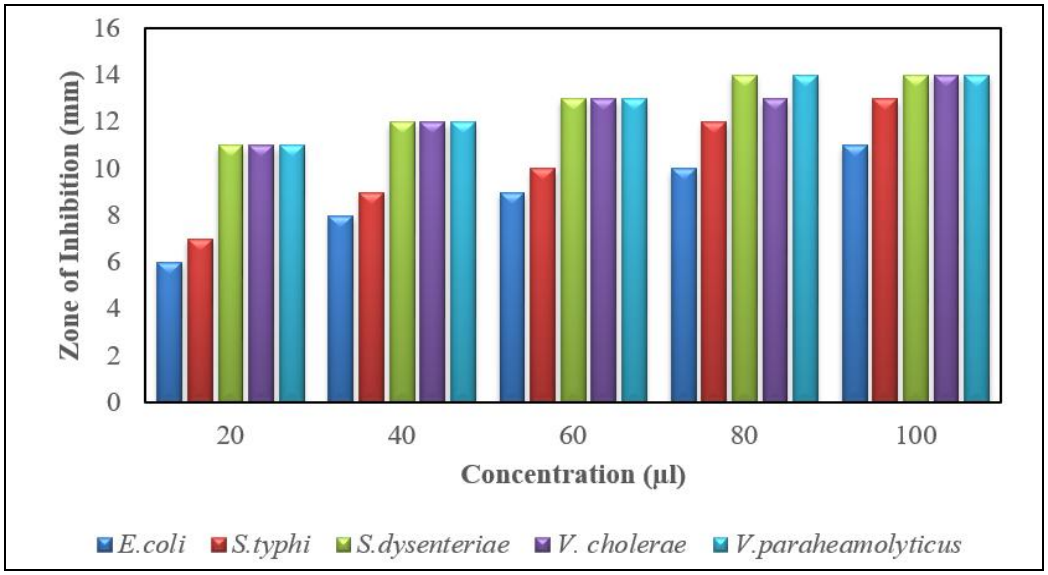

FIG. 8: ANTIBACTRIAL ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES

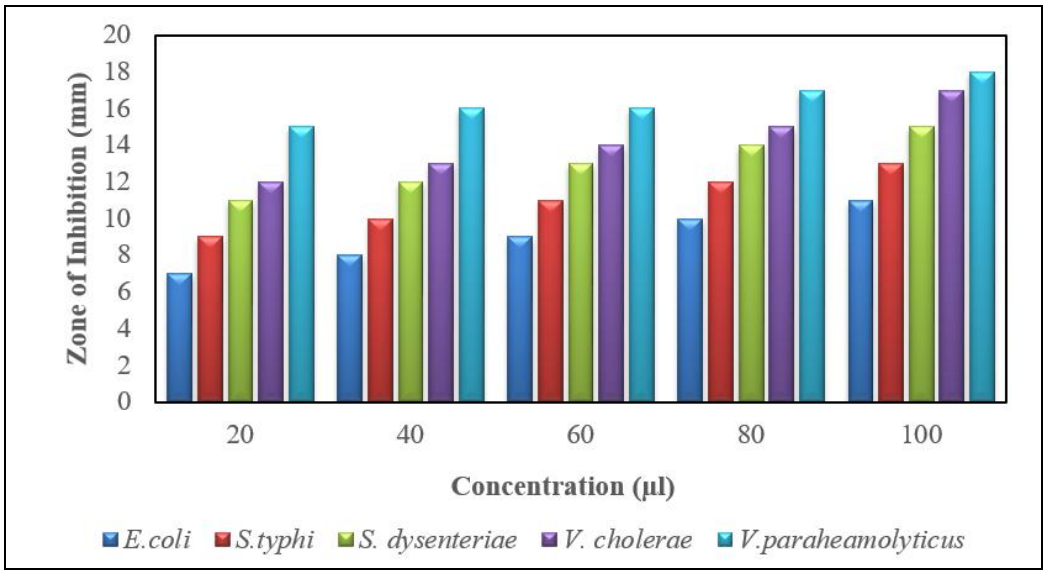

FIG. 9: ANTIBACTRIAL ACTIVITY OG METHANOLIC

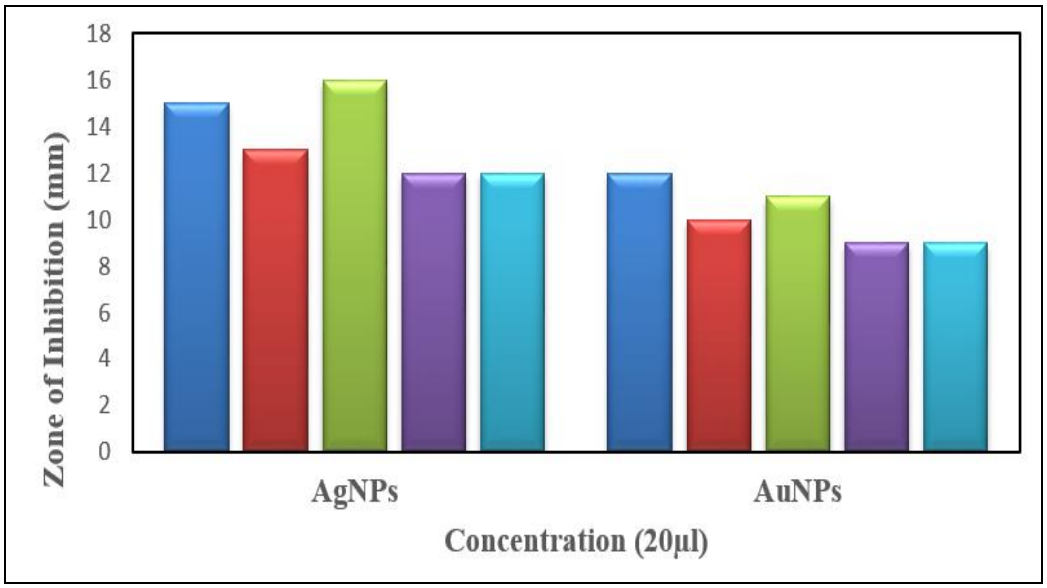

FIG. 10: ANTIBACTRIAL ACTIVITY OF SILVER AND GPLD NPS OF LEAF ETRACT

DISCUSSION: The plant *Lantana camara* Linn family Verbenaceae is habitually present throughout central and south India, especially in most of the dry stony hills and black soil. It had been reported for their use in folk remedies. Most ordinarily, it's been used for ulcers, fever, and tumors. It is a highly richer in secondary metabolites which of potential medicinal values that exhibits several biological activities, such as antibacterial, antiulcer, and antioxidant that was proved by the previous studies [14]. The diverse type of plant extracts and phytochemicals with known antimicrobial properties are of great significance to therapeutic treatments [15]. Extracts of various plants were used for the treatment of various diseases, and this forms the basis for all Indian systems of Medicine. The effect of plant extracts on bacteria and fungi has been studied by a large number of researchers in different parts of the world [16, 17]. Agarry *et al*. have shown the potent antimicrobial activities of the plant against a wide range of bacteria and fungi [18].

