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The antioxidant and antimicrobial activity for some new synthesized Schiff bases derived from Ascorbic acid

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ABSTRACT

This study was carried out to synthesise some new Schiff bases compounds through condensation of ascorbic acid (keto form) with some compounds which have amine group in their structure also these compounds synthesized by using of microwave irradiation and traditional method and compare the results obtained. Microwave irradiation of organic reactions has quickly acquired popularity as it increases the speed of the reaction towards multiple kinds of synthetic transformations, solventless procedures without the use of supporting reagents and therefore eco-friendly, the antioxidant properties were studied for all compounds included in this study, the results indicate that IC50 for the compounds synthesized by using of microwave irradiation method were higher than the traditional method, and also the biological activity of the prepared compounds were also studied to estimate capability of suppressing *Enterococcus, Staphyllo coccus aureus* and *E. Coli* than the starting materials that have biological activity on these bacteria. This study shows that the diameters of inhibitions in Petri dishes have higher and wider at a concentration (10 mg / ml) for used bacteria.

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INTRODUCTION

A Schiff base is a compound (named after Hugo Schiff) which has a structure $R_2C=NR'$ ($R'\neq H$). Schiff bases considered as a sub-class of imines, being either secondary ketimines or secondary aldimines relying on their synthesis (IUPAC, Compendium of Chemical Terminology 2006). Schiff bases have a lot of applications in various fields like inorganic chemistry biological chemistry, organic chemistry and. Altogether, they represent very frequently used and useful scaffold in medicinal chemistry. Schiff bases have an importance in the medical and pharmaceutical field because of an enlargement range of biological activities like inflammatory reducers (Sathe BS *et al.,* 2011), (Chandramouli C *et al.,* 2012), painkiller (Sondhi SM *et al.,* 2006) (Chinnasamy RP *et al.,* 2010), antibiotic (Arif M *et al.,* 2011) (Anacona JR and Ortega G. 2015), spasmolytic (Chaubey AK, Pandeya SN 2012), tuberculosis (Aboul-Fadl T *et al.,* 2003),

Anticancer (Miri R. *et al.,* 2013) (Ali SMM *et al.,* 2012), antioxidants (Wei D *et al.,* 2006), anthelmintic (Avaji PG *et al.,* 2009). Schiff bases are also managed as catalysts, intermediates in organic synthesis, dyes, pigments, polymeric stabilizers (Dhar DN, Taploo CL 1982) and corrosion inhibitors (Li S. *et al.,* 1999).

Antioxidants considered to play as a protector in the human body against deleterious effects of reactive free radicals (Ergul, B K. 2016). They are chemical compounds that can hold back, stop, or minimize the reactive effect of free radicals. These effects contain oxidative damage to membranes and improved susceptibility to lipid peroxidation or enzyme inactivation (Alugoju, P. *et al.,* 2015). Free radicals come from molecules via the homogenous breakage of a chemical bond such that each frag-

ment preserves one electron, by cleavage of a radical to produce another radical and also via redox reactions (Halliwell, B. and Gutteridge, J.M. 2008) (Satish, B. N and Dilipkumar, P. 2015)

Biological free radicals are molecules that are highly unstable who have available electrons to react with multiple organic substrates such as lipids, proteins, DNA and finally progress to oxidative stress (Kaur, C. and Kapoor, H.C. 2001) (Amic, D. *et al.,* 2003)

Antioxidants obtained from the diet or synthesized in the body. These can be divided into two main groups: Endogenous antioxidants: which include glutathione peroxidase-GPX, Glutathione-GSH. Etc. (Ratnam, D.V. *et al.,* 2006), and Exogenous Antioxidants: which include alpha tocopherol-vitamin E, beta carotene, selenium, ascorbic acid-vitamin C. etc. (Halliwell, B. and Gutteridge, J.M. 2008) (Kaur, C. and Kapoor, H.C. 2001).

