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Synthesis, Microbial Growth and Oxidation Inhibitory Properties of Three Mannich Bases

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Abstract
Diseases, infections and unhealthy diet serve as threat to human existence. Bases obtained from Mannich reaction have been found to have pharmacological application as antioxidant, antimicrobial, anti-cancer, anti-hypertensive, antiviral and anti-inflammatory in drugs and as food additives. Biologically active 4-(3-oxo-1,3-diphenyl propylamino) benzoic acid (A1), N-phenyl-3-(phenylamino) propanamide (A2) and 3-(p-tolylamino)-N-phenylpropanamide (A3) were synthesized and their purity confirmed by melting point determination and Thin layer chromatography. Characterization of the Mannich bases by spectroscopic methods: Infrared and Ultraviolet spectroscopy confirmed the presence of C=O, O-H, N-H typical of Mannich bases. They are also readily soluble in organic solvents. Antioxidant activity of A1, A2 and A3 involving the use of 2,2- diphenyl-1-picrylhydrazyl radical (DPPH) and hydrogen peroxide was significant when compared with antioxidant standards: ascorbic acid, butylatedhydroxylanisole and α-tocopherol. Compounds A2 and A3 gave percentage inhibition of 90.37 and 90.11 at 0.0625 mg/ml in the hydroxyl radical scavenging activity, a better percentage inhibition than ascorbic acid and α–tocopherol at the same concentration. Antimicrobial activity of the three compounds against bacterial and fungal strains; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomona aeruginosa, Klebsiellae pneumoniae, Salmonellae typhi*, *Candida albicans, Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium notatum* showed moderate microbial inhibition when activity was compared with standard drugs: gentamicin and tioconazole for bacteria and fungi respectively. The ionization constant (pKa) values at 6.01, 8.15 and 7.23 reported for compounds A1, A2 and A3 respectively indicates that they would be readily metabolized at physiological pH.
Keywords: Mannich bases; spectroscopy; oxidation; antimicrobial; ionization constant

1. Introduction

As part of our continued effort at synthesizing organic compounds of pharmacological importance which can be used as drugs, supplements or as food additives, Mannich reaction has been employed because of its efficiency, short synthetic steps and proceeds by utilizing the reactivity of the functional groups in a molecule (Arend et al., 1998; Delgadado and William, 1998). Mannich bases so formed have found applications in medicine and industry. They are used medically as anti-malarial, antioxidant, anti-inflammatory, anti-platelet, anti-hypertensive, antiviral, vasorelaxing, oxytocic and antimicrobial. They are also used in organic synthesis of natural compounds such as peptides, nucleotides, antibiotics and alkaloids (e.g. tropinone) and in the synthesis of medicinal compounds such as rolitetracycline (Mannich base of tetracycline), fluoxetine (antidepressant), tolmetin (anti-inflammatory drug) and azacyclophanes. They are employed industrially for the production of polymers, resins, surface active agent and detergents. Other applications are in agro chemicals such as plant growth regulators, paint and polymer chemistry, catalysts and main mechanism of formalin tissue crosslinking (Ahlam and Nada, 2009; Saraswathi et al., 2010; Oloyede et al., 2011; Oloyede et al., 2014a; Valarmathi et al., 2011).

This research work is aimed at synthesizing Mannich bases: 4-(3-oxo-1,3-diphenyl propylamino) benzoic acid (A1), N-phenyl-3-(phenylamino) propanamide (A2) and 3-(p-tolylamino)-N-phenylpropanamide (A3), determine their purity by melting point determination and thin layer chromatography and confirm their structures by using spectroscopic methods, infrared and ultraviolet/visible spectroscopy. The ionization constant of the synthesized compounds by non-aqueous titration and solubility in polar and non polar solvents were also determined. Bioassay of the synthesized compounds was carried out by determining the antioxidant activity using the free radical scavenging methods: scavenging effect on 2, 2-diphenyl picryl hydrazyl radical (DPPH) and hydroxyl radical generated from hydrogen peroxide while antimicrobial screening on pathogenic and multi-resistance microbes was determined by Agar well diffusion method. Results of which can make the synthesized compounds to be classified as antioxidant and antimicrobial drugs or food additives.

