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Synthesis, Characterization and Antibacterial Studies of 4-{[(*E*)-Phenylmethylidene] amino}-*N*-(1,3-thiazol-2-yl)benzenesulfonamide and its Mn(II) Complex

[1]Otuokere, I. E., [1]Anyanwu, J. C. and [2]Igwe, K. K.
[1]Department of Chemistry, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria
[2]Department of Vet. Biochemistry and Animal Production, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria
***Correspondence Email:** ifeanyiotuokere@gmail.com

ABSTRACT
A Schiff base, 4-{[(*E*)-Phenylmethylidene]amino}-*N*-(1,3-thiazol-2-yl)benzenesulfonamide (PTSA) was prepared by a reaction between benzaldehyde and sulphathiazole. Mn(II) complex of PTSA was also prepared. The melting point, colour, yield and conductivity of PTSA and [Mn(PTSA)] were determined. The PTSA and [Mn(PTSA)] were characterized by UV-Vis, FTIR and ^{1}H-NMR spectroscopy. Low conductivity value of 13.9 Sm^2mol^{-1} indicated the non-electrolytic nature of the complex. The PTSA behaved as a tridentate ligand towards Mn, it coordinated through NH-group, two C=N groups. PTSA and [Mn(PTSA)] have been screened for their *in vitro* antibacterial activity against four bacterial strains; *Staphylococcus aureus, Ecsherichia coli, Pseudomonas aeruginosa and Salmonella typhi*. It was observed that the complex was more potent than PTSA against the bacterial strains used. In line with the findings, PTSA and [Mn(PTSA)] inhibited *E. coli, P. aerugunosa, S. typhi and S. aureus.* The Mn(II) complex showed enhanced antibacterial activity when compared with PTSA. It was deduced that [Mn(PTSA)] complex have improved antibacterial activity than PTSA alone and it was evident that the overall potency of PTSA was enhanced on coordination with manganese ion.

Keywords: Antibacterial, Complex, Manganese, Schiff base, Spectroscopy

INTRODUCTION
Metal complexes have played important and diverse roles in medicine for thousands of years. They take part in variety of biological processes due to their characteristic electronic features, which generally involve their binding to electron-rich biological components, such as proteins and DNA (Morris, 2014). Schiff bases are among the most important ligands that played important role in the development of coordination chemistry (Burlov *et al*., 2014). This is as a result of their well-known coordinative capability. Several Schiff base compounds have been known to play an important role in revealing the preferred coordination geometries of metal complexes due to their diversity, preparative accessibility and structural variability. Due to a variety of applications, Schiff base compounds have been the subject of great interest. Xishi *et al*., (2003), reported the synthesis and characterization of a novel Schiff base ligand formed from the condensation of 2,2-bis(P-methoxyphenylamine) and Salicylaldehyde and its Mn(II), Co(II) and Cu(II) complexes

In the past many Schiff base derivatives have been prepared and employed for applications in catalysis and enzymatic reactions, luminescent material, magnetism and molecular architectures. However recently Schiff bases metal complexes gain massive attention in the domain of biological chemistry and coordination chemistry. Schiff bases are a special class of ligands with a variety of donor atoms exhibiting interesting coordination modes towards various metals. Schiff bases containing polyfunctional groups produce stable complexes of transition, non-transition, inner-transition and actinide metal ions. Report has it that the biological properties of Schiff base ligands are due to the azomethine (–HC=N–) group (Ren *et al*., 2002). Schiff' bases of different carbonyl compounds showed antimicrobial activity against *B. subtilis, E. coli, P. fluorescence, S. aureus and A. niger* (Mohamed *et al*., 2005; Mohamed *et al*., 2010).