The bioactive constituents that are extracted from the whole plant or from the different parts of the plant have always been an exciting task. Visweswari *et al*., had investigated and reported the extraction and detection or screening of active phytochemical compounds from different extracts of WS root. Generally, plants contain pharmaceutically active compounds, and that the type and concentration of these compounds determine the activity of a plant extract [20]. The phytochemical composition of this plant had been extensively studied in the last few decades. Different parts of the different types of *L. camara* had been reported for their functional constituents like flavonoids, carbohydrates, proteins, alkaloids, glycosides, tannin, phenolic compounds, oligosaccharides, quinine, saponins, steroids, triterpens, phenyl ethanoid, sesquiterpenoides, and essential oils [21]. The phytochemical test done on the leaf extract of *L. camara* revealed the presence of alkaloids, carbohydrates, glycosides, phytosteroids, saponins, flavonoids, tannins, and phenolic compounds as per the previous studies [19].

The formation and stability of metal nanoparticles in an aqueous solution were determined using UV-Vis spectroscopy techniques. After the addition of leaf extract with the respective metal ion suspension, we can have a visual perception of the change in color of the reaction mixture from yellowish to brown and ruby red, indicating the formation of Ag and AuNPs, respectively; their origin is attributed to the collective oscillation of free conduction electrons results in surface plasmon resonance (SPR) induced by an interacting electromagnetic field. The result from UV-Vis absorption is in accordance with the earlier report [22].

