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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF AZINE AND 3, 4, 5 – TRIMETHOXY SUBSTITUTED AZINE

[1] P. O. Osarumwense

[1]*Department of chemical science, Ondo State University of science and technology Ondo State Nigeria.*
Corresponding Author: E–mail. Osarodion.peter@yahoo.com

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ABSTRACT
Azine and the substituted 3, 4, 5 – trimethoxy azine were synthesized by the condensation of the corresponding Benzaldehyde and substituted Acetophenone with hydrazine hydrate. The compounds synthesized were characterized and screened for anti-microbial activity against Bacillus species, Escherichia coli, Klebsiella pneumonia, serratia marcensces and candida albicans.

Key words: Azine, antimicrobial activity, 3, 4, 5 – trimethoxy substituted azine

INTRODUCTION
Azines may be further classified as aldazines or ketazines, depending on the nature of the carbonyl compound. (Moss e.t.al:1995, and IUPAC, compendium of chemical Terminlogy 2006).
Azines may also be named by substitutive or functional class nomenclature. (panico R etral; 1993 and IUPAC, chemical Nomenclature and structure Representation chemistry 2004).
In older nomenclature, the functional class name ‘’ketazinne’’ has been used with the names hydrocarbhy substituents: e.g ‘’methyl, ethyl ketazine’’. In substitutive nomenclature, azines are named as derivatives of hydrazine: hence, “diisoproprylidenehydrazine’’. In the presence of groups of higher seniority, the prefixes ‘’hydrazinylidene” and hydrazinediylidene” are used. (IUPAC, chemical Nomenclature and structure Representation Division, 2004)
Unsymmetrical azines, that is compounds of the type X =N – N = Y with X = Y, are not named as azines: in the absence of other functional groups having higher seniority, they can be named as substituted hydrazones. (Rigaudy *et. al* 1979)

Enormous research work has been carried out on different kinds of azines, both symmetrical and asymmetrical concerning their application in diverse fields (Sarojini etal: 2006, zyss etal 1994 and Nalwa *et al*, 1993).

Azines act as biologically and pharmacologically important molecules. (Bodtke *et al;* 2005.)
Introduction of nitro group in organic molecules, sometimes, enhances its biological profile mainly due to the electronegative nature of the nitro group. (Kumaraswamy *et al*, 2005, Mahadevan *et al*, 2002, Vaidya *et al* 2003) and 2004)

Research and development of potent and effective antimicrobial agents represent one of the most important advance in

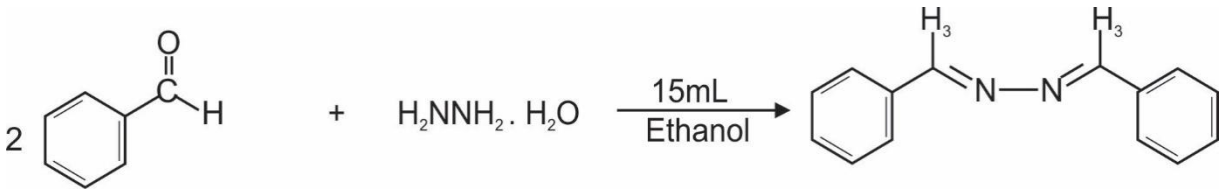
therapeutics: the main aim of these efforts is not only to control the serious infections, but also prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. (Serap Basoghe *et al*, 2013).

This research is thus aimed at synthesizing and testing the antimicrobial activity of azine and substituted 3,4,5 – trimethoxyl azine from phenyl acetone and 1, 4, 6 trimethoxy benzaldehyde respectively.