Three Schiff base compounds of N-substituted benzohydrazide and sulfonohydrazide derivatives: N-(2-hydroxy-3-methoxybenzylidene)-4tert-butyl-benzohydrazide, N-(5-bromo-2-hydroxybenzylidene)-4-tertbutylbenzohydrazide and N-(2-hydroxy-3-methoxybenzylidene)-4methylbenzenesulfonohydrazide were synthesized and characterized by elemental analysis, FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The compounds possessed antibacterial, antifungal, antioxidant and

cytotoxic to enzymatic activities (Sirajuddin, 2013). Salama *et al*., (2015). Synthesized Schiff bases of chitosan by the reaction of chitosan with 3-(4-substitutedphenyl)-1-phenyl-1H-pyrazole4-carbaldehyde. The structure of the prepared chitosan derivatives was characterized by FT-IR spectroscopy, elemental analysis, and X-ray diffraction studies and thermogravimetric analysis (TG). The chitosan and Schiff bases of chitosan showed promising antimicrobial activity against *Streptococcus pneumonia, Bacillis subtilis, Escherichia coli.*

The growing interest in transition metal complexes containing Schiff base antibiotics is derived from their functions and well-established chemical in biological systems as well as their pharmaceutical and catalytic applications (Rehder *et al*., 2003; Rehder, 2003). In recent years, Schiff base ligands have received much recognition and yet there is much scope for thorough probe on metal complexes with such ligands which are also of biochemical interest. Schiff bases are versatile pharmacophore (Kajal *et al*., 2013) that binds metal ions. Their coordination behavior is due to presence of various donor atoms. Schiff bases metal complexes are considered to have medicinal and pharmaceutical importance with broad spectrum of biological significances like anti-tubercular (Bhat and Al-Omar, 2013), anticancer (Chakraborty *et al*., 2010), antimicrobial, analgesic (Chinnasamy *et al*., 2010) and anti-inflammatory actions (Pontiki *et al*., 2008). In search of new and potentially active Schiff base, we selected benzaldehyde and sulphathiazole as starting compounds and augmentation of antibacterial activity was reported by insertion of manganese ion into the synthesized Schiff base. We hereby present the synthesis, characterization and antibacterial studies of 4-{[(E)-Phenylmethylidene]amino}-N-(1,3-thiazol-2-yl)benzenesulfonamide schiff base and its Mn(II) complex.