2. Experimental section

2.1 Materials: Chemicals and Reagents

All solvents used were BDH analar grade; benzaldehyde, methanol, ethanol, acetone, chloroform, p-amino benzoic acid, acetophenone, p-toludine, acetanilide, formaldehyde, aniline, isopropyl ether, diisopropyl ether and hexane, glacial acetic acid, hydrogen peroxide, α-tocopherol, conc. hydrochloric acid, butanol, tetrahydrofuran, isopropanol, diethylether, ethylacetate, acetonitrile 1,4-dioxan, ammonium thiocyanate, perchloric acid, potassium hydrogen phthalate, sodium tetraborate, ethanol, benzene. The following were however purchased from Sigma-Aldrich; 2,2-dipenyl-1-picrylhydrazyl (DPPH), ascorbic acid, iodine and butylated hydroxy anisole (BHA).

2.2 General Experimental Procedure

A standard reflux set-up was used for the reaction and measurement was done on Mettler H18 weighing balance. Assessments of the degree of purity of the final products obtained were achieved by determination of melting point using Gallenkamp Melting point Apparatus Model MFB 595 and also analytical Thin Layer Chromatography. Thin Layer Chromatography was carried out using Silica Gel F_{254} precoated plate (Merck, Germany) as adsorbent and mobile phase hexane: ethylacetate (1:3). Visualization in Ultraviolet light was later aided by the use of iodine vapor and the retardation factor (R_f) was calculated for each of the synthesized compounds. These compounds were further characterized by spectroscopic analysis, UV-Visible and Infra-red to ascertain the structures. The UV/Visible Spectra of 0.01% w/v of the compounds at 190–900 nm were determined with the aid of Spectro UV/Visible double beam Pc scanning spectrophotometer (UVD - 2960). A graph of Absorbance against wavelength (nm) was obtained. $V_{max}(cm^{-1})$ from IR data also confirmed the structures. The Infrared spectra of the synthesized compounds were recorded on Perkin - Elmer FT - IR Spectrophotometer in the range 4000─ 400 cm^{-1} as KBr discs. The relative strength and position of all absorption in the infrared region were determined in the spectrophotometer and intensity (Transmittance) was plotted against wave number. Non-aqueous titration was the technique used for the determination of ionization constant (pKa) which is a physicochemical property which can provide information that can be used to predict the absorption and passage of the drug through cell membranes. The pH (hydrogen ion concentration) was determined using pH meter 7020 (Electronic Instrument Ltd London) and pKa was calculated. Antioxidant and antimicrobial activities of the synthesized compounds were carried out to investigate their pharmacological importance. The same UV/Visible spectrophotometer specified above was used for antioxidant analysis while agar well diffusion method was used for antimicrobial analysis.

2.3 Test Organisms: Micro organisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomona aeruginosa, Klebsiellae pneumoniae, Salmonellae typhi*, *Aspergillus niger*, *Candida albicans, Penicillium notatum* and *Rhizopus stolonifer* collected from the stock of the Dept of Pharmaceutical Microbiology, Faculty of Pharmacy of University of Ibadan were used. They were maintained on nutrient agar slopes and kept in a refrigerator at 4 ^oC. Nutrient broth (100 ml aliquots) were inoculated with the culture of test micro-organisms and then incubated for 24 hrs at 37 ^oC.

