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Synthesis of Piperidine and *p*-Choloroaniline Mannich bases and Investigation of their Antioxidant and Antimicrobial properties

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Abstract: This research was aimed at synthesizing Mannich bases of piperidine and *p*-chloroaniline derivatives and investigating their antioxidant and antimicrobial activities since report has it that Mannich bases possessing electron withdrawing group show good anti-oxidant, antimicrobial, anti-cancer and anti-tumor properties. The synthesized compounds were characterized by Nuclear Magnetic Resonance (NMR), Infra red (IR) and Ultra/Violet-Visible (UV-V) spectroscopy. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and Agar Well diffusion methods were used for the antioxidant and antimicrobial screening respectively. Eight Mannich bases namely N,3-diphenyl-3-(piperidin-1-yl)propanamide **(**MB1), 3-((4-chlorophenyl)amino)-2-hydroxy-1,2,3-triphenylpropan-1-one (MB2), 2-((3-((4-chlorophenyl)amino)-3-phenylpropanoyl)oxy)benzoic acid (MB3), 3-(((4-chlorophenyl)amino)(phenyl)methyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (MB4), 3-((4-chlorophenyl)amino)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylpropan-1-one (MB5), 3-((4-chlorophenyl)amino)-N,3-diphenylpropanamide (MB6), benzoic 2-((4-chlorophenylamino)methyl)benzoic peroxyanhydride (MB7) and 3-(4-chlorophenylamino)-1,3-diphenylpropan-1-one (MB8) were synthesized. NMR confirmed the presence of the N-H aromatic stretch, in the range δ 4.00. IR also confirmed the presence of C=O, O-H and N-H typical of the Mannich bases. The Ultra/Violet-Visible absorption spectra for the synthesized compounds revealed that the compounds are aromatic. *In vitro* antioxidant screening of the compounds by DPPH free radical scavenging method showed that the compounds possessed significant antioxidant activity when compared with standards vitamin C, and butylatedhydroxylanisole (BHA). MB3 (73.56%, 71.17%), MB4 (78.83%, 75.25%), MB5 (73.06%, 70.58%), MB6 (74.55%, 72.47%) and MB8 (78.73%, 74.65%) showed significant inhibition at 1.0 mg/mL and 0.5 mg/mL respectively. MB1, MB2 and MB3 shows potent antimicrobial activity while MB4 – MB8 showed moderate activity, against bacterial and fungal strains when compared with the standards, Gentamicin and Tioconazole for bacteria and fungi respectively. This research work has therefore provided information about the spectroscopic properties, anti-oxidant and antimicrobial activities of new Mannich bases.
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Keywords: Mannich bases, piperidine, *p*-Choloroaniline, antimicrobial, antioxidant

1. Introduction

Organic compounds can be synthesized from smaller subunits that have functional groups. These groups are said to be reactive in comparison to ordinary carbon-carbon or carbon-hydrogen bonds (Cornforth., 1993; Roman et al., 2000). Mannich reaction is one of such reactions utilized in the synthesis of organic compounds. It consists of the condensation of ammonia or primary or secondary amine, with an aldehyde and a compound containing an active hydrogen atom. Mannich reaction has been employed with a wide variety of amine, aldehydes such as formaldehyde, benzaldehyde, acetaldehyde, phenylacetaldehyde and many others. Compounds that contain active hydrogen that have been employed include ketones, esters, aldehydes, ketones, acetylenes, phenols and certain other with the hydrogen atom of usual activity (Oloyede et al.2011, 2015; 2019; Abdullahi and Rajeswari, 2018). The study of Mannich reaction has attracted a great deal of attention to the chemists because of its wide application in pharmaceutical and chemical industry (Komal et al., 2018). Mannich base derivatives with bridge N-atom have been found to be potent drug in medicinal science and possesses wide range of biological activities like antimicrobial, antimalarial (Kotecka *et al.*, 1997; Altintas et al., 2005; Abdul Rahman et al., 2008), anticonvulsant (Aytenir and Calis, 2007; Sheela et al., 2005), analgesic, anti-inflamatory, anticancer (Shivarama, 2003; Bhupendra et al., 2015), and antioxidant (Muthumani et al., 2010; Chakkaravarthi et al., 2013; Oloyede et al., 2014a,b

and c). In 1997, Kotecka *et al*. reported the synthesis of chloroquine analogues, a quinolone-based di-Mannich bases and screened their activity against multi-drug resistant strains of *Plasmodium falciparum*. The anthracycline synthetic analogue 4,11-dihydroxynaphtho [2,3- *f*]indole-5,10-dione (an antitumor molecule) synthesized by Mannich reaction showed significant activity against multi-drug resistant tumor cell lines (Shchekotikhin *et al*., 2005; Chen *et al.*, 2011). Lóránd et al. (2004) studied the antibacterial properties of unsaturated Mannich ketones., The Mannich base derivative of isatin-4-amino-*N*carbamimidoyl benzenesulphonamide Schiff's base was found to be more active than the reference drug sulphaguanidine (Singh *et al.*, 2010; Vishant, 2014). Joshi *et al*. (2004) accomplished the synthesis of non-toxic aminoalkyl substituted isonicotinyl hydrazide by Mannich reaction. The Mannich products were found to be more active against several Gram-positive and Gram-negative bacteria. Mannich bases have also found application in the petrochemical industries, as an additive composition for hydrocarbon fuel and as intermediate in chemical synthesis. In the plastic industry, amine compounds such as aminoamide, aminoimidazoline or polyamine are useful as hardeners for epoxy resins by reacting them with a Mannich base. Tannin Mannich adducts are useful for corrosion resistant primering of metals in metallurgy (Lindert *et al*., 1990). The aim of this research work is to synthesize Mannich bases of piperidine and *p*-choloroaniline derivatives, elucidate their structures by using spectroscopy methods such as NMR, IR and UV/Visible. The antioxidant screening was assayed using 2,2-Diphenyl-1- picryl hydrazyl radical (DPPH) method while the antimicrobial effect on multi- resistance microbes was determined by Agar well diffusion method.