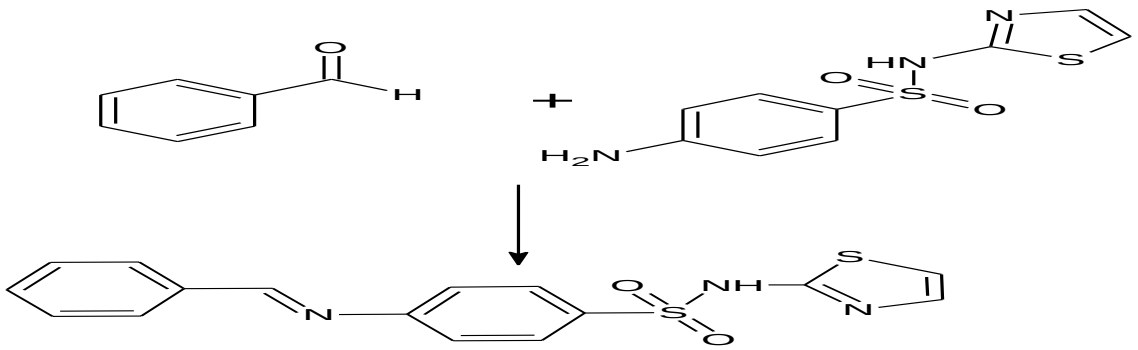
Materials and Methods

The chemicals used in this study were of analytical grade and were used as obtained without further purification. The bacteria, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella typhi.* used in the antimicrobial test were clinical isolates obtained from Federal Medical Centre, Umuahia. The melting points and decomposition temperature of both the ligand and the complex were determined using Gallenkamp melting point apparatus. Their solubility were measured in common organic solvent. Molar conductance was measured at room temperature in a concentration of 10^{-3} M DMSO solution using Jenway Conductivity Meter 4510. Amount of manganese in the complex was obtained by AAS spectrophotometer (bulk 210). The elemental analysis for C, N, H and S was obtained using a Perkin-Elmer 240B elemental analyzer. The stoichiometry of the complex was determined using continuous variation method as described by Tirmizi *et al.,* (2012). The liquid state UV-Vis spectra of the ligand and its Mn(II) complex were recorded on Uv-1800 series using Dimethylsulfoxide (DMSO) as the solvent in the range 200-800 nm. The solid state FTIR spectra of the ligand and its complex were recorded on a Perkin Elmer Spectrum BX FTIR spectrophotometer (4400-350 cm^{-1}) in KBr pellets. The NMR spectral measurement was recorded on nuclear magnetic resonance Bruker spectrophotometer using tetramethylsilane internal standard and DMSO-d6 as solvent.

Synthesis of 4-{[(E)-Phenylmethylidene]amino}-N-(1,3-thiazol-2-yl)benzenesulfonamide Schiff base

The **s**ynthesis was carried out according to the method of Zahid and co-workers (2012) with slight modification. Sulphathiazole (5.11 g; 2 mmol) in ethanol (45 cm^3) solution was added to 2.03 cm^3 (2 mmol) of benzaldehyde. The solution was stirred and refluxed for 3 hours. The product was filtered off, washed several times with H_2O, cold EtOH and dried in a dessicator.

The general synthesis of the Schiff base ligand PTSA is proposed in Scheme 1.



Scheme 1: Synthesis of 4-{[(*E*)-Phenylmethylidene]amino}-*N*-(1,3-thiazol-2-yl)benzene sulfonamide

Synthesis of Mn(II) complex of 4-{[(E)-Phenylmethylidene]amino}-N-(1,3-thiazol-2-yl)benzenesulfonamide

To 45 cm^3 hot ethanol solution of (6.87 g; 2 mmol) PTSA, 45 cm^3 aqueous solution of (3.22 g; 2 mmol) $MnCl_2.2H_2O$ was added and refluxed for 2 hours. The concentrated solutions were left overnight at room temperature which led to the formation of solid product. The product was filtered off, washed several times with H_2O, cold ethanol and dried in a dessicator (Zahid, 2012).

Antibacterial sensitivity test

The organisms used were Gram-negative *Escherichia coli and Pseudomonas aeruginosa, also* the Gram-positive bacterial strains *Staphylococcus aureus and Salmonella typhi were investigated.* The organisms were clinical isolates obtained from Federal Medical Centre, Umuahia, Abia State. Antibacterial activity of samples were determined by using agar well diffusion method and bacterial growth were subcultured on nutrient broth for their *in vitro* testing which were prepared by dissolving (24 g) of nutrient broth. The mixture was autoclaved for 15 minutes at 120 °C. Stock solution for *in vitro* antibacterial activity was prepared by dissolving 5 mg of compound in 9 cm^3 of DMSO. Inoculation was done with the help of micropipette with sterilized tips in which 100 µL of activated strain was placed onto the surface of agar plate. It was spread over the whole surface and then two wells having diameter of 10 mm were made in the media and incubated at 37 °C for 48 hours. Activity was determined by measuring the diameter of zone showing complete inhibition and has been expressed in mm (Jai *et al*., 2018).

RESULTS AND DISCUSSION

Physicochemical properties of PTSA and [Mn(PTSA)] are shown in Table 1. The ligand PTSA and [Mn(PTSA)] have high melting points and the melting point of the complex was higher than that of the ligand showing that coordination occurred. The complex showed higher decomposition temperature because the metals have closed packed structures, so they have strong metallic bond and small atomic radii, they need more energy the bonds (Nadiah and Uwaisulqarni, (2013). The colour change from greenish-brown (ligand) to yellow for [Mn(PTSA)] complex suggested complexation.