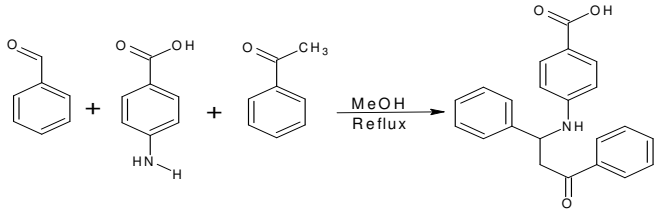
2.4 Reference Standards: Bacteria standard used was Gentamicin (10 mg/ml) while tioconazole (70%) was used as fungi in the antimicrobial screening; α-tocopherol, ascorbic acid and butylated hydroxyanisole (BHA) were used for antioxidant activity.

2.5 Methods

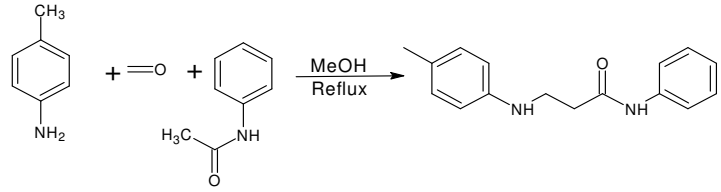
2.5.1 Preparation of Mannich bases

Medicinally active and non-toxic compounds were used as starting materials in the preparation of Mannich bases: 4-(3-oxo-1,3-diphenyl propylamino) benzoic acid (A1), N-phenyl-3-(phenylamino) propanamide (A2) and 3-(p-tolylamino)-N-phenylpropanamide (A3). These were based on procedures previously reported for substituted benzenes (Saraswathi et al., 2010; Oloyede et al, 2011, Oloyede et al, 2014a, b and c).

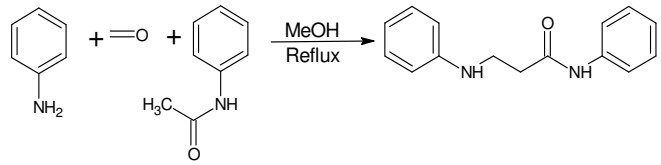
A mixture of 0.04 M benzaldehyde, 0.04 M Para-amino benzoic acid and 0.04 M acetophenone was refluxed for 4 hrs with 0.5 ml of NaOH in 50 ml of absolute methanol for preparation of compound A1; using the same experimental condition, 0.02 M formaldehyde, 0.02 M acetinilide and 0.02 M Para – toludine was used for A2 while 0.02 M formaldehyde, 0.02 M acetinilide and 0.02 M aniline was used for A3; the reaction was monitored using Thin Layer Chromatography. The resulting mixture was cooled and crystals obtained were washed with chloroform, filtered under pressure and recrystallized with warm ethanol. Equations of the reaction are shown in Schemes 1-3.



Scheme 1: Synthesis of 4-(3-oxo-1,3- diphenylpropylamino) benzoic acid - (Compound A1)



Scheme 2: Synthesis of 3-(p-tolylamino)-N-phenylpropanamide (Compound A2)



Scheme 3: Synthesis of N-phenyl-3-(phenylamino) propanamide (Compound A3)

2.6 Antimicrobial Screening of Compounds A1, A2 and A3

2.6.1 Preparation of samples for Antimicrobial analysis

Samples A1, A2 and A3 were weighed separately (0.50 g) and dissolved in 10 ml of DMSO to give 100 mg/ml. Four other test tubes contained 10 ml of the same solvent. From the first test tube, 10 ml of the content was drawn and added to the second test tube to give 50 mg/ml of the content and was done serially up to the fifth test tube to get a concentration of 6.25 mg/ml. Two other test tubes contained the negative control (solvent of dissolution) and the positive control which contained the standard drug Gentamicin (10 mg/ml) for bacteria and tioconazole (70%) for fungi (Oloyede et al., 2014a, b and c).