2. Material and Methods

Materials: Chemicals and Reagents/ Apparatus

Major chemicals and reagents used include; piperidine, p-chloroaniline, acetophenone, vanillin, benzoin, benzoyl peroxide, naringenin, methanol, ethanol, hexane, ethylacetate, concentrated sulfuric acid, acetanilide, benzaldehyde, diethylamine, chloroform, dimethyl sulfoxide (DMSO), acetone, formaldehyde, trioxonitrate (V) acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, vitamin C and butylated hydroxyl anisole (BHA). Mettler weighing balance (OAUS), Gallenkamp melting point apparatus (0.3 amps, 220/40 volts), AVANCE AV-400 spectrometer, Perkin Elmer Infrared spectrophotometer (4000-350cm^{-1}), Spectro UV-Visible double beam Pc scanning spectrophotometer (UVD- 2960).

Degree of purity of the synthesized compounds were assessed by determination of melting point using Gallemkamp Melting point Apparatus Model MFB 595 and also analytical Thin Layer Chromatography (TLC). TLC was carried out using Silica Gel F_{254}, hexane, ethyl acetate 3:1 as the mobile phase. ID Nuclear Magnetic Resonance (NMR), was recorded on AVANCE AV-400 spectrometer operating at 400 MHz for ^{1}H. The chemical shift values (δ) are reported in ppm. The Infrared spectra of the synthesized compounds were recorded as KBr discs on Perkin-Elmer FT-IR Spectrophotometer in the range 4000-400 cm-1. Vmax (cm-1) from IR data confirmed the structures. The UV/Visible Spectra of 0.01% w/v of the samples were determined and the samples were scanned between 190 nm and 1100 nm. Data from chart/recorder gave a graph of Absorbance against wavelength (nm).

Test Organisms: *Escherichia coli*, *Staphylococcus aereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellae pneumoniae, Salmonellae typhi, Candida albicans, Rhizopus stolonifer, Aspergillus niger* and *Penicillium notatum* (Micro organisms were collected from the stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy of University of Ibadan). The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4^{o}C.

Reference Standards: Gentamicin (5 mg/ml) for bacteria and Tioconazole (70%) for fungi both for antimicrobial activity; vitamin C and Butylated hydroxyanisole (BHA) for antioxidant activity.

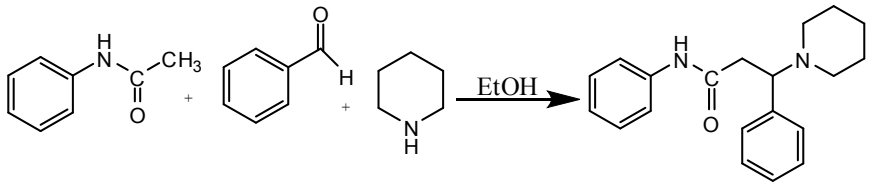
Methods

The preparation of Mannich bases were carried out based on the procedure used for the synthesis of Mannich bases previously described in literature. The lead compounds are medicinally active and non-toxic (Oloyede et al 2014a,b, 2015 and 2019).

Preparation of N,3-diphenyl-3-(piperidin-1-yl)propanamide (MB1)

Acetanilide (0.02 mol) was dissolved in 4 mL of 35% benzaldehyde in ethanol. To this was added 0.02 mol of piperidine gradually with stirring. The mixture was stirred continuously for 30 minutes at room temperature and the crystals formed were filtered under pressure using a suction pump, the crystals were re-crystallized with warm ethanol. The purity of the compound was checked by TLC.

The equation for the reaction is as follows:

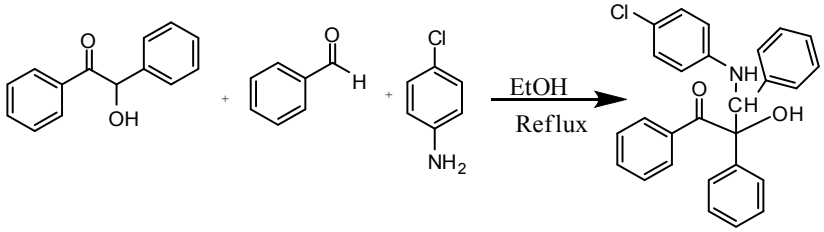


Scheme 1.1: Preparation of N,3-diphenyl-3-(piperidin-1-yl)propanamide **(**MB1)

Preparation of 3-((4-chlorophenyl)amino)-2-hydroxy-1,2,3-triphenylpropan-1-one (MB2)

A mixture of 0.005 mol of benzoin, 0.005 mol of benzaldehyde and 0.005 mol of 4-chloroaniline was refluxed for 8 hours and then cooled at room temperature for 72 hours. The reaction was monitored using TLC. The solid crystals that precipitated were obtained by filtering under pressure and re-crystallized with warm ethanol.

The equation for the reaction is as follows:

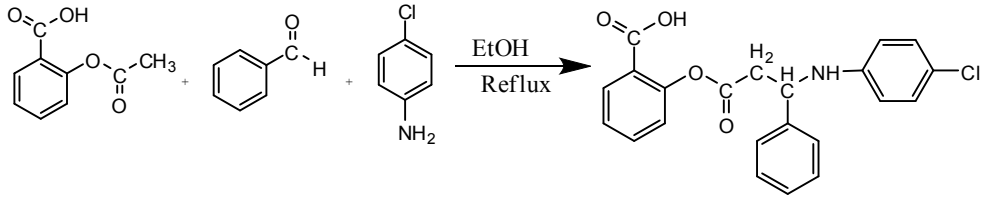


Scheme 1.2: Preparation of 3-((4-chlorophenyl)amino)-2-hydroxy-1,2,3-triphenylpropan-1-one (MB2)

Preparation of 2-((3-((4-chlorophenyl)amino)-3-phenylpropanoyl)oxy)benzoic acid (MB3)

2-acetoxybenzoic acid (aspirin) (0.002 mol) was mixed with 0.002 mol of benzaldehyde and 0.002 mol of 4-chloroaniline in absolute ethanol. The resultant mixture was refluxed for 6 hours and then cooled at room temperature for 72 hours. The precipitated solid were obtained by filtering under pressure and re-crystallized with warm ethanol.