FTIR spectroscopy is used to probe the organic and inorganic composition of the nanoparticles capped by leaf extract biomolecules. FTIR spectrum of green synthesized NPs has been reported by various authors. The strong, intense broad band at 3193 cm^{-1} is assigned to the N-H group vibrational bands from proteins present in the extract, and another intense band at 2347 cm^{-1} is characteristic of C-O stretching vibrations [23]. Also, medium peaks at 1461 and 1328 cm^{-1} are attributed to C-Cstretching mode and C-N stretching vibrations of aromatic amines, respectively [24]. Another couple of bands at 1046 and 980 cm^{-1} implicates C-OH stretching of secondary alcohols and C-O-C vibrations of proteins/polysaccharides, respectively [25]. Small bands at 1635, 811, and 672 cm^{-1} correspond to the carbonyl groups in amide linkages which are involved in AgNPs formation, C-H stretching of alkenes, and C-Cl stretching modes of alkyl halides, respectively.

The IR peaks for amide I and amide II arise owing to carbonyl stretch were reported on the AuNPs synthesized from the same plant [26]. This may be the reason for the reduction of metal ions while using the leaf extract for the synthesis of NPs indicates the binding of the nanoparticles with biomolecules [27]. The FTIR spectrums of silver and gold nano-particles synthesized from *L. camara* leaves extract in our study were more or less similar in the characteristic expect few peaks like -C C- stretch and C-O stretch which stands for the presence of alkynes and alcohols. Apart from this, almost both the nanoparticles reported for the presence of similar peaks at the O-H stretch, N-H stretch, C-C stretch, C-N stretch, =C-H bend and C-Br stretch of respective functional groups like phenols, 1° and 2° amines, amides, aromatics, aliphatic amines, alkenes, and alkyl halides. Ajitha *et al.* [28] had reported the XRD profile of greenly synthesized

NPs with the peaks appeared at 38.1, 44.8, 64.4 and 77.3 indexed as (111), (200), (220), and (311) miller indices, respectively. The AgNPs are crystalline in nature with fcc structure, and no impurity peaks were identified, which indicates the purity of AgNPs synthesized.

In the present study, the XRD analysis of silver nanoparticles using *L. camara* leaf extract showed 2θ values at 27 - 29°, 38°, 40.5°, 44 - 46°, 49°, 64° and 77° that were indexed at (111), (200), (220) and (311) are the major lattice planes. At the same time, gold nanoparticles of the same extract indicated the peaks at the angle starting from 28°, 36°, 38°, 40°,44°, 64° and 77° which indexed at (110), (102), (111), (220) and (311) planes that have been depicted on the above result. This study also indicated the pure crystalline nature of the biosynthesized nanoparticles.

The spherical-shaped silver nanoparticles with fair agglomeration was reported previously by and as $AgNO_3$ concentration is decreased from 0.01 M to 0.001 M, the average particle size was found to be decreased from 37 nm to 29 nm [28]. In the case of gold nanoparticles, the size ranges between 11 and 32 nm were similar to the earlier report of Ghosh *et al* [29]. And the differences in the size of NPs could be due to the reduction of ions to gold being formed at different times [30]. On the characterization study of the silver and gold nanoparticles using leaf extract of *L. camara*, the spherical-shaped particles along with the agglomerates were identified on SEM analysis with a range of 25-48 nm. The EDX reports have confirmed the presence of metallic silver and gold ions and their pure crystalline nature, together with the signals of O and Cl atoms on analysis of nanoparticles which has been represented in the above result.

The antibacterial activity of the *L. camara* extracts varied with the different solvents used for the extraction. The crude preparation of the leaves of the plant generally contains both the active and non-active components to have higher efficacy than semi-crude or pure plant substances [31]. The wide variety of activity of the ethanolic extracts over the water extracts is significant because the leaves of plants are of traditional uses. The antibacterial activity is due to the presence of secondary metabolites existed in the plant. Hence, it is tough to explain the limited spectrum of activity of other extracts compared with the ethanolic extracts since all the extracts had had the secondary metabolites [32]. Sharma and Kumar have reported the antibacterial activity of different varieties of *L. camara* plants' leaves and flowers.