Experimental

MATERIALS

Analar grade sulfamethoxazole, Ascorbic acid, Amoxicillin, Benzocaine and 4-Nitroaniline were gained from Fluka and BDH and used without recrystallization with a 99.9% purity as received.

Spectral measurements and instrumentation

Melting points recorded by using of melting point apparatuses Mettler FP 61, records of IR spectra have been done by using Shimadzu Infrared Spectrophotometer Fourier transform, the FTIR-8400S (KBr Disc) in 4000-400 cm^{-1} in KBr pellet region. Scan of ^{1}HNMR spectra was on a 400MHz Bruker spectrometer TMS as the inner standard and used a solvent (DMSO-d6), microwave oven.

Synthesis

1 mmole of primary amine (amoxicillin, sulfamethoxazole, Benzocaine) dissolved in 10 ml methanol was mixed with 1 mmole of a carbonyl compound (vitamin C) dissolved in 10 ml methanol, 0.5 ml of sulfuric acid used as a catalyst, this mixture refluxed for 2hrs. The solid precipitate was filtered hot and washed by using cold ethanol.

1 mmole of primary amine (amoxicillin, sulfamethoxazole, Benzocaine, 4-Nitroaniline) dissolved in 10 ml methanol was mixed with 1 mmole of carbonyl compound (ascorbic acid) dissolved in 10 ml methanol, 0.5 ml of sulfuric acid used as a catalyst was kept inside a microwave oven operating at (500 W) for five minutes. After the reaction completed, the mixture was poured into water and then allowed to cool to RT. The resulting solid was recrystallized.

In vitro antioxidant assay

The antioxidant activity of (Ascorbic acid, amoxicillin, sulfamethoxazole, benzocaine, 4-nitro aniline, 3-((4-(((2,3-dihydroisoxazol-3-yl) amino) thio) phenyl) imino) -5-(1,2-dihydroxyethyl) -4-hydroxyfuran-2 (3H) -one (traditional method), (2S) -6- ((2S) -2- (((E) -5- (1,2-dihydroxyethyl) -4-hydroxy-2-oxofuran-3 (2H) -ylidene) amino) -2-(4-hydroxyphenyl) acetamido) -3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (traditional method), 3-((4- (((2,3-dihydroisoxazol-3-yl) amino) thio) phenyl) imino) -5-(1,2-dihydroxyethyl) -4-hydroxyfuran-2 (3H) -one (microwave method), (2S) -6- ((2S) -2- (((E) -5-(1,2-dihydroxyethyl) -4-hydroxy-2-oxofuran-3(2H) -ylidene) amino) -2-(4-hydroxyphenyl) acetamido) -3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (microwave method) and 5- (1,2-dihydroxyethyl) -4-hydroxy-3-((4-nitrophenyl) imino) furan-2 (3H) -one were done by using Total Antioxidant Capacity by Phosphomolybdenum Method, which based on the reduction of Mo (VI) to Mo (V) with existence of antioxidant compound to form green phosphate Mo (V) complex at higher temperature and pH less than 7 (acidic) with a maximum absorption at 695 nm (Nabavi, S.M. 2009)

Antimicrobial activity

- Valuation of media activity (pH = 7.2)
- Culture media : *Entero coccus, S. aureus, E. Coli*
- Buffer solution pH = 7.2
- Three various weights (7.5, 5, 2.5) mg took from the starting material.
- 5 ml of absolute ethanol poured to every concentration to dissolve the compounds after that another 5 ml poured to Buffer solution.
- A shaker used to all samples.
- 1 ml of each concentration mixed with 9 ml of Buffer solution.

Preparing the media at pH=7.2 sterilized by autoclave at (45-50) C^0 with addition of the bacterial solutions and poured them into 6 holes with 6 mm diameter in Petri dishes every hole have 3mm thickness, a micropipette used to take 50 Ml from prepared solutions (standard & samples), left for 1 hour put them later in an incubator at (36.5 – 37.5) C^0 for 24 hours after that zone reader used to read inhibition zone.