2.6.2 Agar diffusion: Pour plate method for bacteria

A culture of each organism was prepared by adding 0.1 ml of each of the organism to 9.9 ml of sterile distilled water to give 10 ml of 1: 100 dilutions. 0.2 ml was then taken into the prepared nutrient agar at 45^0C and poured aseptically into sterile plates. The prepared concentrations of the samples, including the positive and negative controls were introduced into the holes with syringe after 45 minutes and allowed to set. Incubation was done at 37^0C for 18 hours. Clear zone of inhibition was measured. The following micro-organism *S. aureus, E. coli, B. subtilis, P. aeruginosa, K. pneumoniae* and *S. typhi* were used. The experiment was carried out in triplicate and the average reading was taken (Oloyede et al., 2014a, b and c).

2.6.3 Agar diffusion: Surface plate method for fungi

Fungal strains: *A. niger*, *C. albicans, P. notatum* and *R. stolonifer* maintained on nutrient agar slopes and kept in a refrigerator at 4^oC were used. Sabouraud Dextrose Agar (SDA): molten and sterile was poured aseptically into the sterile plates and allowed to cool for 45 minutes. The same procedure described for antibacterial activity above was followed. Plates were incubated at 28 ^{o}C for 48 hours and 70 % tioconazole was used as the positive control for fungi. Clear zones of inhibition were measured (Oloyede et al., 2014a, b and c).

2.7 Antioxidant activities of the synthesized compounds

Two methods with relatively different mechanism were carried out to determine the antioxidant activity of the synthesized compounds.

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

Ability to scavenge free radicals was determined using DPPH. A 100 µM solution was prepared by dissolving 3.94 mg of DPPH in 100 ml methanol. 3.0 mg each of the synthesized compounds were separately dissolved in 3 ml methanol to prepare the stock solution and to 3.0 ml of the methanol solution of DPPH was added 0.5 ml of the dissolved samples, shaken and left to stand for 10 minutes. 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, 0.0625 mg/ml) were prepared from the stock solution by serial dilution and analyzed the same way. An average of triplicate analysis was reported. Butylated hydroxyl anisole (BHA), ascorbic acid and α- tocopherol were used as antioxidant standards (Oloyede and Farombi, 2010).

2.7.2 Hydroxyl radical scavenging effect of Compounds A1, A2 and A3

Hydrogen peroxide (H_2O_2) was the source of hydroxyl radical targeted in this assay. A solution of 2 µM H_2O_2 was prepared in phosphate-buffered saline (PBS) at pH 7.4. Synthesized compounds A1, A2 and A3 at the following concentrations: 1.0, 0.5, 0.25, 0.0125 and 0.00625 mg/ml were added separately to the H_2O_2 solution. Reduction in absorbance value of H_2O_2 at 285 nm was determined, 10 minutes later against a blank solution containing the samples in PBS without H_2O_2. All tests were run in triplicate and averaged (Oloyede and Farombi, 2010; Oloyede et al., 2014 c).

2.8 Determination of Ionization constant (pKa) of compounds A1, A2 and A3 via potentiometric titration

The physicochemical properties of drugs greatly influence drug absorption, biological availability, metabolism and excretion through cell membranes. Also of importance is the drug's method of formulation and route of administration.

2.8.1 Procedure

Standard buffer solution of pH 4 (0.05 M potassium hydrogen phthalate) and pH 9 (0.01 M borax solution) were used to standardize the pH meter. Standardized perchloric acid was used to titrate 2.5 ml of solution of compounds A1, A2 and A3 respectively in 60% 1, 4-dioxan. A graph of pH against volume (ml) of titrant was plotted and the pKa was determined using the Henderson–Hasselbalch equation. The pKa results represent mean values of triplicate analysis carried out near pH equivalence at 30^0C. The limit of experimental error was found to be ±0.001 when the results were analyzed statistically (Oloyede et al, 2014 c).

2.9 Statistical analysis

Absorbance measurements were expressed as mean absorbance ± SD of triplicate analysis. Percentage inhibition was calculated from this. Statistical analysis was performed by a one-way analysis of variance (ANOVA) processed on SPSS 15 windows software for more than two means while Student's t-test was used for comparison between two means. Values of $p<0.05$ were taken to be statistically significant.

