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Comparative Analysis of Macrocyclic Mn (II) Nanocomplexes Synthesized using Sonication: Assisted and Conventional Method for Biological Activity

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ABSTRACT

We have reported the synthesis of manganese acetate nanocomplexes from L- aspartic acid and o-phenylenediamine through sonochemical and conventional methods. The FT-IR, LC-Mass, UV-Vis, ESR, FESEM, XRD and CHNS elemental analysis was carried out to confirm the formation, bonding, size and morphology of the synthesized nanocomplexes The purity, yield and crystallinity of Mn(II) nanocomplexes synthesized by the sonochemical method were found to be better than those from the conventional method. Based on these studies, octahedral geometry has been proposed for the synthesized nano complexes. The antimicrobial and antioxidative activity of the synthesized Mn(II) nanocomplexes have been studied against some bacterial and fungal species by two-fold serial dilution method and DPPH Scavenging method respectively. The results confirmed that the sonochemical method enhances the properties of nanocomplexes by effectively regulating and reducing the size of the nanocomplexes and hindering their agglomeration and has better antimicrobial, and antioxidative compared to conventional methods.

Keywords: Nanocomplex; Sonication; Antibacterial; Antifungal; Antioxidative

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INTRODUCTION

The study of macrocyclic incorporated transition metal complexes derived from dioic and diamine have become increasingly interesting in chemistry due to the wide variety of applications in biological, analytical, medicinal and industrial areas [1,2]. The metal binding to these macrocycles backbone is confined in the tetradentate, planar enclosed framework which has unusual stability to the metal complex [3]. The chemistry of poly-azamacrocyclic complexes containing amide groups is an important area of research interest in the coordination chemistry of their ability to react with different metal ions forming stable complexes [4,5]. In the current era, nanocomplexes and nanomaterials have gained much attention due to distinctive applications in the chemical, optical, electrical, and biological field [6-8]. The sonication technique for the synthesis of Nano complexes has emerged as an enchantment in the field of synthetic chemistry arousing the area of 'sonication-assisted chemistry. Sonication assisted synthesis involves sound waves penetrating inside the materials and leading to uniformity in the reaction heating and a

hundred times better in terms of speed, yield and purity than conventional heating methods [9]. It is worth saying that the sonication technique is eco-friendly offering better productivity, faster reactions, less solvent and energy [10].

Nowadays antimicrobial resistance is growing globally and is the major concern to inhibit the growth of these microbes. The interest in transition metal-based drugs has gained attention in the field of bioinorganic chemistry due to their remarkable biological activity [11]. The transition metal macrocyclic complexes can perform remarkable antitumour, anticancer, antifungal and antibacterial activity [12,13]. The nanocomplexes of transition metal would enhance the antimicrobial activity by inhibiting microbial growth through a novel mechanism of action.

In the current work, we have discussed the synthesis of nanocomplex of Mn(II) using macrocyclic ligand containing amide group synthesized from the condensation of L-aspartic acid and o-phenylenediamine as precursors using conventional heating and sonication methods. In this context, the work deals with the synthesis, characterization and biological studies of the nanocomplexes. The core of this work is the comparison of antimicrobial and antioxidative studies of the nanocomplexes synthesized from two different protocols *viz.* conventional and sonication-assisted methods.