RESULTS AND DISCUSSION

Prepared Schiff bases have various colours, powders, stability in the air, accurate melting points ‹ 150, >250°C, have a solubility in DMSO, DMF but they don't soluble very well in current organic solvents like ethanol.

Table 1: Physical properties of prepared compounds

Comp. No.	Chemical Formula	Compounds name	m.p °c	color	Yield %
1	$C_{18}H_{17}N_3O_8S$	3-((4-(((2,3-dihydroisoxazol-3-yl) amino) thio) phenyl) imino) -5-(1,2-dihydroxyethyl) -4-hydroxyfuran-2(3H) -one	208	yellow powder	64%
2	$C_{24}H_{25}N_3O_{10}S$	(2S) -6-((2S) -2-(((E) -5-(1,2-dihydroxyethyl) -4-hydroxy-2-oxofuran-3(2H) -ylidene) amino) -2-(4-hydroxyphenyl) acetamido) -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	211	Brown powder	71%
3	$C_{15}H_{14}O_6N$	Ethyl-4-((3-(1,2-dihydroxyethyl) -2-hydroxy-5-oxocyclopent-2-en-1-ylidene) amino) benzoate	83	White crystal	76%
4	$C_{12}H_{10}O_7N_2$	5-(1,2-dihydroxyethyl) -4-hydroxy-3-((4-nitrophenyl) imino) furan-2(3H) -one	142	Yellow crystal	82%

Table 2: IC50 for compounds

Compounds No.	Compounds name	IC50 mg/ml
1	Ascorbic acid	0.025
2	Amoxicillin	1.658
3	Sulfamethoxazole	1.578
4	Benzocaine	0.0249
5	4-nitroaniline	0.0241
6	Compound 1 (traditional method)	1.251
7	Compound 2(traditional method)	0.048
8	Compound 1 (microwave method)	0.024
9	Compound 2(microwave method)	0.047
10	Compound 3	0.046

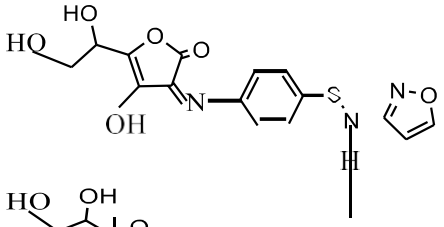
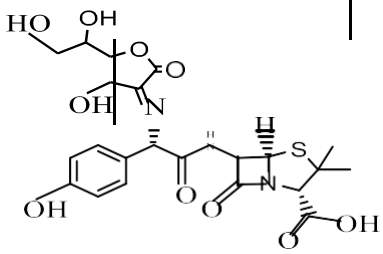
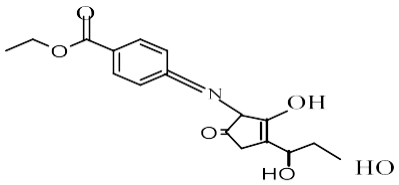
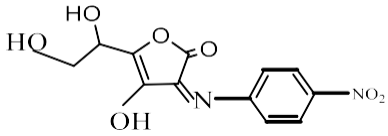
Table 3: biological activity of starting materials toward S. aureus, E. Coli and E.Coccus

Comp. No	Name	Conc. mg/ml	*Enterococcus*	*E. Coli*	*Staphylococcus aureus*
1	Sulfamethoxazole	7.5	8	4	14
		5	13	7	11.3
		2.5	18	11	9
2	Ascorbic acid	7.5	20	19	16.2
		5	17.6	14.7	14
		2.5	14	12	11
3	Amoxicillin	7.5	21	20	15
		5	16.8	18.1	12.8
		2.5	15	16	11
4	Benzocaine	-	-	-	-
		-	-	-	-
		-	-	-	-
5	4-Nitro aniline	-	-	-	-
		-	-	-	-
		-	-	-	-