The equation for the reaction is as follows:

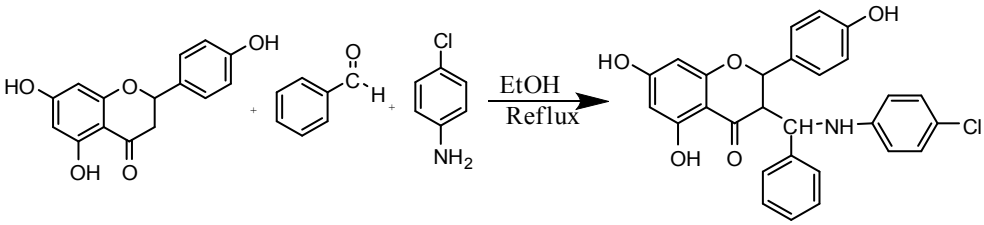


Scheme 1.3: Preparation of 2-((3-((4-chlorophenyl)amino)-3-phenylpropanoyl)oxy)benzoic acid (MB3)

Preparation of 3-(((4-chlorophenyl)amino) (phenyl)methyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (MB4)

Naringenin (0.002 mol) was mixed with 0.002 mol of benzaldehyde and 0.002 mol of 4-chloroaniline in absolute ethanol. The mixture was refluxed for 6 hours and thereafter cooled at room temperature for 72 hours. The precipitated solid were obtained by filtering under pressure and re-crystallized with warm ethanol.

The equation for the reaction is as follows:

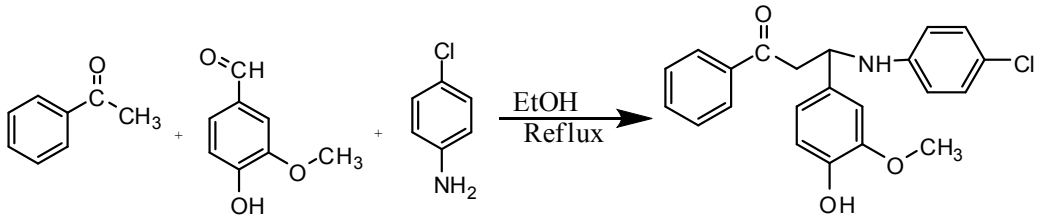


Scheme 1.4: Preparation of 3-(((4-chlorophenyl)amino) (phenyl)methyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (MB4)

Preparation of 3-((4-chlorophenyl)amino)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylpropan-1-one (MB5)

Acetophenone (0.005 mol) was mixed with 0.005 mol of vanillin and 0.005 mol of 4-chloroaniline in absolute ethanol. The resultant mixture was refluxed for 3 hours and the mixture obtained was cooled and left at room temperature overnight. The solid which separated–out was filtered, dried and recrystallized from ethanol.

The equation for the reaction is as follows:

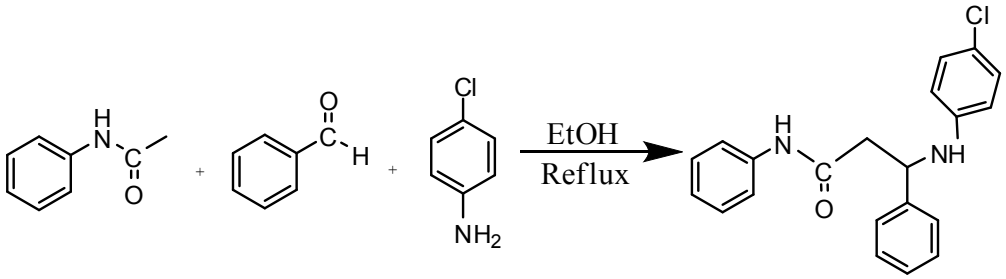


Scheme 1.5: Preparation of 3-((4-chlorophenyl)amino)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylpropan-1-one (MB5)

Preparation of 3-((4-chlorophenyl)amino)-N,3-diphenylpropanamide (MB6)

Acetanilide (0.002 mol) was dissolved in 4 mL of 35% benzaldehyde in ethanol. To this was added 0.002 mol of 4-chloroaniline. The mixture was refluxed for 7 hours and later cooled at room temperature for 72 hours. The solid crystals were obtained by filtering under pressure and re-crystallized with warm ethanol.

The equation for the reaction is as follows:

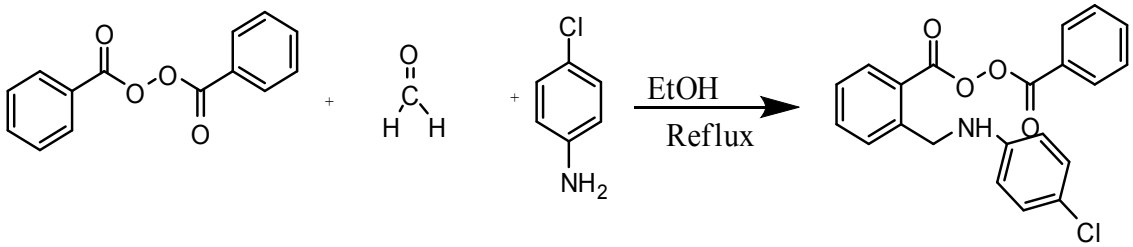


Scheme 1.6: Preparation of 3-((4-chlorophenyl)amino)-N,3-diphenylpropanamide (MB6)

Preparation of benzoic 2-((4-chlorophenylamino)methyl)benzoic peroxyanhydride (MB7)

A mixture of 0.002 mol of benzoylperoxide, 0.002 mol of formaldehyde and 0.002 mol of 4-chloroaniline was refluxed for 6 and half hours. The mixture obtained was cooled at room temperature for 72 hours. The solid crystals were obtained by filtering under pressure and re-crystallized with warm ethanol.

The equation for the reaction is as follows:

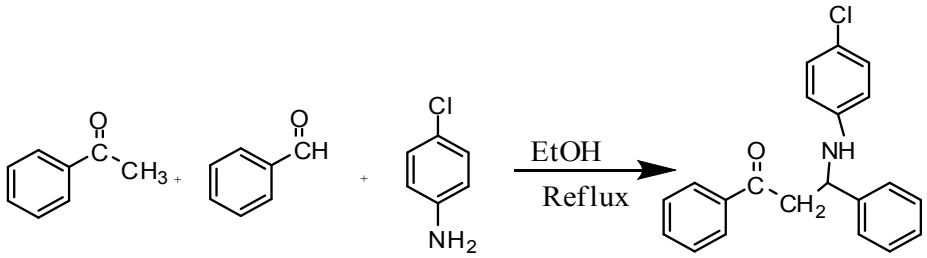


Scheme 1.7: Preparation of benzoic 2-((4-chlorophenylamino)methyl)benzoic peroxyanhydride (MB7)

Preparation of 3-(4-chlorophenylamino)-1,3-diphenylpropan-1-one (MB8)

A mixture of 0.002 mol of acetophenone, 0.002 mol of benzaldehyde and 0.002 mol of 4-chloroaniline were reacted together in absolute ethanol. The resultant mixture was refluxed for 7 hours and then cooled at room temperature for 72 hours. The solid crystals were obtained by filtering under pressure and re-crystallized with warm ethanol

The equation for the reaction is as follows:



Scheme 1.8: Preparation of 3-(4-chlorophenylamino)-1,3-diphenylpropan-1-one (MB8)

Analysis of the synthesized compounds

Spectroscopic analysis of the synthesized compound was carried out to ascertain the functional groups and structure using NMR, Infrared and UV/Visible Spectrophotometry. The antioxidant and antimicrobial activities of the synthesized compounds were also determined.