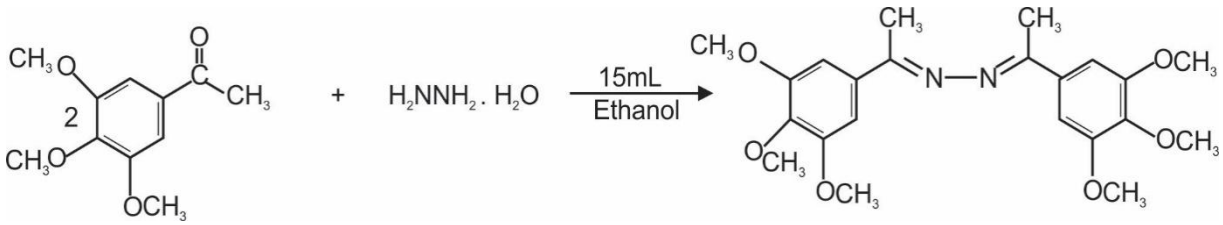
MATERIALS AND METHODS

General Experimental Procedure

All reagents and solvents were purchased from sigma-Aldrich chemical suppliers in Germany. Melting point were determined of a koffer hot stage apparatus and were uncorrected. IR spectra were recorded on a Buck. Scientific IR M500 instrument. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO at 400 MH_Z with HAZ. VOLATILES V_2: M. Chemical shift are reported in ppm relative to tetra-methyl-silane ($(CH_3)_4Si$ as a standard. Gas chromatography Mass spectra were obtained on a HAZ VOLATILE V2. M ($400MH_2$) and chemical shifts are reported in ppm relative to tetramethylsilane as reference standard. Elemental analysis agreed favourable with the calculated values. Analytical thin layer chromatography (TLC) was used to monitor the reactions .



SCHEME 1 **1**



SCHEME 2 **2**

Synthesis of Azine and substituted Azine

A mixture of Benzaldehyde (15ml, 1.02g)(compound 1), 3,4,5 Trimethoxy Acetophenone(compound 2) and hydrazine hydrate (5mL, 0.1mol) was heated and refluxed with stirring using magnetic stirrer in ethanol (15mL) for 20minuts (monitored by TLC). Ethanol was removed from the mixture under reduced pressure. The product on recrystalization from proper solvent gave yellow crystals. Compound 1

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Yield 11.5374g (91%); Compound 2 Yield 8.243g (70%);

Evaluation of Antimicrobial Activity

Agar well diffusion method was utilized for the antimicrobial activity. (Okeke M . E).Six species: *Staphylococcus aureus*(ATCC10145),*Bacillus species* (NCTC 8236), *Escherichia coli*(ATCC 25922),*Klebsiella pneumonia* (NCTC 10418), *Pseudomonas aeuriginosa,* (ATCC 10145))and *Candida albicans* (ATCC24433) stock cultures were used. The test organisms were obtained from the Pharmaceutical Microbiology Department of the University of Benin, Benin City, Nigeria. The test organisms were cultured overnight in nutrient broth, diluted to the turbidity of 0.5 McFarland standard. Broth culture (0.2 mL) were seeded on nutrient agar (for bacterial organisms)or Sabouraud dextrose agar (for the fungus) andallowed to dry. The various concentrations of the compounds (20 – 640 mg/mL) were introduced. The culture plates were incubated at 37^{o}C for 24h (for bacterial organisms) or at room temperature (28^{o}C) for 48 h (for the fungus). The results were taken by considering the zones of inhibition by the test compounds. Ciprofloxacin (20 mg/mL) was used as positive control while the vehicle (10% DMSO) was used as negative control. Activity and inactivity were observed in accordance with standard and accepted method. (Mackie .R.).