The FTIR spectrum of compound 1 (Fig. 1) shows stretching band at 3411 Cm^{-1} that belongs to the hydrogen bonded alcoholic (- OH) group another band appears in 3236 Cm^{-1} attributed to (-NH) group, in 3035 Cm^{-1} a band appeared attributed to aromatic (C-H) and another band in 1618 Cm^{-1} for (C=C). A strong band is illustrated at 1681 Cm^{-1} which belongs to (-C=N) group; this stretching band describes the participation of the azomethine groups in Schiff bases and passing of stretching band at 3300-3500 Cm^{-1} for ($-NH_2$) group for primary amines and stretching band in ketones and aldehydes (carbonyl compounds) at 1680-1790 Cm^{-1}

The FTIR spectrum of compound 2 (Fig. 2) shows broadband at 3411 Cm^{-1} l belongs to alcoholic (-OH) group, the band in 3236 Cm^{-1} belongs to (-NH) group, another band in 2925 Cm^{-1} for aromatic (-C-H), a band appeared in 1681 Cm^{-1} belongs to (C=N methine) which existing in Schiff bases in the same time the band belongs to (-C=O) group at 1720

Table 4: biological activity of prepared compounds

Comp. No	Structure	Conc. mg/ml	*Entero coccu*	*E. Coli*	*Staphylococcus aureus*
1		7.5	23.6	19	18
		5	21	16	15
		2.5	20	15.3	12
2		7.5	21	19	17.6
		5	18.4	17	15
		2.5	15	14	13
3		7.5	23	21	15
		5	19	18	13.2
		2.5	14	15	10
4		7.5	-	-	-
		5	-	-	-
		2.5	-	-	-

Cm^{-1} disappeared, and the band in 3500 Cm^{-1} for (-NH_2) group in primary amines disappeared also.