MATERIALS AND METHODS

Materials and Measurements

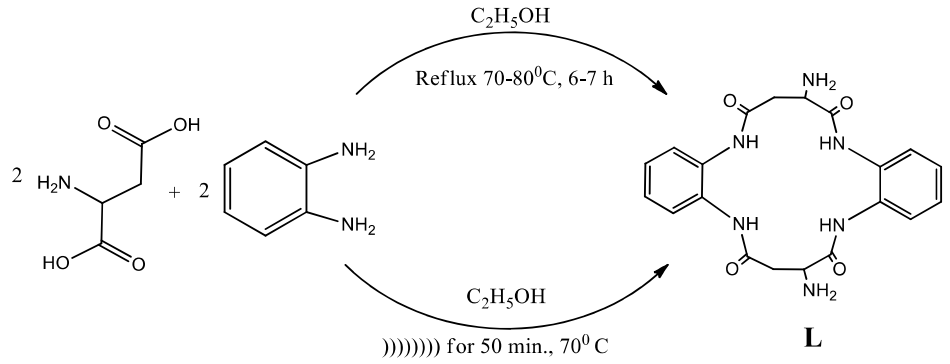
All the solvents and chemicals used were of AR grade with high purity and further used without purification. L-aspartic acid, o-phenylenediamine, and metal salt i.e. manganese acetate were purchased from received from Sigma-Aldrich (Germany) and used as such. The FT-IR spectra was measured in the solid-state using a Model-spectrum Two make-Perkin Elmer which was operated in the wavenumber range of 4000-400 cm^{-1}. CHNS element analysis was done using EuroEA elemental analysis and metal content was estimated volumetrically. The molecular weight was determined by mass spectrometry Using waters Q-Tof micromass (LC-Ms). The UV-Vis absorption spectra was recorded using a Shimadzu spectrophotometer (UV-1800PC, and the wavelength range of 200-800 nm was employed. The Field Emission scanning electron microscopy (FE-SEM) images were taken on Carl Supra 55. The EDS/EDX images were taken along with FE-SEM on Oxford Instruments software Aztec. The XRD pattern was taken on X'Pert Pro XRD equipped with X'celerator solid-state detector, target copper with secondary monochromate. The tested bacterial and fungal strains were purchased from the Institute of Microbial Technology, Chandigarh.

Preparation of macrocyclic ligand: The macrocyclic ligand was synthesized using two different methods.

Sonication-assisted method: The macrocyclic ligand was prepared from the equimolar quantities of L-aspartic acid (0.002 mol) and o-phenylenediamine (0.002 mol) in 25 mL of ethanol in presence of a few drops of Conc. HCl and reaction mixture was placed in a sonicator for 50 minutes at 70°C at the frequency of 40 KHz. The resulting product was washed and recrystallized from ethanol and finally dried under an IR lamp.

Conventional method: In this method, the macrocyclic ligand was synthesized by the condensation reaction of a hot ethanolic solution (25 ml) of aspartic acid (0.002 mol) and a hot ethanolic solution (25 ml) of o-phenylenediamine (0.002 mol) were mixed slowly with constant stirring. This mixture was refluxed at 70°C-80°C for 6-7 hours in presence of a few drops of concentrated hydrochloric acid. On cooling, the solution at room temperature precipitates was formed. The products obtained were then washed and recrystallized from ethanol and dried under an IR lamp.

The schematic procedure has been shown in Scheme 1.



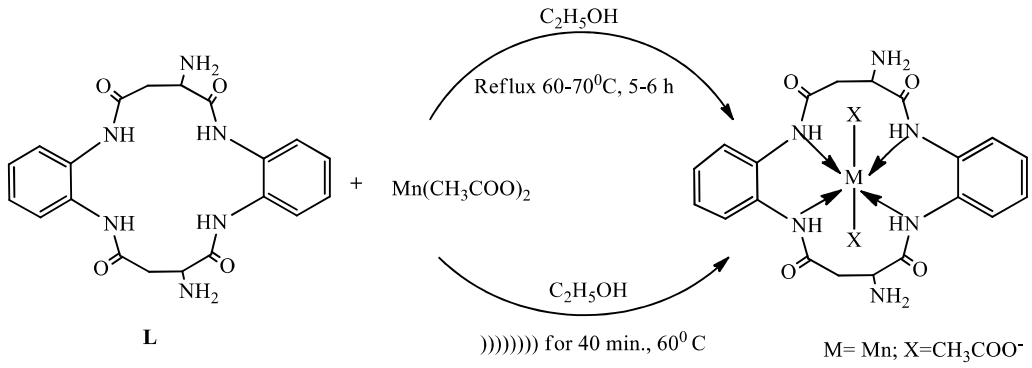
Scheme 1: Schematic representation of synthesis of macrocylic ligand L (7,18-diamino-7,8,17,18-tetrahydrodibenzo[b,j][1,4,9,12]tetraazacyclohexadecine-6,9,16,19(5H,10H,15H,20H)-tetraone)

Preparation of Mn(II) nanocomplexes: The Mn(II) nanocomplexes were synthesized using sonication and conventional method:

Sonication-assisted method: In this method reaction mixture of manganese acetate (0.002 mol) and macrocyclic ligand (0.002 mol) in 20 ml of ethanol in presence of a few drops of Conc. HCl was placed in a sonicator for 40 minutes at 60 °C at a frequency of 40 kHz. The product was filtered, washed with ethanol, recrystallized from methanol and finally dried under an IR lamp.