3. Results

3.1.1 Mannich base - 4-(3-oxo-1,3- diphenylpropylamino) benzoic acid (A1): Brown crystals; yield: 71 %; m.pt: 162ºC. Soluble in methanol and ethanol; sparingly soluble in chloroform, and isopropylether, insoluble in water, di-isopropyl ether and hexane, R_f 0.59 (Silica gel $F_{254,}$ hexane: ethylacetate (1:3). Molecular weight (calc): for $C_{22}H_{19}NO_3$, 345.39 g. pKa= 6.01. UV nm (EtOH, λ_{max}nm): 299.00 (0.362), 419.00 (0.008), 423.00 (0.008), 425.00 (0.007), 432.00 (0.007), 473.00 (0.009), 487.00 (0.008), 501.00 (0.008), 660.00 (0.009), 826 (0.010). IR (KBr) V_{max} cm^{-1}: 3460 (O-H Hydrogen bonded), 3362 (N-H 2^0 amine), 3232 (C-H aromatic stretch), 2827, 2669 (C-H aliphatic stretch), 1692 (C=O stretch), 1522 (N-H bending), 1442 (C=C stretch Aromatic),1292 (C-N stretch of amine), 1172 (C-O stretch), 842,771 (C-H aromatic, out of plane bend).

3.1.2 Mannich base - 3-(p-tolylamino)-N-phenylpropanamide (A2): White crystals; yield 73.0 %; m.pt: 113 ^{0}C. Soluble in methanol and ethanol; sparingly soluble in chloroform, di-isopropyl ether and isopropylether, insoluble in water and hexane, R_f 0.52 (Silica gel $F_{254,}$ hexane: ethylacetate (1:3). Molecular weight (calc): for $C_{16}H_{18}N_2O$ 254.32 g, pKa= 8.15. UV (EtOH, λ_{max}nm): 192.00 (0.000), 221.00 (0.001), 266.00 (0.002), 301.00 (0.005), 356 (0.002), 397 (0.001), 421 (0.032), 437 (0.032), 449 (0.005). IR (KBr) V_{max} cm^{-1}: 3252 (N-H 2^0 amine), 3060 (C-H aromatic stretch), 2928, 2975 (C-H aliphatic stretch), 1650 (C=O stretch), 1556 (N-H bending), 1434 (C=C stretch Aromatic), 1319 (C-N stretch of amine), 1263 (C-O stretch), 754,838 (C-H aromatic out of plane bend).

3.1.3 Mannich base - N-phenyl-3-(phenylamino) propanamide (A3): White crystals; yield 74.8 %; m.pt: 114 ^{0}C. Soluble in methanol, ethanol and chloroform; sparingly soluble in di-isopropyl ether and isopropylether, insoluble in water and hexane, R_f 0.57 (Silica gel $F_{254,}$ hexane: ethylacetate (1:3). Molecular weight (calc): for $C_{15}H_{16}N_2O$ 240.30 g, pKa= 7.23, UV (EtOH, λ_{max}nm): 198.00 (0.001), 203.00 (0.001), 207 (0.001), 220 (0.001), 236 (0.002), 243 (0.001), 252 (0.001), 305 (0.003), 340 (0.000), 349 (0.000), 472 (0.037). IR (KBr) V_{max} cm^{-1}: 3260 (N-H 2^0 amine), 3020 (C-H aromatic stretch), 2927, 2856 (C-H aliphatic stretch), 1650 (C=O stretch), 1600 (N-H bending), 1426 (C=C stretch Aromatic), 1322 (C-N stretch of amine), 1262 (C-O stretch), 838,750 (C-H aromatic out of plane bend).