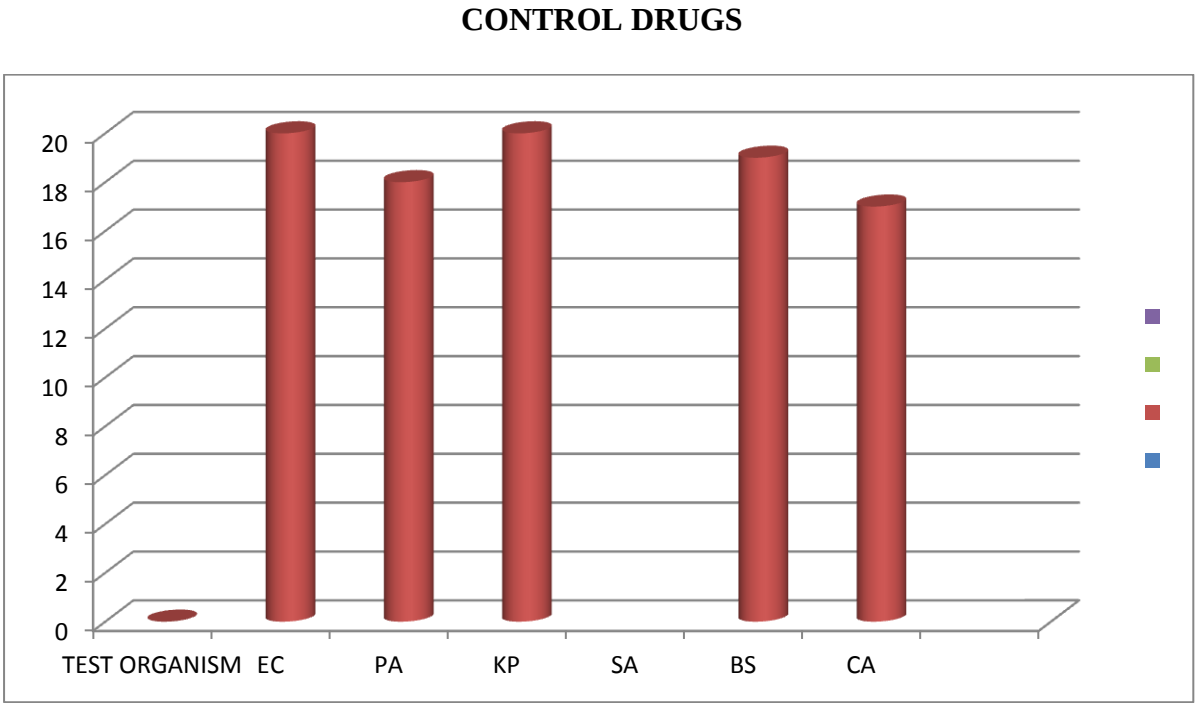

Figure 1: The effect of Control Drugs : CIPROFLOXOCIN(for bacterial), KETOKONAZOL(for fungus), toward studied bacteria. SA=Staphylococcus aureus, BS= Bacillus species, EC=Escherichia coli, KP=Klebsiella pneumonia, PA= Pseudomonas aereginosa and CA=Candida albicans
Significantly different from Ligand at P<0.05, Values are in mm.

COMPOUND

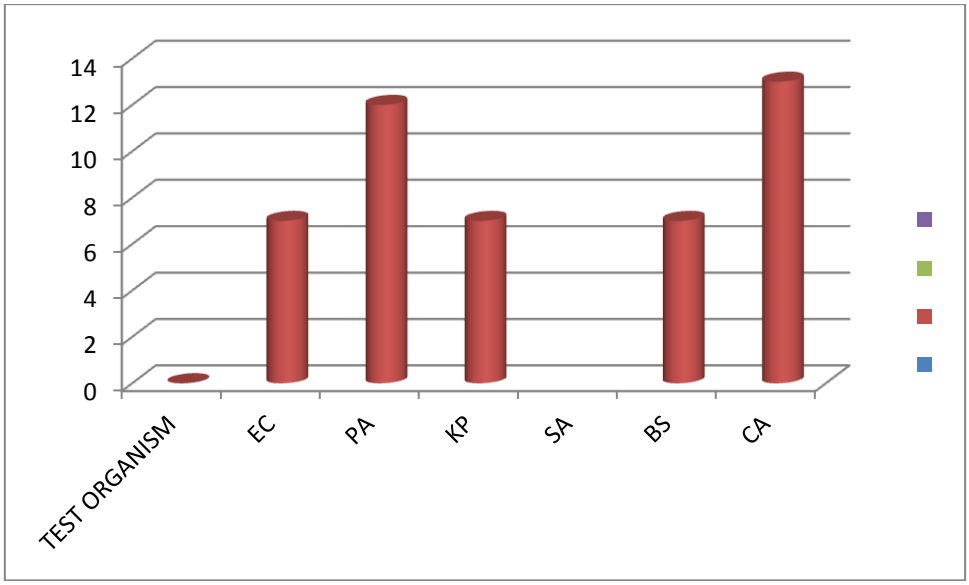

Figure 2. The effect of the compound 2 toward studied bacteria. SA=Staphylococcus aureus, BS= Bacillus species, EC=Escherichia coli, KP=Klebsiella pneumonia, PA= Pseudomonas aereginosa and CA=Candida albicans
Significantly different from Ligand at P<0.05, Values are in mm.