Table 1: Some physicochemical properties of PTSA and [Mn(PTSA)]

Ligand/ Complex	Colour	Dec. temp (°C)	Yield (%)	Conductance Sm^2mol^{-1}	Metal: ligand ratio	C Found (Calc.)	H Found (Calc.)	Mn Found (Calc.)	N Found (Calc.)	O Found (Calc.)	S Found (Calc.)
PTSA	Greenish-brown	244-246	96.1	-	-	55.92 55.96	3.79 3.82	- -	12.21 12.24	9.30 9.32	18.63 18.67
[Mn(PTSA)]	Yellow	276-278	50.0	13.9	1:1	48.35 48.36	3.01 3.04	3.82 3.83	10.56 10.57	8.03 8.05	16.14 16.14

PTSA = 4-{[(E)-Phenylmethylidene]amino}-N-(1,3-thiazol-2-yl)benzenesulfonamide Dec = decomposition

PTSA and [Mn(PTSA)] showed low conductivity value, which indicated the non-electrolytic nature of the ligand and its Mn(II) complex. Geary (1971) reported that coordination compounds with molar conductance above 100 Ohm^{-1} mol^{1} cm^{-1} are electrolyte. The elemental analysis results were in good agreement with the calculated value. Based on the continuous variation method, metal: ligand ratio is 1:1 was proposed. PTSA and [Mn(PTSA)] were slightly soluble in n-hexane and very soluble in DMSO (Table 2). The solubility profile showed that the Schiff base and its Mn(II) complex are weakly polar compounds. DMSO is an important polar aprotic solvent that dissolves both polar and non- polar compound, hence the Schiff base and its Mn(II) complex were very soluble in it.

Table 2: Solubility Profile of PTSA and [Mn(PTSA)] in various solvents

Ligand/Complex	n-Hexane	Methanol	Petroleum ether	DMSO
PTSA	SS	IS	SS	VS
[Mn(PTSA)]	SS	IS	SS	VS

Key: IS-Insoluble, SS-Slightly Soluble, VS-Very soluble
PTSA = 4-{[(*E*)-Phenylmethylidene]amino}-*N*-(1,3-thiazol-2-yl)benzenesulfonamide

The IR spectral comparison of the novel Schiff base ligand and its Mn(II) complex have been used to ascertain the binding mode of the manganese ions at donor sites of the ligand. The FTIR spectra of PTSA and [Mn(PTSA)] are shown in Figure 1. The FTIR of the PTSA schiff base showed absorption band at 1697.90 cm^{-1} which was assigned to azomethine υ(C=N) stretching mode. On comparison with [Mn(PTSA)] it was found that this peak was shifted to lower wavenumber 1598.09 cm^{-1} indicating the participation of the azomethine nitrogen in coordination. Similar shift was reported by Narendra and Parashuram, (2014) in the formation of metal complexes of Schiff base derived from streptomycin and amoxicillin. Canpolat, (2014) reported that in the IR spectrum of the dioxouranium (VI) Schiff base complexes, the υ(C=N) azomethine stretching band appearing at ca.1617-1644 cm^{-1} in the ligands are shifted ca.1599-1619 cm^{-1} for the complexes. The absence of band characteristics of υ(C=O) confirms the formation of the proposed Schiff base framework. The band at 3361.79 cm^{-1} was assigned to υ(N-H) stretch in the PTSA ligand. In the complex the υ(N-H) stretch shifted to a higher wavenumber 3398.00 cm^{-1} which suggested the involvement of N-H in coordination to Mn(II) ion. The vibrational frequency observed at 1133.07 cm^{-1} was assigned to υ(S=O) of the Schiff base. This functional group showed no significant change, 1136.97 cm^{-1} in the spectrum of [Mn(PTSA)] suggesting that this group was not involved in coordination to Mn(II) ion.