Antioxidant Analysis of the Synthesized Compounds

Free radical scavenging effect on 2, 2 – diphenyl-1-picrylhydrazyl (DPPH) radical

A 100 µM solution was prepared by dissolving 3.94 mg of DPPH in 100 mL methanol. 2.0 mg each of the synthesized compounds were separately dissolved in 2 mL methanol to prepare the stock solution (1.0 mg/mL) and to 3.0 ml of the methanol solution of DPPH was added 0.5 mL of the dissolved samples, shaken and left to incubate for 30 minutes at room temperature. DPPH absorption at 517 nm was measured using the UV/Visible spectrophotometer. Reduction in absorbance values induced by the samples was calculated by subtracting from control value. Other concentrations (0.500, 0.250, 0.125 mg/mL) were prepared from the stock solution by serial dilution and analyzed the same way. An average of triplicate analysis was reported. Vitamin C and Butylated hydroxyl anisole (BHA) were used as positive control while a negative control (blank) contained methanol and DPPH radical solution (Oloyede et al., 2019). Percentage (%) inhibition was calculated from absorbance values.

Antimicrobial Screening of the Samples

Preparation of samples for Antimicrobial Analysis

Sample (0.2 g) was weighed and dissolved into 2 mL of methanol to give 100 mg/mL. Four other test tubes containing 1mL of methanol were prepared. From the first test tube containing 100 mg/mL, 1mL of the content was drawn and added to the second test tube to give 50 mg/mL of the content and this was done serially to the fifth test tube which gave a concentration of 6.25mg/mL. The sixth test tube was used to prepare the negative control which contained the solvent of dissolution while the seventh test tube served as positive control which contained gentamicin (5 mg/ml) for bacteria and 70% tioconazole for fungi.

Agar Diffusion: Pour plate method for bacteria

The culture of each of the bacteria strains used: *Bacillus subtilis, Escherichia coli, Klebsiellae pneumonae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonellae typhi* was prepared overnight. Preparation of 10 mL of 1:100 (10^2) dilution was done by taking 0.1mLof each of the organisms into 9.9 mL of sterile distilled water. Then 0.2 mL was taken into prepared molten Nutrient Agar at 45^0C and was poured aseptically into the sterile plates and allowed to set on the bench for 45 minutes. The stock was maintained on nutrient agar slant and sub- cultured in nutrient broth for incubation at 37 °C prior to each antimicrobial testing. Using a sterile cork borer of 8 mm diameter, the wells were made according to the number of graded concentration of the sample including the controls. Different graded concentrations of the sample were introduced in each well in duplicates. The plates were allowed to stay on the bench for 2hrs to allow pre-diffusion. The plates were incubated uprightly in the incubator for 24 hrs at 37^0C (Oloyede et al., 2014a, b and c).

Surface Plate Method for Fungi

The fungal strains used were *Aspergillus niger, Candida albicans, Penicillium notatum* and *Rhizopus Stolonifer*. Molten sterile Sabouraud Dextrose Agar (62 g/L) was prepared accordingly and aseptically poured into the sterile plates in duplicates and allowed to set. 0.2 mL of the 10:2 dilution of the agar was used to cover all the surface of the agar using a sterile spreader. The wells were made using a sterile cork borer of 8 mm diameter. In each well, the graded concentrations of the sample were introduced into the wells including the controls. The plates were left on the bench for 120 minutes to allow proper pre-diffusion. The plates were incubated uprightly in the incubator for 48hrs at 28^0C.

The bacteria and fungi plates were observed after 24hrs and 48hrs of incubation respectively. Clear zones of inhibition were observed and the diameters of zones of inhibition were measured in millimeter (Oloyede et al., 2014a, b and c).

Statistical analysis

Data (Absorbance measurements) are expressed as mean absorbance ± SD of triplicate analysis. Values of $p<0.05$ were taken to be statistically significant.

3. Results and Discussions

N,3-diphenyl-3-(piperidin-1-yl)propanamide (MB1)- Light brown; yield: 67% (on dry weight basis); m.pt: 79.3 - 79.7^{0}C; Soluble in methanol, ethanol, chloroform, acetone, ethylacetate. Rf 0.58 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 381.00 (0.066), 333.00 (0.42), IR (KBr) Vmax cm-1: 3404 (N-H stretch, 2^0 amine), 3060 (=C-H aromatic stretch), 2928 (C-H aliphatic stretch), 1670 (C=O stretch), 1599 (N-H bending), 1448 (C=C aromatic), 1259 (C-N stretch of amine), 754, 710 (=C-H (aromatic, out of plane bending). ^{1}H NMR (400 MHz; CD_3OD): δ 10.08 (NH -secondary amide), 7.20-7.65 (10x CH -benzene), 4.10 (CH -methine), 2.40 (2xCH_2- piperidine), 1.45-1.60 (3xCH_2 - pipeidine), 2.70 (CH_2 -methylene)

3-((4-chlorophenyl)amino)-2-hydroxy-1,2,3-triphenylpropan-1-one (MB2)- White; yield: 89% (on dry weight basis); m.pt: 123.8 - 124.2 ^{0}C; Soluble in methanol, ethanol, chloroform, acetone, ethylacetate. R_f 0.47 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 381.00 (0.035), 333.00 (0.386), 240.00 (0.013), 226.00 (0.019). IR (KBr) Vmax cm-1: 3760 (N-H stretch, 2^0 amine), 3380 (O-H stretch), 3060 (=C-H aromatic stretch), 2933 (C-H aliphatic stretch), 1681 (C=O stretch), 1577 (N-H bending), 1449 (C=C Aromatic), 1262 (C-N stretch of amine), 855 (=C-H (aromatic, out of plane bending), 698 (C-Cl). 1H NMR (400 MHz; CD_3OD): δ 6.98 (OH, alcohol), 4.09 (NH -aromatic), 6.50-7.65 (19x CH -benzene), 4.97 (CH -methine).