3.2 Antimicrobial screening of Compounds A1, A2 and A3

Agar well diffusion methods: pour plate method for bacteria and surface plate method for fungi showed that all the synthesized compounds possessed protective effect against gram positive/negative bacteria and fungi. The compounds were moderately active at 6.25 - 100 mg/ml although lower than the activities of the controls at the same concentration. Compound A1 showed pronounced activity on *E. coli, S. aureus* and *C. albicans* while compound A2 inhibited *S. aureus, K. pneumonia, S. typhi*, *C. albicans* and *P. notatum* better than the rest of the organism. Compound A3 was the most active on *E. coli* and *S. aureus.* Little or no activity was observed at 6.25

mg/ml. However, compound A1 inhibited the growth of *E. coli, S. aureus* and *C. albicans* at that concentration while A2 and A3 inhibited *K. pneumonia.* Compound A3 too inhibited the growth of *S. aureus* at 6.25 mg/ml (Table 1).

Table 1: Antimicrobial screening of compounds A1, A2 and A3*

Conc (mg/ml)	Zones of inhibition (mm)									
	B.sub	*E.coli*	*S.a*	*Ps.a*	*Kleb*	*Sal.*	*A.n.*	*C.a*	*Rh.s*	*Pen.*
Compd	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3	A1 A2 A3
1	16 14 14	18 16 20	20 20 22	16 14 14	16 20 20	14 16 14	16 14 14	18 18 18	16 14 14	14 16 14
2	14 12 12	16 14 18	18 18 20	12 12 12	14 18 18	12 14 12	14 12 12	16 14 14	14 12 12	12 14 12
3	12 10 10	14 12 14	16 16 16	10 10 10	12 14 16	10 12 10	12 10 10	14 12 12	12 10 10	10 10 10
4	10 - -	12 10 12	14 14 14	- - -	10 12 12	- 10 -	10 - -	12 - 10	10 - -	- - -
5	- - -	10 - -	12 - 10	- - -	- 10 10	- - -	- - -	10 - -	- - -	- - -
-ve control	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
+ve control	40 40 40	38 38 38	38 38 38	38 38 38	40 40 40	40 40 40	28 28 28	28 28 28	28 28 28	28 28 28

* Integers 1–5 represent the concentrations of compounds A1, A2 and A3 at 100, 50, 25.5, 12.5 and 6. 25 mg/ml respectively. The negative control is dimethylsulfoxide (DMSO) while the positive control is gentamicin at 10 mg/ml for bacteria and Tioconazole (70%) for fungi. ‘‘–’’ Represents no inhibition. *S.a = Staphylococcus aureus*, *E.coli = Escherichia coli*, *B.sub. = Bacillus subtilis*, *Ps.a = Pseudomonas aeruginosa*, Kleb = *Klebsiella pneumoniae*, *Sal = Salmonellae typhi*, *C.a = Candida albicans*, *A.n. = Aspergillus niger*, *Rh.s = Rhizopus stolonifer* and *Pen. = Pencillium notatum.*

3.3 Antioxidant analysis

The reduction in absorbance of 2,2-diphenylpicryl hydrazyl radical (DPPH), a stable free radical at 517 nm and hydrogen peroxide at 285 nm caused by reactive substances indicated that the synthesized compounds showed moderate activities as free radical scavengers when compared with butylated hydroxylanisole (BHA), ascorbic acid and α –tocopherol which are standard antioxidants (Figures 1-2). Compound A1 had 50% inhibition at 1.0 mg/ml in the DPPH free radical scavenging method; lower than the standards used (Figure 1) but compounds A2 and A3 gave percentage inhibition of 90.37 and 90.11 at 0.0625 mg/ml in the hydroxyl radical scavenging activity, a better inhibition percent than those of ascorbic acid and α–tocopherol at the same concentration (Figure 2). Oxidative stress is reported to be caused by excess of hydroxyl radicals because of their participation in free radical chain reactions. Compounds A2 and A3 showed good activity as hydroxyl radical scavengers and can therefore act as antioxidants.