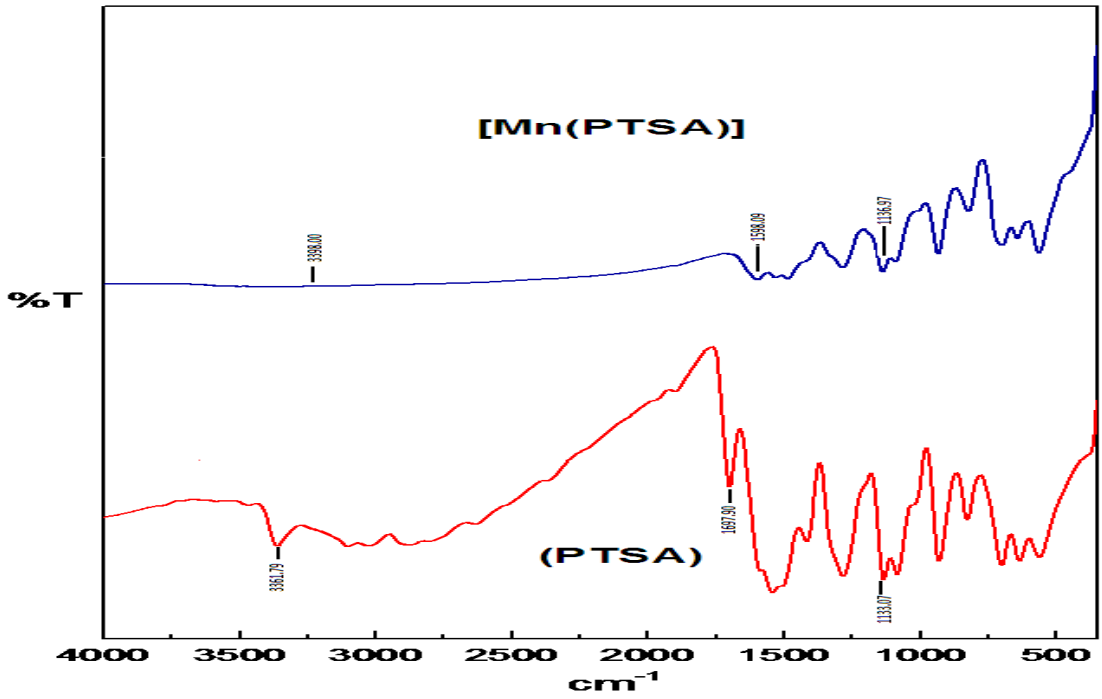


Figure 1: FTIR spectra of PTSA and [Mn(PTSA)]

The UV-Vis spectra of PTSA and [Mn(PTSA)] are shown in Figure 2. Comparison of the Uv-Vis spectrum of PTSA and [Mn(PTSA)] were made. The electronic spectrum of PTSA and [Mn(PTSA)] absorbed at the λ = 207.50, 216.50, 229.50, 244.50, 250.50, 254.50, 261.50, 271.50, 280.50 and 284.50 nm and these absorptions were as a result of the chromophores presents in the PTSA and [Mn(PTSA)]. These transitions have been assigned π-π* and n-π* and are known as Intra-Ligand Charge Transfer (ILCT). In the Uv-visible spectrum of the Mn(II) complex, the ligand to metal charge transfer (LMCT) was observed at 313.00 nm. The ligand to metal charge transition band suggested formation of complex (Amna *et al*., 2009).