Three different solvent extract of leaves and flowers of four different varieties of *L. camara* exhibited significant antibacterial activity against *E. coli*, *Bacillus subtilis*, and *P. aeruginosa* and a very poor antibacterial activity against *Staphylococcus aureus*. Ethanolic extracts of *L. camara* leaves and roots exhibited antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Víbrio cholerae*, *Escherichia coli*, and two multidrug resistant strains *E. coli* and *S. aureus*. Methanolic extracts of different parts of *L. camara* screened for antimicrobial activity against 10 bacteria and 5 fungi by disk diffusion method, and broth micro dilution method showed the highest activity against S. typhi [33].

There is an evidence from the past results that leaf extracts have exhibited a high degree of activity. Especially the crude and methanol extracts of *Lantana camara* L. exhibited potential antibacterial activity against the tested pathogens, suggesting that methanol will be the appropriate solvent for extraction of antibacterial principle comparing to other solvents. Almost methanol was found to be the most effective against all of the bacteria except *E. coli* [34]. The same plant has been previously studied for its antibacterial and antifungal activities, but still, the literature was insufficient.

In the current investigation, *L. camara* showed its antimicrobial potential against enteric pathogens, which are being involved in a number of human diseases. The aqueous (Crude) leaf extract of *L. camara* repressed the growth of all the pathogens with the maximum zone of inhibition on *Shigella dysenteriae* followed by *E. coli*. At the same time, the zone of inhibition of methanolic extract showed the topmost activity against Vibrio's followed by the *Shigella dysenteriae* and other pathogens, when comparing to the ethanolic extract of the same plant. Probably increase in the concentration of the extracts has increased the antibacterial activity. The green method of nanoparticle synthesis employing

plant extracts is a humble and feasible alternative rather than the chemical and physical methods. The ancient traditional biosynthesis method using plant sources offers several advantages like cost-effectiveness, eco-friendliness and at the same time they eliminate high energy, temperature, and toxic chemicals.

The weed, *L. camara* Linn, has various medicinal applications which were contributed with earlier synthesized nanoparticles by several researchers [35, 36, 37]. In our study, the silver and gold nanoparticles were biologically synthesized using the leaf extract of *L. camara* and characterized with appropriate analysis, at laston antibacterial study, the minimum concentration (20 µl) of silver nanoparticles (AgNPs) synthesized from the leaf extract revealed the highest activity against *Shigella dysenteriae* followed by *E. coli* and other enteric pathogens when comparing to gold nanoparticles (AuNPs).

CONCLUSION: An efficient one-step biosynthesis of silver and gold nanoparticles using a leaf extract of *L. camara* was reported in the current study. According to our research, we conferred about the various and their efficiency over the enteric pathogens. The crude and methanolic extracts of this particular plant showed the highest activity against the pathogens comparing to the ethanolic extract. Apart from this, the leaf extracts are highly efficient on reducing the silver and gold ions and stabilize the synthesis of Ag and AuNPs. On characterizing the nano-particles, the spherical shaped particles of about 25-48 nm with agglomerates have been reported along with the pure crystalline nature on EDX and XRD analysis.

At the same time, the FTIR spectrum of the nanoparticles also proved the presence of various peaks representing the compounds like alcohols, aromatics, amines, proteins, aliphatic compounds, alkanes, and alkyl halides. From the antibacterial activity against enteric pathogens, the crude and the solvent extracts exhibited good activity against Vibrios and Shigella spp. It is also inferred that the biosynthesized AgNPs revealed the highest activity against the pathogens rather than the AuNPs at the minimum concentration. Thus, we conclude that at higher concentrations, the nanoparticles might also increase the activity. This study highlights the use of the leaf extracts in medicinal applications which open ups the route of synthesizing a new drug using nanotechnology during the enteric outbreaks.

ACKNOWLEDGEMENT: The research was financially granted under the University Research Fellowship, Periyar University, and the FIST grants of the Department of Science and Technology (Ref No. SR/FST/LSI- 640/2015(C) Dt. 30/5/2016).

CONFLICTS OF INTEREST: The authors declared no conflict of interest.

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How to cite this article:
Gowdhami M, Balagurunathan R and Ayyasamy PM: Anti-bacterial efficacy of phytogenic silver and gold nanoparticles of *Lantana camara*. Linn against enteric pathogens. Int J Pharm Sci & Res 2021; 12(3): 1903-14. doi: 10.13040/IJPSR.0975-8232.12(3).1903-14.