2-((3-((4-chlorophenyl)amino)-3-phenylpropanoyl)oxy)benzoic acid (MB3)- Reddish brown; yield: 66% (on dry weight basis); m.pt: 84.8 - 85.1^{0}C; Soluble in methanol, ethanol, chloroform, acetone, partially soluble in ethylacetate. R_f 0.45 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 381.00 (0.107), 333.00 (0.495). IR (KBr) Vmax cm-1: 3881 (N-H stretch, 2^0 amine), 2851 (O-H stretch), 3051 (=C-H aromatic stretch), 1682 (C=O stretch), 1562 (N-H bending), 1487 (C=C aromatic), 1305 (C-N stretch of amine), 808,757 (=C-H (aromatic,out of plane bending), 691 (C-Cl). ^{1}H NMR (400 MHz; CD_3OD): δ 12.08 (OH, carboxylic acid), 4.01 (NH -aromatic), 6.50-8.15 (14x CH -benzene), 2.80 (CH_2 -methylene).

3-(((4-chlorophenyl)amino) (phenyl)methyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (MB4)- Yellow; yield: 75% (on dry weight basis); m.pt: 135.2- 136.1^{0}C; soluble in methanol, ethanol, chloroform, acetone, ethylacetate. R_f 0.38 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 365.00 (0.009), 306.00 (0.038), 295.00 (0.045), 276.00 (0.052), 254.00 (0.059). IR (KBr) Vmax cm-1: 3786 (N-H stretch, 2^0 amine), 3051 (O-H stretch), 2604 (=C-H aromatic stretch), 1620 (C=O stretch), 1566 (N-H bending), 1496 (C=C aromatic), 1309 (C-N stretch of amine), 823,611 (=C-H (aromatic, out of plane bending), 637 (C-Cl). ^{1}H NMR (400 MHz; CD_3OD): δ 9.40-11.89 (3xOH, aromatic), 4.01 (NH -aromatic), 5.89-7.41 (15x CH -benzene), 4.45-5.52 (3xCH -methine).

3-((4-chlorophenyl)amino)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylpropan-1-one (MB5)- Reddish; yield: 77% (on dry weight basis); m.pt: 180.3 - 186.5^{0}C; soluble in methanol, ethanol, chloroform, acetone, ethylacetate. R_f 0.14 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 679.00 (0.347), 615.00 (0.338), 526.00 (0.339), 503.00 (0.429), 306.00 (0.058), 296.00 (0.091), 249.00 (0.117). IR (KBr) Vmax cm-1: 3898 (N-H stretch, 2^0 amine), 3198 (O-H stretch), 3057 (=C-H aromatic stretch), 2950 (C-H aliphatic stretch), 1612 (C=O stretch), 1517 (N-H bending), 1492 (C=C aromatic), 1257 (C-N stretch of amine), 832,610 (=C-H (aromatic, out of plane bending), 637 (C-Cl). ^{1}H NMR (400 MHz; CD_3OD): δ 9.80 (OH, aromatic), 4.01 (NH -aromatic), 6.50-7.99 (12x CH -benzene), 4.45 (CH -methine), 3.80 (CH_3-methyl), 3.23 (CH_2-methylene).

3-((4-chlorophenyl)amino)-N,3-diphenylpropanamide (MB6)- Greyish; yield: 52% (on dry weight basis); m.pt: 145.2 - 147.3^{0}C; Soluble in methanol, ethanol, acetone. R_f 0.53 (Silica gel F254, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 381.00 (0.060), 333.00 (0.313). IR (KBr) Vmax cm-1: 3406 (N-H stretch, 2^0 amine), 2993 (=C-H aromatic stretch), 2605 (C-H aliphatic stretch), 1620 (C=O stretch), 1542 (N-H bending), 1495 (C=C aromatic), 1289 (C-N stretch of amine), 821,747 (=C-H (aromatic, out of plane bending), 697 (C-Cl). ^{1}H NMR (400 MHz; CD_3OD): δ 10.04 (NH, stretch 2^0 amide), 4.01 (NH -aromatic), 6.50-7.61 (14x CH -benzene), 4.15 (CH -methine), 2.83 (CH_2-methylene).

Benzoic 2-((4-chlorophenylamino)methyl)benzoic peroxyanhydride (MB7)- White; yield: 63% (on dry weight basis); m.pt: 121.3-121.5^{0}C; Soluble in methanol, ethanol, chloroform, acetone, ethylacetate. R_f 0.71 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 381.00 (0.072), 333.00 (0.902), 244.00 (0.014). IR (KBr) Vmax cm-1: 3721 (N-H stretch, 2^0 amine), 3064 (=C-H aromatic stretch), 1757 (C=O stretch), 1597 (N-H bending), 1450 (C=C Aromatic), 1318 (C-N stretch of amine), 1178-1072

(C-O anhydride), 847,793 (=C-H (aromatic, out of plane bending), 699 (C-Cl). ^{1}H NMR (400 MHz; CD_3OD): δ 6.69 (NH, stretch, aromatic), 6.50-8.25 (13x CH -benzene), 4.39 (CH_2-methylene).

3-(4-chlorophenylamino)-1,3-diphenylpropan-1-one (MB8)- Light yellow; yield: 54% (on dry weight basis); m.pt: 144.0-144.4^{0}C; Soluble in methanol, ethanol, chloroform, acetone, ethylacetate. R_f 0.69 (Silica gel F_{254}, Hexane:Ethylacetate 3:1). UV nm (MeOH, λ_{max}nm): 381.00 (0.031), 333.00 (0.269). IR (KBr) Vmax cm-1: 3894 (N-H stretch, 2^0 amine), 2924 (=C-H aromatic stretch), 2604 (C-H aliphatic stretch), 1620 (C=O stretch), 1542 (N-H bending), 1495 (C=C Aromatic), 1286 (C-N stretch of amine), 820 (=C-H aromatic, out of plane bending), 633 (C-Cl). ^{1}H NMR (400 MHz; CD_3OD): δ 4.03 (NH, stretch, aromatic), 6.50-7.95 (14x CH -benzene), 4.48 (CH-methine) 3.30 (CH_2-methylene).