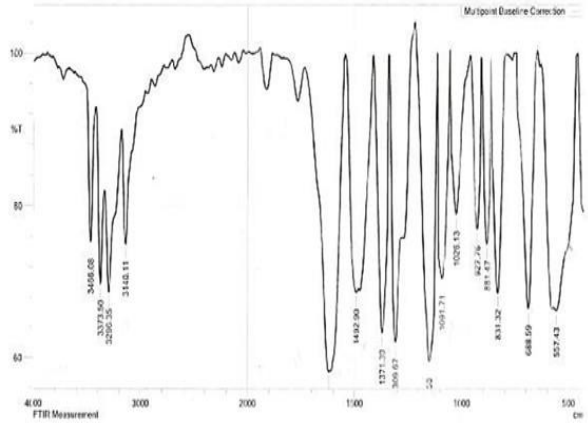


Figure 1: FTIR for compound Comp.1

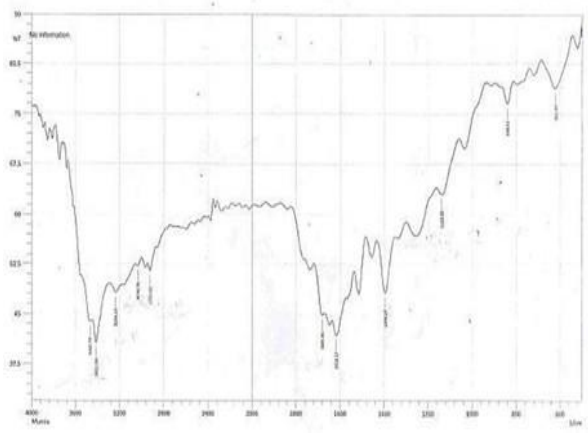


Figure 2: FTIR for Comp. 2

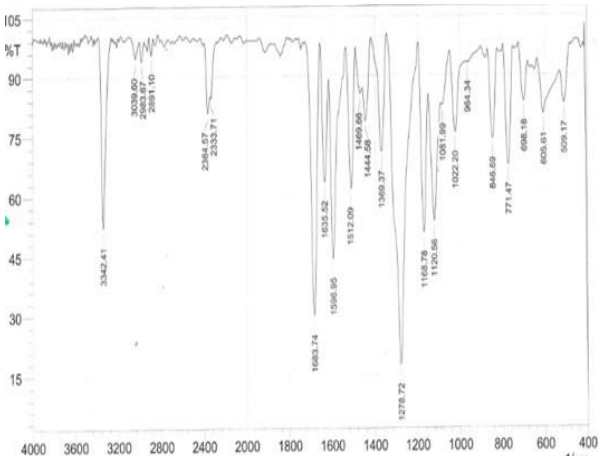


Figure 3: FTIR for Comp. 3

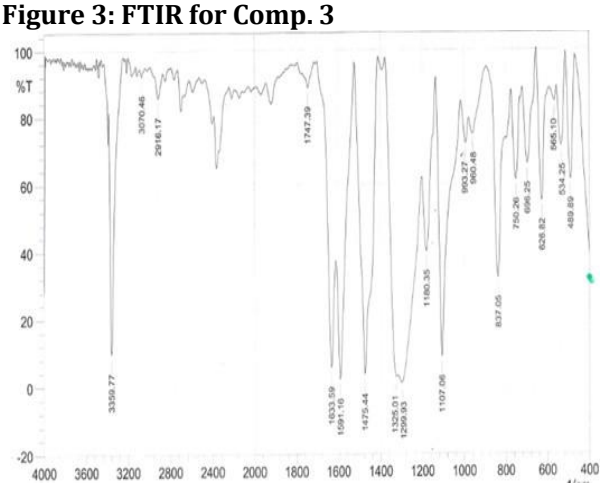


Figure 4: FTIR for Comp. 4

The FTIR spectrum of compound 3 (Fig. 3) a broad-band appears at 3324 Cm^{-1} belongs to the hydrogen bonded alcoholic (-OH) group another band appear in 3039 - 2891 Cm^{-1} belongs to aromatic (-CH), in 1683 Cm^{-1} a band appeared belongs to (-C=N) group this band describes the azomethine groups participation in Schiff base compounds with stretching band passing at 3300-3500 Cm^{-1} for ($-NH_2$) group for primary amines and stretching band for carbonyl compounds (aldehydes and ketones) at 1690-1780 Cm^{-1} and another band in 1683 Cm^{-1} for (C=C). A band is described at 1278 Cm^{-1} which is assigned to (-C-C-O) stretching in esters.