Conventional method: Solution of manganese acetate (0.002 mol) in hot ethanol (20 ml) was added dropwise to a hot ethanolic solution (20 ml) of the ligand (0.002 mol) with continuous stirring. The resulting solution was refluxed at 60°C-70°C for 5-6 hours in presence of a few drops of Conc. HCl and placed overnight in a refrigerator. The products obtained were filtered, then washed and recrystallized from ethanol and dried under an IR lamp. The complexes were ground manually in pestle mortar so grinding is done to achieve the nanosized complexes.

The schematic procedure has been shown in Scheme 2.



Scheme 2: Schematic representation of synthesis of Mn(II) nanocomplex with macrocylic ligand L

Antimicrobial activity: The Mn(II) nanocomplexes were examined for their *in vitro* antibacterial activities against four Gram-positive bacteria i.e *Staphylococcus aureus* (MTCC No-6845), *Enterococcus faecalis* (MTCC No-441),

Bacillus subtilis (MTCC No- 4214), Listeria (MTCC No- 902) four Gram-negative bacteria *Escherichia coli* (MTCC No-448), *Salmonella enterica* (MTCC No-1165), *Acinetobacter calcoaceticus* (MTCC No-1948), *Serratia marcescens* (MTCC No-2645), and two fungal strains i.e, *Aspergillus niger* (MTCC No-9933), *Candida albicans* (MTCC No-227) using standard two-fold serial dilution method in 96-well micro-test plates. Chloromycin was used as a controlled drug for antibacterial activity and fluconazole for antifungal activity.

Biological assays procedures: Minimal inhibitory concentration (MIC, µg/mL) is defined as the lowest concentration of target compounds that completely inhibit the growth of microbes [14]. To check the effect of solvent on microbial growth DMSO was inoculated with microbial having not any medicine as a positive control. The microbial suspension was adjusted with sterile saline to a concentration of 1 × 105 CFU/mL. The stock solutions were prepared by dissolving complexes in DMSO solvent. The complexes and reference drugs were prepared in Nutrient broth by two-fold serial dilution to obtain the required concentrations of 800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.56 µg/mL. These dilutions were incubated at 37 ± 2°C for 24 h. All the bacterial and fungal growth was monitored visually and spectrophotometrically, and the experiments were performed in triplicate.

Antioxidant activity: The antioxidant activity of the synthesized Mn(II) nanocomplexes was evaluated with the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [15] and was recorded by measuring the change in molar absorbance value of DPPH at 517 nm upon various concentrations i.e. (25-100 µg/mL) in DMSO and ascorbic acid used as a standard drug. Methanolic solution of DPPH (0.004%) was prepared. The stock solution for each complex was prepared in methanol (10 mg/10 ml) and further dilution was done to prepare the required concentrations. Now, 2 ml of the complex mixture was added to 1 ml DPPH-methanol solution. The resulting mixture was wrapped with aluminium foil and kept in dark for 30 minutes. After 30 minutes the scavenging activity was performed spectrophotometrically. The molar absorbance of the DPPH-methanol solution used as a reference was observed at 517 nm using a UV-Vis Spectrophotometer and the decrease in DPPH values of molar absorbance for different sample concentrations at 517 nm and change in colour of the solution from purple to colourless after reduction confirmed the DPPH radical scavenging by the antioxidant by donation of hydrogen radical or electron to form a stable DPPH-H molecule. The DPPH scavenging ability of the compounds was determined by the following equation:

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% inhibition of DPPH activity = $[(A_{control}-A_{sample})/A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the control (reference), A_{sample} is the absorbance of the sample.

RESULTS AND DISCUSSION

Physical measurements data

The Mn(II) nanocomplexes were synthesized by using two different methods, i.e., sonication-assisted and conventional methods. The sonication method has increased the yield of the final product with reduced reaction time, enhanced purity along with lesser use of solvent.

The comparison between the two methods has been tabulated in Table 1.

Table 1: Comparison between sonication and conventional methods

S.No.	Complexes	Reaction period		Solvent (mL)		Yield (%)	
		Sonication (min.)	Conventional (h)	Sonication	Conventional	Sonication	Conventional
1	$C_{20}H_{22}N_6O_4$	50	6.5	25	50	85	63
2	$C_{24}H_{28}MnN_6O_8$	40	5.5	20	40	88	70

The elemental analysis results of the macrocyclic ligand and its Mn(II) nanocomplex agreed with their empirical formulae as shown in Table 2. The ligand and its metal complexes were reacted in 1:1 stoichiometry, which indicates the formation of mononuclear complexes. The complexes formed are solid, coloured and air-stable at room temperature. The Complexes were soluble in common organic solvents like DMSO and DMF only but insoluble in ethanol, methanol, acetone, hexane, chloroform and both hot and cold water.