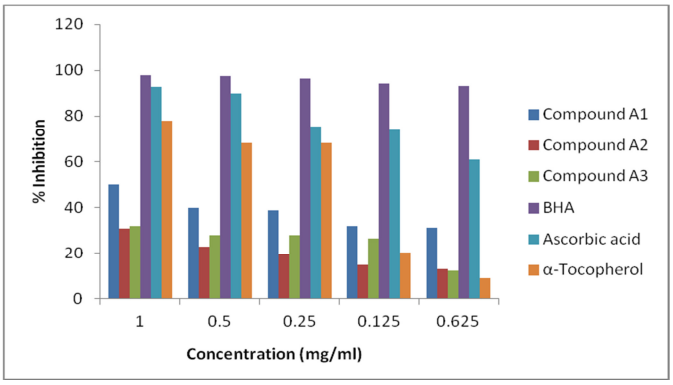


Figure 1: DPPH Free radical scavenging activity of compounds A1, A2 and A3.

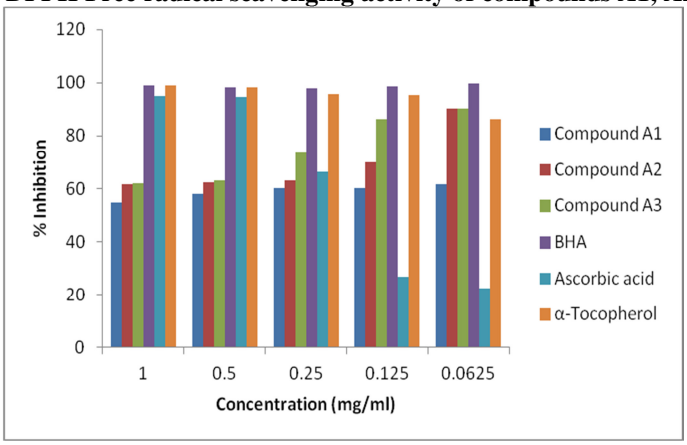


Figure 2: Hydroxyl radical scavenging activity of compounds A1, A2 and A3

4. Ionization constant (pKa) of compounds A1, A2 and A3

Results obtained from potentiometric titration showed that compounds A1, A2 and A3 had pKa values of 6.01, 8.15 and 7.23 respectively indicating that protonation can occur at physiological pH (7.4)

