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Synthesis, Characterization and Antimicrobial of Schfiff Base from 5-Bromo – Salicylaldehyde and P-Toluidine

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ABSTRACT: As pathogens in recent times have developed resistance to the existing antibiotics, the demand for new and more effective antimicrobial agents is a continuous phenomenon. The study involves the synthesis of schiff base from 5-bromo-salicylaldehyde and para-toluidine. The synthesized compounds (5-Bromo – Salicylaldehyde and the Schiff base-N-(5-bromo-2-hydroxybenzylidene) Para-toluidine) were evaluated for antimicrobial activities against different bacteria strains using the agar well diffusion method. The 5-bromosalicylaldehyde and its Schiff base indicated dose dependent activity as concentration increases from 125mg/ml to 1000mg/ml and inhibitory zones were found to increase mostly for *Escherichia coli*. The two compounds synthesized have indicated high antimicrobial activities on the selected germs and as such can be a drug precursor to microbial infections with further research.

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Key words: schiff base, 5-bromo- salicylaldehyde, para-toluidine, antimicrobial activity

Salicylaldehyde is a major precursor to aspirin, used in the synthesis and technology of drug manufacturing and as an important intermediate in the manufacture of herbicides and pesticides (Jane and Kreft, 2008). Salicyladehyde has been identified as a characteristic aroma component of buckwheat, (Fagopyrum esculentum) and oil from other plants like spiraea (Fillip erdula), Ulmaria (rosoceae) and sweet smelling flowers have been found to contain Salicylaldehyde and Methylsalicylate (Jane and Kreft, 2008).Schiff bases are typically formed by the condensation of an amine and an aldehyde, ketone, amino acids which involves the use of organic solvents such as methanol, Tetrahydrofuran (THF), and 1, 2-dichloroethane (DCE) (Sunita et al., 2013). The chemistry of Schiff base compounds has been studied extensively. They have various applications as coordinating ligands, as catalysts, in electrochemistry and medicinal values (Abdullahi et al., 2014). Schiff bases possess antimicrobial and anti-inflammatory activity (Abdullahi et al., 2014). It has been discovered that the biological activity of some Schiff base ligands became enhanced upon chelation with metal ions (Wang et al., 2011). Schiff bases are useful ligands because of their synthetic accessibility, diversity and structural varieties (Gomathi et al., 2015). Over the past few years, there have been many reported applications of Schiff base in medicine (including anti-bacterial, anti-fungal, anti-cancer, anti-

oxidant, anti-inflammatory, anti-malarial and antiviral activity) and agriculture (as plant growth regulators (Mohamed *et al.* 2010). The objective of this work is to synthesize, characterize and evaluate the antimicrobial activities of schiff Base from 5-Bromo – Salicylaldehyde and P-Toluidine.

MATERIALS AND METHOD

All chemicals salicyaldehyde, paratoluidine, bromine were obtained from Aldrich (Germany). The solvents: ethanol, methanol, chloroform, acetic acid were of analytical and spectroscopic grade and used without further purification. IR spectra were recorded as nujol mulls on a Shimadzu FT-IR 157 Spectrophotometer.. Melting points were determined using a Koffer melting point apparatus (England)

Synthesis of Compound I (5-bromo-salicylaldehyde): Bromine solution (13.0ml) was measured in 12ml acetic acid. 10g of Salicylaldehyde was placed in 10ml acetic acid. The bromine solution was in a burette dispensed into a mixture of 10g Salicylaldehyde and 100ml acetic in a drop wise manner in a fume cupboard. The reaction was carried at room temperature for duration of 1hour. The solvent was removed under reduced pressure and solid obtained was re-crystallized in cold ice water (Dieter, 2000). The equation for the reaction is presented as follows