Table 2: Analytical and Physical data recorded for all synthesized nanocomplexes

Complexes	Colour	Melting point (°C)	Elemental analysis found (calculated)%			
			C	H	N	Mn
$C_{20}H_{22}N_6O_4$	Creamy white	196-198	56.01	5.29	20.4	-
			-58.53	-5.4	-20.48	-
$C_{24}H_{28}MnN_6O_8$	Light Brown	251-253	48.99	4.8	14.29	9.33
			-49.41	-4.84	-14.4	-9.42

FT-IR analysis: The FT-IR absorption bands provided information about the mode of coordination in the synthesized complexes. The FT-IR spectra of macrocyclic Ligand (Figure S1), the characteristic band at 3169 cm^{-1} can be assigned to stretching vibration of ν (N-H) and the appearance of three bands in the region 1643 cm^{-1}, 1250 cm^{-1} and 681 cm^{-1} could be assigned to the three different modes of the amide group suggesting the formation of the closed macrocyclic enclosed region [16]. For Mn(II) complex (Figure S2), the ν (N-H) band shifts to lower frequency of 3158 cm^{-1}, the appearance of three bands in the region 1634 cm^{-1}, 1245 cm^{-1} and 661 cm^{-1} could be assigned to the three different modes of the amide group and a strong band at 515 cm^{-1} appeared in the metal complex corresponding to M-N vibration indicated that the coordination of metal occurs through the nitrogen atom of the amide group in the macrocyclic complex [17].

UV-Vis analysis: The UV-Vis spectra of Mn (II) nanocomplex were recorded in DMSO solvent (Figure 1). It exhibits a strong d-d band around 540 nm assignable to 6A1 g→ 4T1 g suggested octahedral geometry and a band around 334 nm transitions can be attributed to π–π^* transition and around 260 nm due to intra-ligand charge transfer [18].

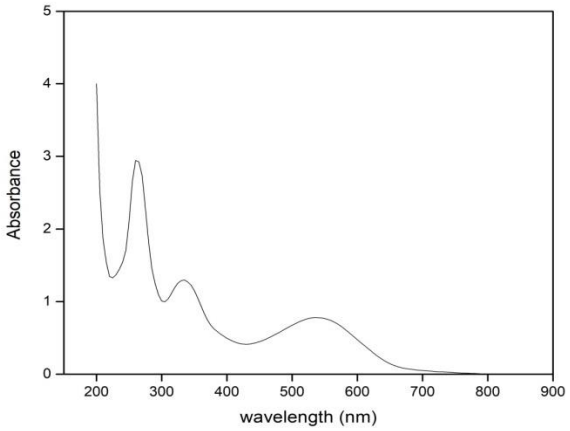

Figure 1: UV-VIS spectra of Mn(II) complex

Mass spectra: The LC-MS spectra of macrocyclic ligand and Mn(II) complex were studied at room temperature. The mass spectrum of macrocyclic ligand L (Figure S3) shows a peak at m/z=410.38 which is in good agreement with its formula molecular weight (410.43). The mass spectrum of the Mn(II) nanocomplex (Figure 2) shows a peak at m/z=587.03 for the molecular ion peak, which is very close to its formula molecular weight (587.17) and the base peak was observed at m/z 212.62.