The purity of the synthesized compounds was confirmed by single spot in Thin Layer Chromatography (Silica gel F_{254}, and Hexane: Ethylacetate 3: 1) and sharp melting point. MB1 and MB3 had melting point below 100^{0}C, while others show melting point above 100^{0}C. The percentage yield (on dry weight basis) of MB2, MB4, MB5 was moderately high while others show reasonable yield. All the compounds were completely soluble in methanol, ethanol, chloroform and acetone. The compounds show absorption peak for MB1-MB8 at 3404, 3760, 3881, 3786, 3898, 3406, 3721, 3884 cm^{-1} respectively which was assigned to secondary N-H stretch frequency. V_{max} at 1670, 1681, 1682, 1620, 1612, 1620, 1757, 1620 cm^{-1} for MB1 - MB8 respectively indicated the presence of C=O stretching frequency and also 1448, 1449, 1487, 1496, 1492, 1495, 1450, 1495 cm^{-1} is indicative of C=C aromatic stretching frequency. The N-H bending for the synthesized compounds was observed around 1599-1542 cm^{-1}. The presence of absorption peaks at 3380, 2851, 2875, 3196 cm^{-1} for MB2, MB3, MB4 and MB5 is indicative of O-H stretching frequency. The presence of absorption band at 698, 691, 637, 637, 697, 699, 633cm^{-1} for MB2, MB3, MB4, MB5, MB6, MB7 and MB8 respectively shows the presence of C-Cl bending vibration. The Ultra-Violet/Visible absorption spectra for the synthesized compound shows that the synthesized compounds are highly aromatic with a chromophore at wavelength band around 381 nm and 333 nm for MB1, MB2, MB3, MB6, MB7 and MB8 while MB4 and MB5 shows absorption at wavelength 306 nm, 254 nm, 306 nm and 249nm respectively. The n- π* is due to the presence of n-electrons which are non-bonding electrons, such as those of the lone pair on oxygen and nitrogen which are present in the synthesized compounds. The π- π* transition is due to the presence of double bonds and conjugation. The proton NMR of the compounds gave characteristic NH -secondary amide at δ 10.08, 6.98 for OH (alcohol) and 4.09 for aromatic NH for MB1. Signal in MB2 at δ 6.98 is assigned to OH (alcohol) and 4.09 to aromatic NH. Signal in MB3 at δ 12.08 is assigned to the OH of carboxylic acid and 4.01 to aromatic NH. δ 9.40-11.89 represent signals for the three aromatic OH while 4.01 is due to aromatic NH in MB4. MB5 showed signal at δ 9.80 and 4.01 for aromatic OH and NH respectively. δ 10.04 is due to the NH stretch of the 2^0 amide and 4.01 for aromatic NH in MB6. MB7 and MB8 showed peaks at δ 6.69 and δ 4.03 for aromatic NH stretch respectively.

Antioxidant Analysis of the Samples (Free Radical Scavenging Effect on DPPH)

The reduction in absorbance of 2,2-diphenyl-1-picrylhydrazine radical, a stable free radical at 517 nm caused by reactive substances indicates that some of the synthesized compounds showed good activities as free radical scavengers when compared with vitamin C and Butylated hydroxylanisole (BHA) which are standard antioxidants. 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It can be deduced (Table 1) that MB3 (73.56%, 71.17%), MB4 (78.83%, 75.25%), MB5 (73.06%, 70.58%), MB6 (74.55%, 72.47%) and MB8 (78.73%, 74.65%) shows significant antioxidant activity at 1.0 mg/mL and 0.5 mg/mL respectively.

These results also confirmed the report of antioxidant activities of some synthesized Mannich bases of 4- Piperidones, 1-Phenyl-3-(phenylamino) propan-1-one and Phenyl (2-[phenyl amino) methyl] phenyl) peroxyanhydride (Ali and Sharharyar, 2007; Shivananda and Shet Prakash, 2011; Oloyede and Omisakin, 2014).

Antimicrobial Analysis

Tables 2-9 show the result of the antimicrobial analysis of the synthesized compounds. MB1, MB2 and MB3 showed potent antimicrobial activity while MB4 - MB8 shows moderate activity. MB 5 shows no activity against the fungi strains. MB 2 was the most active against both the bacteria and fungi strains compared to others. Little or no activity was observed at 12.5 mg/mL and 6.5 mg/mL for MB4, MB5, MB7 and MB8. MB3 shows no activity against the fungi strain of *Rhizopus stolonifer* while MB 4 shows no activity against the fungi strain of *Rhizopus stolonifer* and *Penicillium notatum.*

Table 1: Percentage (%) Inhibition obtained in the Antioxidant Screening*

Concentration	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL
Vitamin C	96.02	95.63	95.23	94.63
BHA	95.43	95.23	94.83	94.23
MB 1	17.14	15.37	14.59	13.23
MB 2	18.89	18.00	15.12	12.82
MB 3	73.56	71.17	68.69	66.80
MB 4	78.83	75.25	67.50	64.41
MB 5	73.06	70.58	67.00	60.14
MB 6	74.55	72.47	69.89	67.01
MB 7	32.40	27.23	24.53	18.43
MB 8	78.73	74.65	71.12	69.88