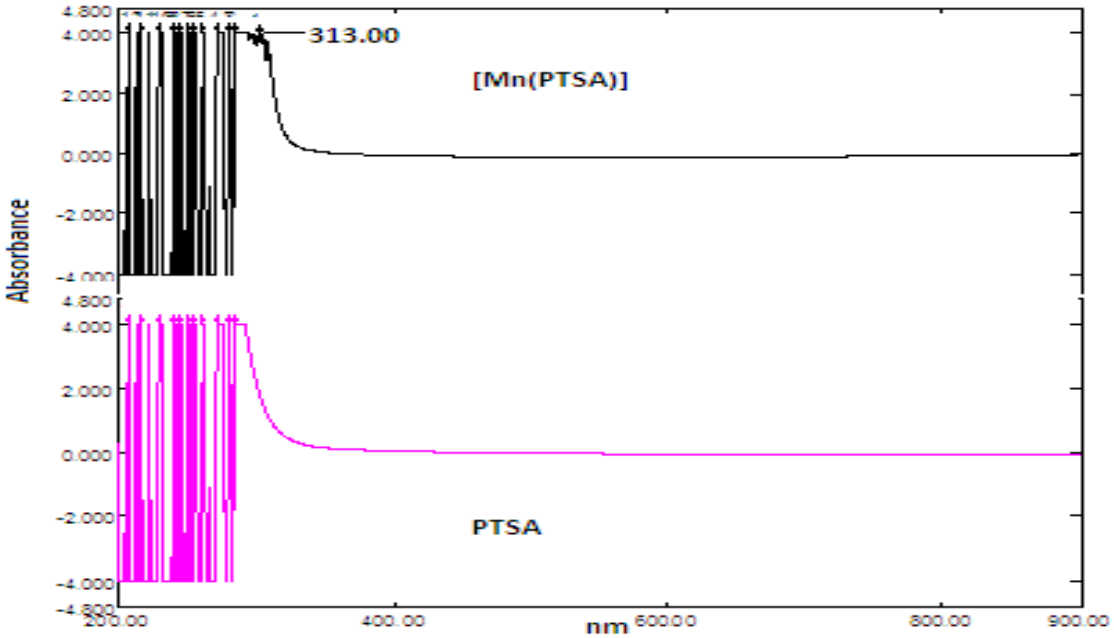


Figure 2: Uv/Visible spectra of PTSA and [Mn(PTSA)]

The ^{1}H-NMR spectra of PTSA and [Mn(PTSA)] are represented in Figure 3. ^{1}H-NMR spectral comparison of novel Schiff base ligand and its manganese complex was made to confirm the binding nature of ligand with metal ion. The resolutions of signals in the ^{1}H-NMR spectra of ligand and metal ion complexes were different due to paramagnetic and diamagnetic nature of the respective substances. The ligand showed resonance peak at 10.02 ppm assignable to the protons of -SO_2NH group. This signal was absent in the spectrum of [Mn(PTSA)]. The signals of aromatic protons and thiazole protons observed as multiplets between 6.71 - 7.59 ppm and 5.82 - 6.58 ppm in PTSA were also observed in the complex.