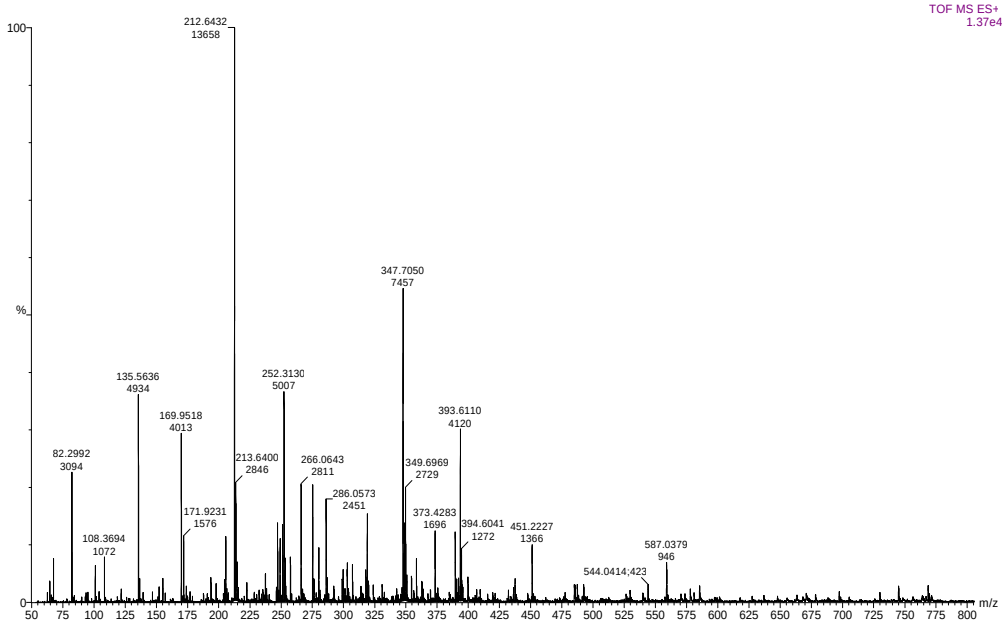

Figure 2: Mass spectrum of Mn (II) complex

EPR spectra: The solid-state EPR spectra of the nanosized complexes were recorded at room temperature on the X-band frequency of 9.719 GHz. The ESR spectra (Figure 3) of Mn(II) in a solid-state at room temperature show a

strong signal centred at g=2 which is associated with an I=5/2 nuclear spin of 55 Mn which confirmed that Mn(II) ions in an environment close to octahedral geometry [19].

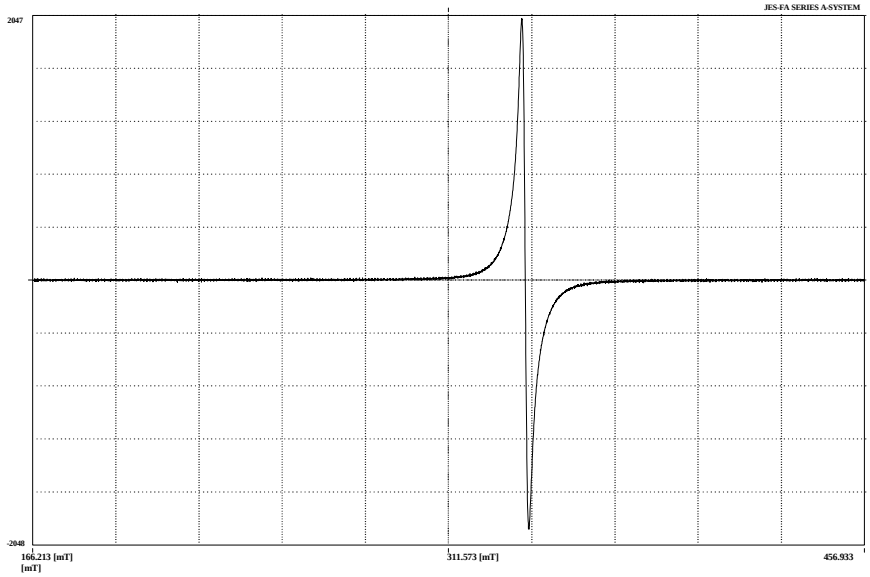

Figure 3: EPR spectrum of Mn(II) complex

XRD studies: Figure 4 shows the X-ray powder diffraction pattern of Mn(II) nanocomplexes synthesized by two different methods i.e. sonication-assisted and conventional methods. The data was recorded by using Cu-Kα radiation. The intensity data were collected over a range of 5-800. The average crystalline size of the nanocomplexes was estimated with the help of the Debye-Scherrer equation (1).