5. Discussion

The preparation of mannich bases: 4-(3-oxo-1, 3- diphenylpropylamino) benzoic acid, N-phenyl-3-(phenylamino) propanamide 3- (p- tolylamino)-N-phenylpropanamide and 3-(p-tolylamino)-N-phenylpropanamide was achieved using medicinally active and non-toxic compounds as lead and was based on procedures previously reported for substituted benzenes using an active hydrogen compound, aldehyde and amine. Single spots observed, aided by visualization in ultraviolet light and iodine vapor as well as R_f calculated form Thin layer chromatography (TLC); 0.59, 0.52 and 0.57 using Silica gel $F_{254,}$ as adsorbent; hexane: ethylacetate (1:3) as mobile phase confirmed the purity of the compounds. Sharp melting point of compounds A1, A2 and A3 at 162^0C, 113^0C and 114^0C respectively also confirmed the purity of the compounds. Spectroscopic data obtained from their IR showed that compounds A1, A2 and A3 respectively showed peaks at 3362, 3252 and 3060 cm^{-1} due to N-H stretch of secondary amines, 1692, 1650 and 1600 cm^{-1} (C=O stretch), 1292, 1319 and 1322 cm^{-1} (C-N stretch), and only Compound A1 showed a signal at 3460 cm^{-1} assignable to O-H . The presence of chromophore and conjugation were observed in the UV bands at λ_{max} 299, 192 and 221, 198 and 203 nm for compounds A1, A2 and A3 respectively. Many other bands were observed corresponding to unsaturation, π-π^* and n-π^* transitions associated with aromatic and carbonyl compounds. The various absorption observed in the visible region for A1 showed that the compound was colored. Biological significance of the synthesized compounds as antimicrobials was established by screening them against *S. aureus, E. coli, B. subtilis, P. aeruginosa, K. pneumoniae, S. typhi, C. albicans, A. niger, R. stolonifer* and *P. notatum* and the activities were compared with standard drugs, gentamicin and tioconazole for bacteria and fungi respectively. Compound A1 showed pronounced activity on *E. coli, S. aureus* and *C. albicans* while compound A2 inhibited *S. aureus, K. pneumonia, S. typhi*, *C. albicans* and *P. notatum* better than the rest of the organism. Compound A3 was the most active on *E. coli* and *S. aureus.* Little or no activity was observed at 6.25 mg/ml. However, compound A1 inhibited the growth of *E. coli, S. aureus* and *C. albicans* at that concentration while A2 and A3 inhibited *K. pneumonia.* Compound A3 too inhibited the growth of *S. aureus* at 6.25 mg/ml. This result therefore is in agreement with the antimicrobial activity of Mannich bases earlier reported (Manikpuri, 2010; Saraswathi et al, 2010; Oloyede et al, 2011; Jameel,2012; Oloyede et al., 2014a, b and c). The results obtained from antioxidant screening also confirmed the report of antioxidant activities of some synthesized Mannich bases (Shivananda & Shet Prakash, 2011, Oloyede et al., 2014 b and c). The free radical scavenging activity of the synthesized compounds was determined by two methods: against 2, 2 – diphenyl-1-picrylhydrazyl radical (DPPH) and hydroxyl radical generated from H_2O_2 at 517 and 285 nm respectively. Compound A1 had 50% inhibition at 1.0 mg/ml in the DPPH free radical scavenging method; lower than the standards used (Figure 1) but compounds A2 and A3 gave percentage inhibition of 90.37 and 90.11 at 0.0625 mg/ml in the hydroxyl radical scavenging activity, a better percentage inhibition than that of ascorbic acid and α–tocopherol at the same concentration. This shows that compounds A2 and A3 are good scavengers of hydroxyl radical. The ionization constant (pKa) influences both absorption and passage of a drug through the cell membrane, and distribution, metabolism, biological availability and excretion of drugs can be readily predicted. Also biological half –lives of drugs may increase or decrease with changes in pH of urine, therefore pKa value is important for the quantitative treatment of systems involving acid – base equilibrium in solution. The pKa values of 6.01, 8.15 and 7.23 were reported for compounds A1, A2 and A3 respectively indicating that protonation can occur at physiological pH (7.4) (Bhasin et al., 2005; Oloyede et al., 2011; Oloyede et al., 2014b and c). A healthy diet is a necessary part of human life. These Mannich bases can be used as antioxidant food additives or as starting material in drugs production.

6. Conclusion

The newly synthesized Mannich bases: 4-(3-oxo-1, 3-diphenylpropylamino benzoic acid) (A1), N-phenyl-3-(phenylamino) propanamide (A2) and 3-(p-tolylamino)-N-phenylpropanamide (A3) were reported to inhibit oxidation and microbial growth *in vitro*. Significant antioxidant activity was observed for compounds A2 and A3 as hydroxyl radical scavengers when activity was compared with butylated hydroxylanisole (BHA), ascorbic acid and α-tocopherol. The Mannich bases also moderately inhibited gram negative/positive bacterial and fungal growth. These compounds can therefore be used as precursors for the synthesis of medicinally active compounds used as antioxidant food additive or antimicrobial agents. The ionization constant which is a constant that gives information on absorption and passage of the drug through cell membranes showed that the compounds can be readily metabolized in cell membranes.

7. Acknowledgment
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There is no conflict of interest associated with this work.

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