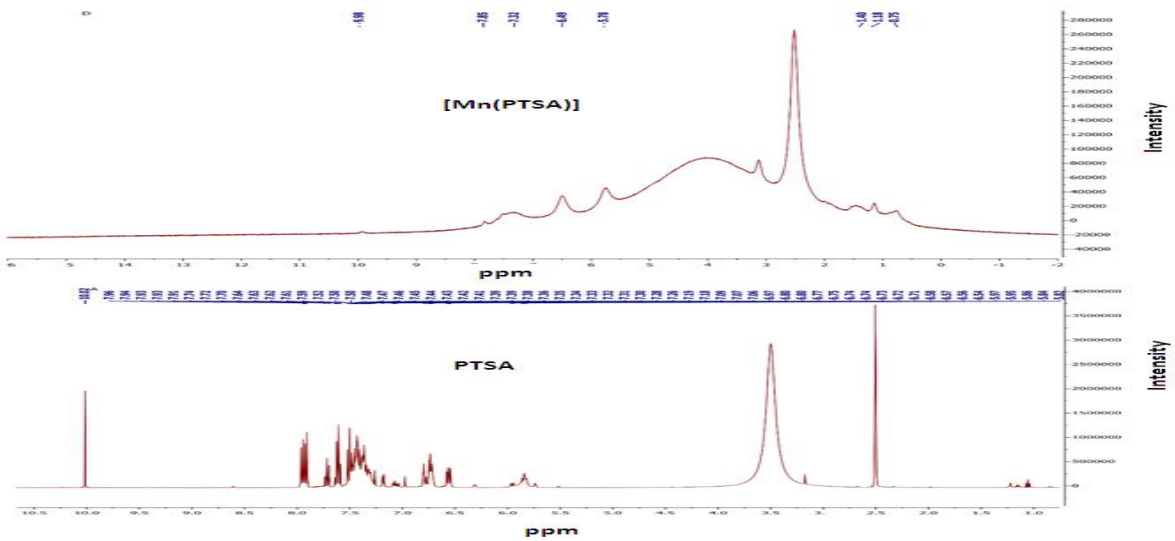


Figure 3: ^{1}H NMR spectrum of PTSA and [Mn(PTSA)]

Based on continuous variation method, elemental analysis, FTIR and ^{1}H-NMR spectroscopy, the structure in Figure 5 has been proposed for [Mn(PTSA)]. PTSA behaved as a tridentate ligand.

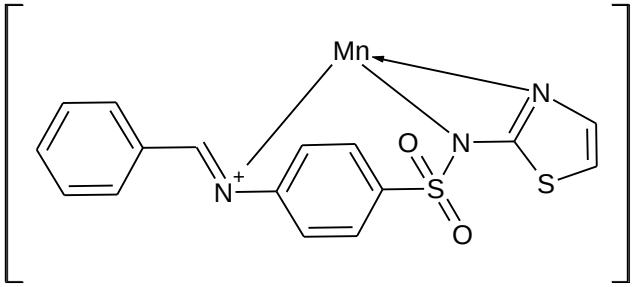


Figure 5: Proposed structure for [Mn(PTSA)]

The antibacterial effect (Table 7) showed that both compounds exhibited varying degree of inhibitory activity on the growth of different tested bacterial strains. It was observed that the inhibitory activity of [Mn(PTSA)], was significantly higher ($p<0.05$) compared to PTSA against *E. coli, S. typhi and S. aureus.* However, the inhibitory activity of [Mn(PTSA)] was significantly lower ($p<0.05$) when compared to PTSA against *P. aeruginosa.* The higher antibacterial activity was as a result of chelation of the ligand with metal ions. It has been reported that coordination could augment the lipophilic character of the central metal atom, which favours its permeability through the lipid layers of the cell membrane and disturbing the metal binding sites on enzymes of the organism, hence the enhanced antibacterial activity (Angelo, 2020; Angelo *et al*., 2020).

Table 7: Zone of inhibition (mm) of PTSA and [Mn(PTSA)] on some bacterial strains

Ligand/complex	Bacteria			
	Gram Positive		Gram Negative	
	E. coli	*P. aeruginosa*	*S. typhi*	*S. aureus*
PTSA	13.00 ±0.02^{a}	16.00±0.04^{b}	14.00±0.01^{a}	17.00±0.03^{a}
[Mn(PTSA)]	20.00±0.02^{b}	10.00±0.03^{a}	20.00±0.02^{b}	18.00±0.04^{b}

Values are mean ±SD of 3 replicates. Means within the rows with different superscripts differ significantly ($P<0.05$). PTSA = Phenylmethylidene-(1-3-thiazole-2ylmethyl)sulfonymethylaniline

CONCLUSION

PTSA and its Mn(II) complex have been synthesized. The decomposition temperature of the complex when compared to the ligand was higher. Low conductivity value indicated the non-electrolytic nature of the Mn(II) complex. Thus, the ability of PTSA to coordinate Mn, ion have been ascertained. The ligand showed and its Mn(II) complex showed significant inhibitory activity against *E. coli, P. aeruginosa, S. typhi and S. aureus* bacterial strains. The complex showed enhanced antibacterial activity. It was deduced that [Mn(PTSA)] complex have improved antibacterial activity than PTSA alone and it was evident that the overall potency of PTSA was enhanced on coordination with manganese ion.

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