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$$D = K\lambda/(\beta \cos\theta) \quad (1)$$

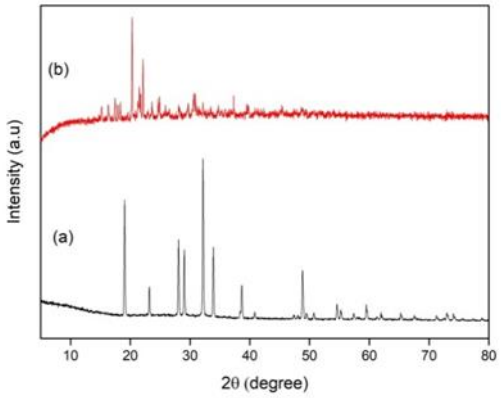

Figure 4: The XRD pattern of Mn(II) nanocomplex synthesized by (a) sonication-assisted method (b) conventional heating method

Where D is the average crystallite size in nm, β is the breadth of the observed diffraction line at its half-maximum intensity, K is the shape factor, which usually has a value of 0.89, λ=0.15406 nm, is the wavelength of the X-ray

radiation for Cu Kα, and θ is the corresponding incidence angle [20]. The average crystalline size of the Mn(II) nanocomplexes synthesized by sonication and conventional method was found to be 42.29 nm and 85.63 nm respectively. The crystalline area found in sonication- assisted method was more than the conventional heating method due to rapid micromixing during sonication, well-defined nanocomplexes were formed in less time, on the other hand, less crystalline nature of Mn(II) nanocomplexes formed by the conventional method that could be since the complex was exposed for a longer duration of time in comparison to sonication method. Visualizing the Figure 4 depicts that the assistance of sonication irridation has significantly decreased the size of Mn(II) complexes when compared to the conventional method. The unit cell parameters were calculated using the hit and trial method and that matched with software JCPDF and found to be hexagonal for Mn(II) nanocomplexes with unit cell parameters a=5.2350 Å, b=5.2350 Å, c=9.9610Å, α=β=90°, γ=120°.

FE-SEM studies: The morphology of Mn(II) nanocomplexes synthesized by two different methods was investigated by FE-SEM and the results are depicted presented in Figure 5. The FE-SEM image for the Mn(II) nanocomplexes synthesized by the conventional method as shown in Figure 5(a) depicts the formation of nanosized particles with irregular morphology whereas that of sonication irradiation, the SEM image shown in Figure 5(b) exhibited that the size of the particles gets reduced and was obtained without any agglomeration. It is concluded that the sonication irradiation method facilitates the formation of Mn(II) nanocomplex which provides an improved surface morphology when compared to the conventional heating method [21].

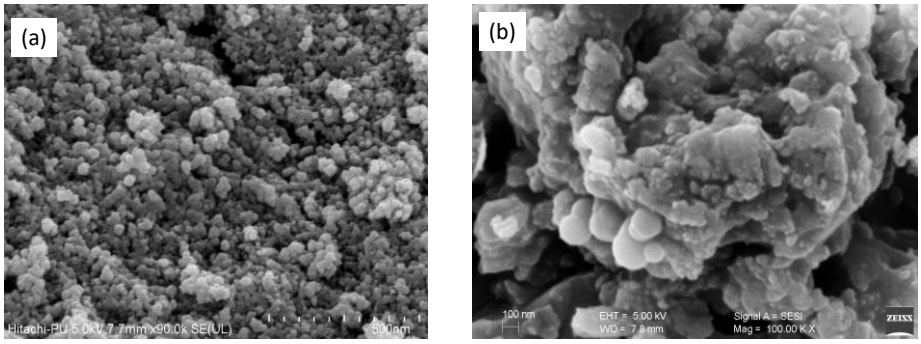

Figure 5: (a) FE-SEM images of Mn(II) nanocomplex synthesized by sonication-assisted method (b) FE-SEM images of Mn(II) nanocomplex synthesized by conventional heating method

Antimicrobial assay: In the present study, the antimicrobial study of macrocyclic ligand and its Mn(II) nanocomplexes synthesized by two different methods were tested against four gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Listeria*) four Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica*, *Acinetobacter calcoaceticus*, *Serratia marcescens*) and two fungal strains (*Aspergillus niger*, *Candida* albicans). The results revealed that nanocomplex obtained from the sonication method exhibited powerful antimicrobial activity as compared to those nanocomplexes prepared by the conventional method. It is attributed to the reduced size and even morphology of Mn(II) nanocomplexes obtained from the sonication method that enhanced their reactivity and activity to kill the microbial growth *via* deeply interventing with cell wall resulting in altered cell permeability leading to cell death (Tables 3a and 3b) [22].