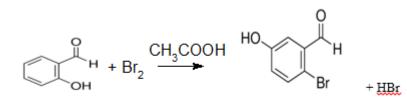


Fig 1: (a) Salicylaldehyde

(b) 5-bromo-salicyaldehyde

mmol) in ethanol (10ml). (1:1) (Dieter, 2000). Two (2)

drops of concentrated sulphuric acid were added. The

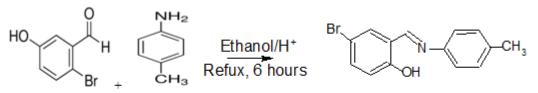
solution was then stirred and warm to observe for any

precipitate. Thereafter, the reaction mixture was

refluxed for 6 hours and the precipitate collected by

filtration and recrystallized from ethanol-hexane

Synthesis of Compound Ii (N-(5-bromo-2hydroxybenzylidene) Para-toluidine): A solution of 5bromo -2-hydroxybenzaldehyde (4g: 0.02mol or 4g: 20mmol) in ethanol (10ml) were added to a stored solution of P-toluidine (2.14g: 0.02mol or 2.14g: 20



N-(5-bromo-2-hydroxybenzylidene) Para-toluidine

Fig 2: Synthesis of Schiff base

Antimicrobial Activity: Micro-organism: The microorganisms employed in this study obtained from the University of Benin Teaching Hospital, Benin City includes clinical isolates of *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and*Candida albican*

Media: Nutrient broth and Nutrient agar, all product of Himedia Laboratories Mumbai (India) were used in this study. The composition of the medium was Beef extract (3.0g), Peptone (5.0g), sodium chloride (8.0g) and agar (15.0g).

Antimicrobial agents: Arnoxicillin (1µg/ml), Streptomycin (1.5µg/ml), Ampicillin (2µg/ml) Gentamycin (1.5mg/ml)

Agar well diffusion assay: The antimicrobial activity of the extracts was determined by using the agar well diffusion technique (Monica, 2003). Nutrient agar plates were each seeded with 0.1 ml of an overnight culture of each bacterial $(10^6$ cfu/ml).The 24 hours broth culture of each bacterium were used to seed sterile molten nutrient agar at 45°C, allowed to set and well made by sterile standard cork borer (6.0mm in diameter) and 200µl (0.2ml) of 15mg/ml solution of extract added into each well. Then bacterial plates incubated at 37°C for 24 hours, after which diameter of zones of inhibition were measured (Monica, 2006).

Determination of Minimum Inhibitory Concentration (MIC): The MIC values of each synthesized compound were determined using two-fold micro-

dilution to prepare concentrations of I000mg/mol, 500mg/mol, 250mg/mol and 125mg/mol of each synthesized compound and a drop of the bacterial suspension that had been previously diluted to about 10^{6} cfu/ml were aseptically incorporated into molten Nutrient agar and allowed to set. The plates were incubated at 37° C for 24 hours. The lowest concentration preventing visible growth for each of the test organisms was recorded as the MIC. The experiment were carried out in triplicate for each synthesized compound concentration and controls against the bacterial isolates were amoxicillin, streptomycin, gentamycin and ampicillin as the positive control.

RESULT AND DISCUSSION

Physical Parameters of Synthesized Compounds: The synthesized compounds (5-bromo-salicyaldehyde) (I) N-(5-bromo-2-hydroxybenzylidene) and paratoluidine (Schiff base, compound II) were screened for their antimicrobial activities using the agar well diffusion method.In table 3, compound I, a bromo derivative of Salicylaldehyde had minimum inhibition zones of (11.80, 125mg/ml) and (10.60, 125mg/ml) as MIC values in methanol and water solvents on bacterial isolates, which are specific inhibition for E. coli. The general trend of inhibition of microbial isolates by compound I indicated a dose dependent activity. As the concentration increases from 125mg/ml to 1000mg/ml, inhibitory zones was found

to increase especially for *E. coli* 125mg/ml (11.80mm), 250mg/ml (13.60mm), 500mg/ml (15.70mm) and 1000mg/ml (18.40mm) using methanol as solvent for dilution while with water as solvent the same trend was observed. MBC for compound I were observed at 125mg/ml for all isolates indicating activity even at a low dose. The antimicrobial activity of compound II (Schiff base) also indicated a dose dependent activity as concentration increases from 125mg/ml to 1000mg/ml

for all clinical isolates. However, high inhibition was observed for *E. coli* ranging from 125mg/ml (11.85mm); 250mg/ml (12.10mm) and 1000mg/ml (17.00mm) using methanol as solvent. The antimicrobial results suggest that the Schiff base is a potential candidate for antimicrobial formulations and this work support the findings of Fasina *et al.* (2013) who asserted that most Schiff bases are potential pharmacological agents.