COMPOUND 2

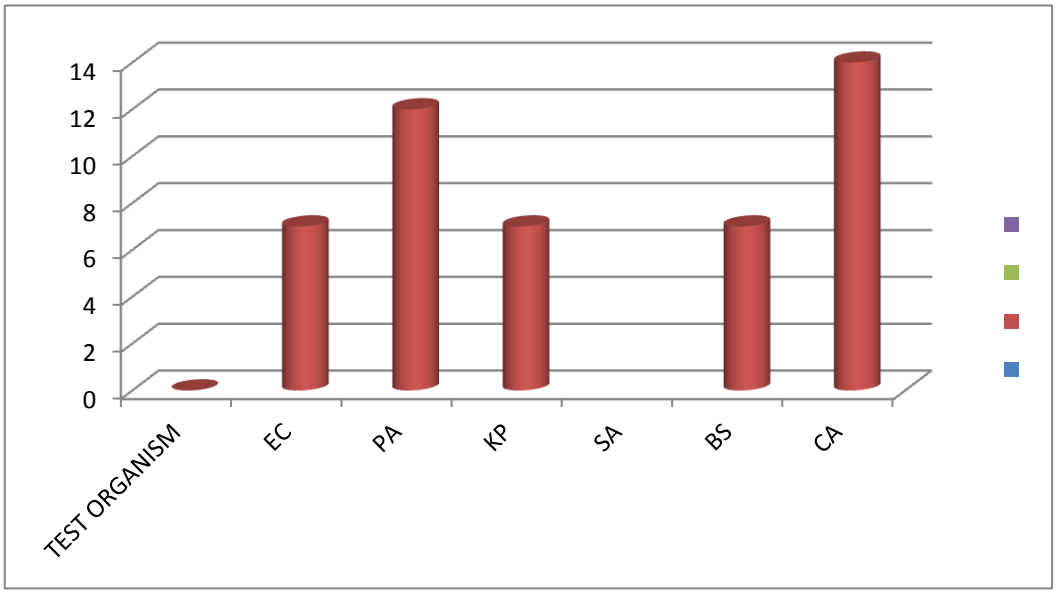

Figure 3. The effect of the compound 2 toward studied bacteria. SA=Staphylococcus aureus, BS= Bacillus species, EC=Escherichia coli, KP=Klebsiella pneumonia, PA= Pseudomonas aereginosa and CA=Candida albicans
Significantly different from Ligand at P<0.05, Values are in mm

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Table 3: Minimum inhibitory concentrations (MIC) in mg/mL of tested compounds against tested standard microorganisms

TEST ORGANISM	COMPOUND	
	1	2
Escherichia coli	7.00	7.00
Bascillus species	7.00	7.00
Staphylococcus aureus	-	-
Klebsiella pneumonia	6.00	6.00
Pseudomonas aureus	6.00	6.00
Candida albicans	7.00	8.00

Azine and 3,4,5-Trimethoxyl Azine

Compound 1: Yield 93% mp – 92-94^{0}C IR (RBr, cm^{-1}): 3067 (ArCH), 1607, 1626, 1549 ^{1}H NMR (400 MH_2, DMSO) ppm 4.72 (S,2H), 4.54 (S,3H), 7.71 (d,3H),7.71(d.3H), 7.82 (d,3H) ,8.41(d,2H). ^{13}C NMR (400 MH_z, DMSO) ppm 56.14, 110.54, 128.74, 139.85, 146.19. anal cal for $C_{16}H_{16}N_2$. C. 81.34, H. 6.78
Found: C. 81.40, H. 6.81

Compound 2; yield 70% mp 210-212^{0}C. IR (KBr, cm^{-1}): 3091 (ArCH), 1609, 1628, 1553, ^{1}H. NMR (400MH_2, DMSO) ppm 4.74 (S,2H) 4.53 ((S, 2H), 7.13 (d, 3H), 7.31 (d, 3H),7.41 (d, 3H), 7.71(d,3H),7.81 (d, 3H),7.84(S, 2H), 7.78 (d,3H), 8.17 (d,3H),8,32(d,3H). ^{13}CNMR (400 MN_2, DMSO) ppm 46.74, 12.19, Anal cal for $C_{20}H_{30}N_2$. C. 80. 5 4 H. 10. 5 0, Found. C. 80. 50, H. 10.09

RESULTS AND DISCUSSION

The present study reported the synthesis of two compounds, Azine (1) and substituted 3,4,5-trimethoxyl Azine (**2).** The azine and the substituted 3,4,5-trimethoxyl azine were synthesized by the condensation of benzaldehyde and 3,4,5-trimethoxyl Acetophenon with hydrazine hydrate.

The compounds were investigated for their antimicrobial activity. The compounds synthesized exhibited promising antimicrobial activity against *Pseudomonas aureus and Candida albicans*. The antimicrobial activity of the compounds showed that compound 1 had an inhibition zone of 12mm and 13mm for *Pseudomonas aereginosa* and *Candida albicans,* while compound 2 had an inhibition zone of 12mm and 14mm against *Pseudomonas aereginosa Candida albicans respectively.* This suggest that compound 2 (Azine from trimethoxyl Acetophenone) had a higher inhibitory action against *Candida*

. Table 3 Showed the MIC of both compounds against the susceptible organisms. Compound 1 had MIC between (6 and 7 mg/mL) then compound 2 between (6 and 8 mg/kg) against the various microorganisms (table 3). This indicated that compound 2 is slightly more active compared to compound 1.

The present work showed that compound 2 (Azine from trimethoxyl Acetophenone) had higher inhibitory activity against *Candida albicans* microbes than compound 1.

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