Table 3a: Comparative analysis of Gram-positive bacterial screening of synthesized complexes (Expressed as MIC in µg/ml)

Complexes	*Enterococcus faecalis*		*Bacillus subtilis*		*Listeria sp.*		*Staphylococcus aureus*	
	Sonication	**Conventional**	**Sonication**	**Conventional**	**Sonication**	**Conventional**	**Sonication**	**Conventional**
$C_{20}H_{22}N_6O_4$	200	400	100	400	200	400	200	400
$C_{24}H_{28}MnN_6O_8$	1.56	25	25	50	6.25	25	1.56	25
Chloromycin	3.125		1.56		1.56		3.125	

Table 3b: Comparative analysis of Gram-negative bacterial screening of synthesized complexes (Expressed as MIC in µg/ml)

Complexes	*Escherichia coli*		*Salmonella enterica*		*Acinetobacter calcoacetius*		*Serratia marcescens*	
	Sonicaon	**Conventional**	**Sonication**	**Conventional**	**Sonication**	**Conventional**	**Sonication**	**Conventional**
$C_{20}H_{22}N_6O_4$	200	400	400	400	200	400	200	400
$C_{24}H_{28}MnN_6O_8$	50	125	100	400	50	200	50	200
Chloromycn	3.125		50		3.125		1.56	

However, the antimicrobial activity of the nanocomplexes can be explained by overtone's concept and Chelation theory [23]. As per the theory, the polarity of transition metal ion reduces due to chelation, the partial sharing of its positive charge with the donor groups in the cyclic background of the macrocycle and π-electron delocalization over the whole chelation ring which rises the lipophilic character of the macrocyclic complex, subsequently plays role in the metabolic pathways of these microbes (Table 4).

Table 4: Comparative analysis of Fungal screening of synthesized complexes (Expressed as MIC in µg/ml)

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Complexes	*C. albicans*		*Aspergillus niger*	
	Sonication	**Conventional**	**Sonication**	**Conventional**
$C_{20}H_{22}N_6O_4$	100	400	200	400
$C_{24}H_{28}MnN_6O_8$	6.25	25	25	50
Fluconazole	1.56		25	

Antioxidant activity: The antioxidant activities of macrocyclic ligand and its Mn(II) nanocomplexes were synthesized by two different methods i.e. sonication and the Conventional method was done by the DPPH Scavenging method. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable purple coloured radical in organic solvents and has a single electron. It helps to measure the antioxidant activity of synthesized complexes by acting as a free radical scavenger or hydrogen donor and reacts with complexes that release a hydrogen atom or an electron, resulting in a colour change from purple to colourless [16]. Table 5 revealed the inhibitory effects of macrocyclic Ligand (L) and its nano metal complexes on the DPPH radical. The inhibitory effects of the tested complexes against the DPPH radical were observed in an increasing dose-dependent manner and the suppression ratio increased with increasing concentrations (25-100 µg/ml) of the complexes using ascorbic acid as reference. The data revealed that the Mn(II) nanocomplex synthesised by the sonication method has shown better DPPH radical scavenging activity than the conventional heating method.

Table 5: Antioxidant activity in terms of concentration on scavenging activity (%) of macrocyclic ligand and its Mn(II) nanocomplexes

S.No.	Conc.(µg/ml)	$C_{20}H_{22}N_6O_4$		$C_{24}H_{28}MnN_6O_8$		Ascorbic acid
		Sonication	Conventional	Sonication	Conventional	
1	25	23.8	39.1	34	52.8	60
2	50	26.5	42.5	35.6	57.6	63.3
3	75	30.2	45.8	39	62.7	68.2
4	100	35.8	48.9	42.8	65.7	70.7

CONCLUSION

In the present paper, we describe the comparative analysis of the sonication-assisted and conventional synthesis of Mn(II) nanocomplexes to study their yield biological Potency. The reason for selecting the sonication technique is due to its major benefits such as increased reaction rate, and product yield with high purity, proving it to be magic in the area of synthetic chemistry. The synthesized complexes have been characterized using different techniques such as FT-IR, electronic spectra, mass spectra, ESR, FE-SEM and X-ray diffraction studies. Based on the spectral data, it is confirmed that the metal complex possesses octahedral geometry and FE-SEM images revealed the regular morphology of the nanocomplexes synthesized from the sonication method than the conventional method. The antimicrobial screening data against different bacterial and fungal strains indicate the enhanced activity of nanocomplexes from the sonication method. Hence, the Mn(II) nanocomplex synthesized by a sonication method could be a better material for biological applications.

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CONFLICTS OF INTEREST

The authors have no conflicts to declare.

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