*Percentage inhibition calculated from Absorbance values

Table 2: Antimicrobial screening of MB1*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	24	24	20	17	24	20	20	18	18	16
50	22	22	19	16	23	19	18	16	16	14
25	20	18	18	15	20	17	15	14	12	12
12.5	18	16	15	13	19	14	14	12	10	-
6.25	14	14	12	12	16	10	10	10	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 3: Antimicrobial screening of MB2*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	28	26	24	18	26	22	20	18	16	18
50	24	22	19	17	23	19	18	14	14	14
25	20	18	16	15	19	16	16	12	12	12
12.5	18	15	13	12	17	13	14	10	10	10
6.25	14	12	11	10	13	10	10	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 4: Antimicrobial screening of MB3*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	22	20	18	16	18	16	16	16	14	-
50	18	16	16	14	14	14	14	14	12	-
25	14	14	14	12	12	12	12	12	10	-
12.5	12	10	12	10	10	10	10	10	-	-
6.25	10	10	10	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 5: Antimicrobial screening of MB4*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	18	18	24	16	14	14	14	14	-	-
50	16	16	18	14	12	12	12	10	-	-
25	14	14	16	12	16	16	10	-	-	-
12.5	12	12	14	16	-	-	-	-	-	-
6.25	10	10	10	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 6: Antimicrobial screening of MB5*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	14	20	16	20	14	12	-	-	-	-
50	12	18	12	18	12	10	-	-	-	-
25	10	16	10	16	10	-	-	-	-	-
12.5	-	14	-	10	-	-	-	-	-	-
6.25	-	10	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 7: Antimicrobial screening of MB6*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	18	16	24	20	18	16	18	18	14	16
50	16	14	20	18	16	14	15	16	12	14
25	14	12	18	16	12	12	13	14	10	12
12.5	12	10	16	14	10	10	10	12	-	10
6.25	10	-	14	-	-	-	-	10	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 8: Antimicrobial screening of MB7*

	Zones of inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	16	16	14	16	18	16	12	14	12	14
50	14	14	12	14	14	14	10	10	10	12
25	10	10	10	10	12	10	-	-	-	10
12.5	-	10	-	-	10	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

Table 9: Antimicrobial screening of MB8*

	Zones of Inhibition (mm)									
Conc(mg/mL)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Sal*	*Kleb*	*C.a*	*A.n*	*Pen*	*Rhi*
100	20	22	18	16	18	16	16	16	14	18
50	18	20	16	14	14	14	14	14	12	16
25	14	18	14	12	12	12	12	12	10	14
12.5	12	16	12	10	10	10	10	10	-	12
6.25	10	14	10	-	-	-	-	-	-	10
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	40	38	40	38	40	28	28	28	30

* -ve= negative control (DMSO), +ve = positive control {Gentamicin at 5 mg/mL for bacteria or Tioconazole (70%) for fungi}, "–'' = no inhibition, *S.a= Staphylococcus aureus, E. c= Escherichia coli, B. Su= Bacillus subtilis, Ps.a= Pseudomonas aeruginosa, Sal= Salmonellae typhi, Kleb= Klebsiellae pneumoniae, C.a= Candida albicans, A.n= Aspergillus niger, Pen= Penicillium notatum, Rhi – Rhizopus stolonifer*.

The result of the antimicrobial screening of the eight synthesized Mannich bases showed that MB1 to MB3 possessed significant antimicrobial activity when compared with the standards Gentamicin and Tioconazole. MB2 with zone of inhibition 28 mm at 100 mg/mL was the most active. Other synthesized Mannich bases have also been observed to have antimicrobial, anticonvulsant, filaricidal and antihelminthic activities (Ali and Sharharyar, 2007; Muthumani et al., 2010; Saraswathi et al, 2010; Oloyede et al, 20014a, b and c, 2011; Priya, et al., 2013).

Conclusion

Eight Mannich bases namely N,3-diphenyl-3-(piperidin-1-yl)propanamide **(**MB1), 3-((4-chlorophenyl)amino)-2-hydroxy-1,2,3-triphenylpropan-1-one (MB2), 2-((3-((4-chlorophenyl)amino)-3-phenylpropanoyl)oxy)benzoic acid (MB3), 3-(((4-chlorophenyl)amino)(phenyl)methyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (MB4), 3-((4-chlorophenyl)amino)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylpropan-1-one (MB5), 3-((4-chlorophenyl)amino)-N,3-diphenylpropanamide (MB6), benzoic 2-((4-chlorophenylamino)methyl)benzoic peroxyanhydride (MB7) and 3-(4-chlorophenylamino)-1,3-diphenylpropan-1-one (MB8) were synthesized by reacting piperidine or *p*-choroaniline and benzaldehyde with different lead compound (compound with active hydrogen). Structure and functional groups C=O, N-H, C-H and C-Cl were confirmed by UV/Visible, IR and NMR spectroscopy. Significant antioxidant activity was observed for MB3, MB4, MB5, MB6 and MB8 when activity was compared to vitamin C and butylatedhydroxylanisole (BHA). The result obtained from antimicrobial analysis shows that MB1, MB2 and MB3 has potent antimicrobial activity. Therefore, this present study has supported evidence on the prospect of Mannich bases as lead compounds for drugs used as antioxidant and antimicrobial agents.

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References

1. Abdullahi, M R., Rajeswari, S. Synthesis, characterization and biological activity of new Mannich base 1-((4-chlorophenyl) (2,5-dioxopyrrolidin-1-YL) methyl) urea. Academic Journals, 2018; 11: 112-117.
2. AbdulRahman M., Balakrishina A, Kalluraya M. Synthesis, Characterization, Antimicrobial and Anthelmintic Activity of Some Sydnone-N-Mannich Bases. Journal Indian Council of Chemistry 2008; 25(1): 10-14.
3. Ali MA, Sharharyar M. Oxadiazole Mannich bases. Synthesis and antimycobacterial activity. *Bioorganic and medicinal chemistry letters* 2007; 17: 3314-3316.
4. Altintas H., Ates O., Birteksoz S., Otuk G., Uzun M, Santana D. Synthesis of Mannich Bases of some 2, 5-Disubtituted 4-thiazolidinones and Evaluation of their Antimicrobial Activities. Turkish. Journal of Chemistry. 2005; 29: 425-435.
5. Aytenir MD, Calis U. Synthesis of some Novel Mannich bases derived from Allomaltol and Evaluation of their Anticounvulsant activities. Hacettepe University Journal of the faculty of Pharmacy. 2007; 27: 1-10.
6. Bhupendra M, Rahul, V, Young-Soo, K, Kim, D. Synthesis of N-Mannich bases of berberine linking piperazine moieties revealing anticancer and antioxidant effects. Saudi Journal of Biological Sciences, 2015; 24; 36–44.
7. Chakkaravarthi K., Gokulakrishnan K., Sumgeoan T, Tamilvendan, D,. Synthesis, Spectral, Antimicrobial and Antioxidant studies of Diamide Mannich base derivatives. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 6(1); 492-495.
8. Chen YL., Huang ST., Sun FM., Chiang YL., Chiang CJ., Tsai CM., Weng CJ. Transformation of cinnamic acid from trans- to cis- form raises a notable bactericidal and synergistic activity against multiple drug resistant *Mycobacterium tuberculosis*. European Journal of Pharmaceutical Science 2011; 43: 188-194.
9. Cornforth, JW. The Trouble with Synthesis. *Australian Journal of Chemistry,* 1993; 46(2):157-170.
10. Joshi S, Khosla N, Tiwari P. *In Vitro* study of some medicinally important Mannich bases derived from antitubercular agent. Bioorganic Medicinal Chemistry 2004; 12: 571-576.
11. Komal, Y., Rashmi, B., Poonam P., Bardhan, NB. Synthesis, characterization and application of Mannich base. Galore International Journal of Applied Sciences&Humanities,2018;2(2);19-24.
12. Kotecka, BM., Barlin, GB., Edstein, MD.,