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C:/Program Files/LabSolutions/IR/Data/Compd 11.ispd

Fig 3: FT-IR spectrum of compound II (N-(5-Bromo-2-hydroxybenzylidene) para-toluidine)

S/N	Wave number	Appearance	Band	Functional group
	(cm ⁻¹)			
1	823.60	Strong	C - H	1,4 Disubstituted benzene
2	906.54	Strong	C – H bend of CH ₂ =CH	Alkene group
3	1382.96	Medium	C – H bend	Methyl group
4	1471.89	Strong	C=C stretch	Arene
5	1608.63	Strong	C=C stretch	Conjugated alkenes
6	1772.58	Short	C=O stretch	Acid halide
7	2374	Short	_	Nitrile
			C N stretch	
8	2856	Medium, Strong	C – H stretch	Alkyl
9	3030.17	Medium	C – H stretch	Alkene, Arene
10	3435.22	Medium	N – H stretch	Amines

Table 2: Infra-red band for compound II (Schiff base)

		E.coli			P. aureginosa			S. aureus			C.albican						
Compound	solvent	*10	5	2.5	1.25	10	5	2.5	1.25	10	5	2.5	1.25	10	5	2.5	1.25
	Water	11.50	11.30	10.90	10.60	10.70	10.40	10.30	10.10	11.40	11.10	10.80	10.50	10.40	10.10	9.80	9.30
	Methanol	18.40	15.70	13.60	11.80	11.80	11.50	10.90	10.60	13.50	12.70	11.30	11.10	11.20	10.40	10.80	10.30
	Water	13.10	10.20	10.15	10.10	14.40	11.20	11.10	10.80	11.50	11.10	10.60	10.10	12.70	13.50	12.80	10.90
[Methanol	17.00	10.80	12.10	11.85	15.00	13.10	12.70	10.80	12.40	11.10	11.80	10.30	14.80	16.10	15.60	11.70
	[*] Concentration in mg/ml x 10 ²																

Table 3: Antimicrobial activity of compound 1 and II

The infra-redband for compound I, indicated 829.39 cm⁻¹ (C – H bend) a disubstituted benzene which suggest the presence of CHO and OH group on the benzene ring of Salicylaldehyde; 1274.95 cm⁻¹ (C – O stretch) of phenolic group which suggest the presence of phenolic - OH in the compound I. The presence of acid halide functional group observed at 1784.15cm⁻¹ suggest the success of the bromination of the Salicylaldehyde in compound I. Thus compound I possess the needed functional group in 5-Bromosalicylaldehyde. The infra-red band for compound II were observed at 823.60cm⁻¹ (C - H bend) of disubstituted benzene which confirms the use of 1,4 - disubstition reagent (Para-toluidine) in synthesizing the Schiff base (N-(5-bromo-2hvdroxvbenzvlidene) Para-toludine).

Table 4: Antibiotic Sensitivity Testing

ANT IBIOTIC DISC (mm)									
Test organism	CPX	OFX	PEF	CN	SXT	AU			
	10 µg	10 µg	10 µ g	10 µg	30 µg	30µg			
E. coli	12	10	15	9	5.20	-			
P. aeroginosa	16	-	18	-	-	13			
P. vulgaris	11	-	10	-	-	-			
S. aureus	18	15	11	-	14	10			
C. albicans	18	11	-	13	9	10			

Key: CPX-Ciprofloxalin, OFX-Tarivid. PEF –pefloxacin, CN-Gentamycin, SXT- septrin, augmentin

The peak 1471.89cm⁻¹ (C=C) suggests the presence of double bond as in arenes (benzene). This also confirms that the Schiff base is from a benzene compound. Also, the peak at 1772.58cm⁻¹ (C = O stretch) suggest that aldehyde functional groups was detected which could be as a result of unreacted benzaldehyde derivative present as impurity in the compound. The peak at 3435.22cm⁻¹ indicating (N – H) stretch suggests the presence of an amine derivative which is related to the Imine (Schiff base) produced.

Conclusion: Compounds I and II were active at inhibiting the selected microorganisms when compared to the standard positive controls in this study. Hence, these compounds can be used in formulation of narrow spectrum antibiotics for treatment of infections caused by bacteria particularly *E.coli*.

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