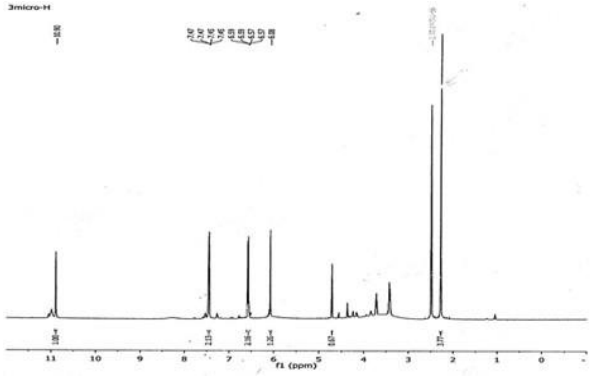


Figure 5: H1NMR for comp. 1

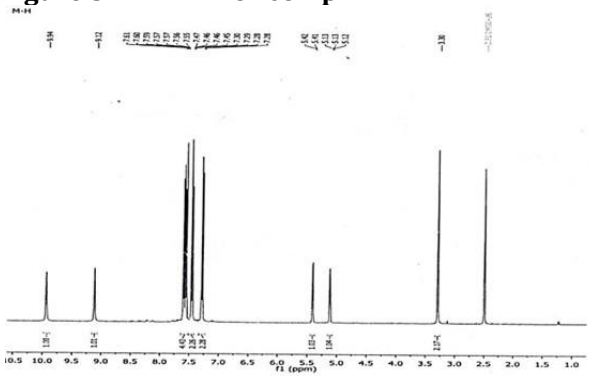


Figure 6: H1NMR for comp. 2

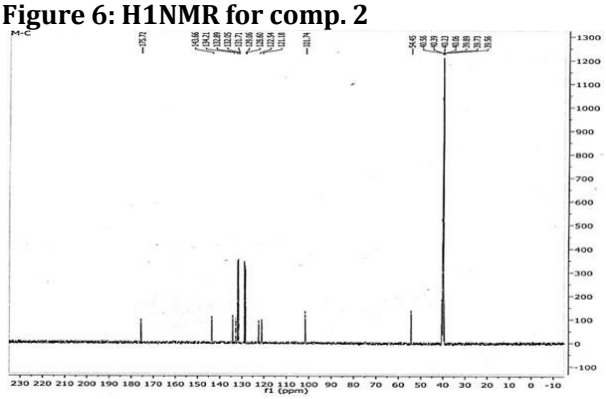


Figure 7: C13 NMR for comp. 2

The FTIR spectrum of compound 3 (Fig. 3) shows stretching band at 3395 Cm^{-1} attributed to alcoholic hydrogen bonded (-OH) group another band appear in 3070 - 2916 Cm^{-1} belongs to aromatic (-CH), in 1633 Cm^{-1} a band appeared belongs to (-C=N) group this band belong to azomethine groups which found in Schiff base compounds with stretching band passing at 32800Cm^{-1} for ($-NH_2$) group which found in primary amines and stretching band belongs to carbonyl compounds (aldehydes and ketones) at 1690-1780 Cm^{-1} and a band in 1683 Cm^{-1} for (C=C). A band is described at 1278 Cm^{-1} which is assigned to (-C-C-O) stretching in esters.

The 1HNMR spectral data of compound 2 prepared in microwave (Fig.6) shown DMSO protons at (2.5) ppm, protons at (3.21) belongs to olefinic protons, protons of aliphatic alcohol at (5.1), protons of benzene rings at (7.27.44) ppm, -CH=N protons at (9.1) ppm, proton of aromatic –OH at (10) ppm.

The C^{13} spectral data of compound 2 prepared in microwave (Fig.7) shown DMSO carbon at (40) ppm, aliphatic -C-H group at (54) ppm, carbon for phenyl group at (110-132) ppm, carbon of –HC=N group at (144) ppm and carbon-related in –OH group at (177) ppm.

The results indicated that IC50 (IC50 value is the concentration of the sample required to inhibit 50% of radical) for the compound under investigation were 0.025, 1.658, 1.578, 0.0249, 0.0241, 1.251, 0.048, 0.024, 0.047 and 0.046 for the compounds in table 1 labelling with 1-10.

The present study demonstrated that benzocaine and 4-nitro aniline exhibited the highest antioxidant capacity for phosphomolybdate reduction as compared with ascorbic acid as reference for antioxidant capacity, and also the compound 8 prepared by microwave method have highest antioxidant capacity as compared with the same compound prepared by traditional method, while the compounds 7 and 9 which they have the same structures but prepared by traditional and microwave methods have the approximately the same antioxidant capacity.

Biological activity

The results show higher inhibition effect of biological activity for compounds that contains sulfamethoxazole, amoxicillin, ascorbic acid, Benzocaine and 4-nitro aniline shown higher inhibition effect compared to the starting materials, that is maybe happened as a result of the existence of carbonyl group and OH group in ascorbic acid.

Antimicrobial activity of sulfamethoxazole, amoxicillin, benzocaine was evaluated through the agar diffusion method. Prepared Schiff bases reveal antimicrobial activity against tested microorganism strains gram-positive bacteria (*Enterococcus, Staphyllo coccus aureus, E. Coli)*

The preparation of Schiff bases throws microwave irradiation showed better results than traditional technique also the prepared compounds showed

higher levels of antioxidant and antimicrobial features than the starting materials.

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