Rieckmann KH. New quinolone di-Mannich base compounds with greater antimalarial activity than chloroquine, amodiaquine or pyronaridine. Antimicrobial Agents Chemotherapy 1997; 41:1369-1374.
13. Lindert A, Wolpet A., Stephen M. Tannin Mannich Adducts for Corrosion Resistance Pripmering of Metals PCT. Int. Appl. W090 05, 794 (cl L 23c22/00) 1990 USb Appl. 272, 172, 1988 23pp.
14. Lorand, T., Kispal, G., Nagy, G., Weckert, E., Luebbert, D., Meents, A., Kocsis B. Prókai L., Diastereoselective reduction of cyclic bioactive Mannich Ketones. *Arkat Usa Inc. ARKIVOC vi*i, 1424-6376, 2004; pp 34-52.
15. Muthumani P., Neckmohammed1 R., Vekataraman1 S., Chidambaranathan N., Devis P., Suresh Kumar CA. Synthesis and Evaluation of Anticonvulsant and Antimicrobial Activities of Some Mannich Bases of Substituted Aminophenol and Acetophenone. International Journal of Pharmaceutical and Biomedical Research. 2010; 1(3): 78-86.
16. Oloyede, GK, Onocha PA, Oke, JM. Mannich Synthesis of 2- (Thiazol-4-yl)-1H-benzimidazole-2-methylene Piperazinyl (2,5 - Diamino-1,3,4- thiadiazolyl) Oxamide and Evaluation of the Filaricidal Activity American-Eurasian Journal of Scientific Research 2011; 6(2): 116-122.
17. Oloyede, GK, Fisayo-Bambi. E, Nwachukwu, TE. Spectroscopic properties and antimicrobial activity of synthesized Mannich bases: 1-Phenylaminomethyl-naphthalen-2-ol and (2-{ [2 – hydroxy ethyl) amino] methyl}phenyl) phenyl peroxyanhydride American J. of Drug Discovery and Development 2014a; 4(2): 113-120.
18. Oloyede, GK, Omisakin OO. Synthesis, ionization constant, toxicity, antimicrobial and antioxidant screening of 1-Phenyl-3-(phenylamino) propan-1-one and Phenyl (2-[phenyl amino) methyl] phenyl) peroxyanhydride Mannich bases Chemistry and Materials Research Journal 2014b; 6(2): 78-91.
19. Oloyede, GK., Willie IE, Adeeko, OO. Synthesis of Mannich bases: 2-(3-Phenylaminopropionyloxy)-benzoic acid and 3-Phenylamino-1-(2, 4, 6-trimethoxy-phenyl)-propan-1-one, their toxicity, ionization constant, antimicrobial and antioxidant activities. Food Chemistry Journal 2014c; 165:515-521.
20. Oloyede GK, Onocha, PA, Olisa DI. Synthesis, microbial growth and oxidation inhibitory properties of three Mannich bases. Chemistry and Materials Research 2015; 7 (8): 63-69.
21. Oloyede GK., Ogunsile BO.,. Ali MS, and. Ngouonpe WA. Antioxidant activity of Lignin - Mannich bases Synthesised by Amination reactions Journal Chemical Society of Nigeria, 2019; 44(7): 1276 -1282.
22. Priya, VF, Mahesha, MP., Naral, D., Chandrashekhar, C. Synthesis and antimicrobial studies of some Mannich bases carrying imidazole moiety. Acta Pharmacy 2013; 63; 231–239.
23. Roman G., Nanu D., Comanita E., Comanita B. Synthesis and Reactivity of Some Mannich Bases. *Turkish Journal of Chemistry* 2000; 24: 67-71.
24. Sarawathi M., Rohini RM., Ranaprathapsingh A., Naira N. Synthesis and Evaluation of Mannich Bases of Benzimidazo [1, 2, c] Quinazolin- 6(5H)- Thione for Antimicrobial Activity. Pakistan Journal of Pharmaceutical. Science. 2010; 23: 159-462.
25. Shchekotikhin, AE., Shtil AA., Luzikov, YN., Bobrysheva, TV., Buyanov VN., Preobrazhenskaya MN., 3-aminomethyl derivatives of 4,11-dihydroxynaptho [2,3-f]indole-5,10-dione for circumvention of anticancer drug resistance. Bioorganic. and Medicinal Chemistry 2005; 13: 2285-2291.
26. Sheela J., Navita K., Deepak K, Rakesh S. Synthesis and *In vitro* study of Novel Mannich Derived from Allomaltol and Evaluation of their Anticonvulsant Activities. *Hacettepe University Journal of Faculty of Pharmacy* 2005; 27: 1-10.
27. Shivarama, B., Veerendra, B., Shivananda, MK., Boja, P. Synthesis, characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. *European Journal of Medicinal Chemistry.,* 2003; 38(7-8): 759-767.
28. Singh, UK., Pandeya, SN., Singh, A., Srivastava, BK Pandey, M., Synthesis and antimicrobial activity of Schiff and N-mannich bases of Isatin and its derivatives with 4-amino-N-carbamindoyl benzene sulfonamide. *International Journal of Pharmaceutical Science and Drug Research*. 2010; 2: 151-154.
29. Vishant, P., Tarulata, S., Akshay, G, Synthesis, characterization and antimicrobial activity of Mannich bases of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo [3,2-a]pyrimidine-6-carboxylate derivatives. *Pelagia Research Library, Der Pharmacia Sinica*, 2014; 5(2); 63-70.