Synthesis and Biological Activity Study of New Some Schiff Bases Derived From D-Erythroascorbic Acid

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Abstract

New Schiff bases derived from D-erythroascorbic acid were prepared by condensing pentulosono-γ-lactone-2,3-enedianisoate (V) with 3-amino phenol, (4-iodo, 3-methoxy, 4-methyl, 4-nitro) aniline in the presence of glacial acetic acid and dry benzene. The products were characterized by spectral methods FTIR, and some by (UV-Vis), ^{1}H and ^{13}CNMR, mass spectra and measurement of its physical properties and evaluation of the biological activity for them.

Keywords**:** Schiff bases, imine, biological activity, erythroascorbic acid.

Introduction

The formation of carbon–nitrogen double bond plays important role in organic synthesis. This can be achieved by the reaction of aldehydes and amines in acidic medium which leads to synthesis of Schiff bases (imines). Schiff bases have attracted considerable attention of organic chemists due to their significant biological activities like anticancer [1], antitumor [2], anti-inflammatory agents [3], insecticidal [4], antibacterial [5], antituberculosis [6], antimicrobial [7], anticonvulsant [8] activity. The Schiff bases are also used as versatile components in nucleophilic addition with organometallic reagents [9] and in cycloaddition reactions [10,11]. The reaction of primary aromatic amines with aryl aldehydes is found to be catalyzed by lemon juice as natural acid under solvent-free conditions to give the corresponding Schiff bases in good yields. This eco-friendly reaction has many advantages like economical, environmental, mild reaction conditions and simple work-up with high product yield [12].

Experimental

Melting points were determined by electrothermal Stuart melting point apparatus and are uncorrected. IR spectra (in KBr) were recorded on 8400s Shimadzu FT infrared spectrophotometer. ^{1}HNMR spectra were recorded on Ultra Shield (300 MHz) spectrometer with tetramethyl silane as internal standard. ^{13}CNMR spectrum was recoded on a Varian Mercury plus 100 MHz spectrometer. Electronic spectrum was obtained using a (U.V-Vis) spectrophotometer type CECl 7200 England. Mass spectrum for the compound (V) was recorded on IEOLJMS-7high resolution instrument. Thin layer chromatography (TLC) was performed on aluminum plates coated with layer of silica gel, supplied by Merck. The spots were detected by iodine vapor. All chemical were obtained from Fluka or BDH.

Synthesis of 5,6-*O*-isopropylidene-L-ascorbic acid (II)

Dry hydrogen chloride was rapidly bubbled with stirring for 20 minutes into a (250ml) flask containing (10g, 57mmol) of powdered L-ascorbic acid (I) and (100ml) of dry acetone.

After addition of (80ml) *n*-hexane, stirring and cooling in an ice-water, the supernatant was decanted. The precipitate was washed four times with (154ml) of acetone-hexane mixture (4:7)

(v/v), cooling in an ice-water and removal of supernatant after each addition. The last precipitate was dried under reduced pressure to give (II) (95%) as a white crystalline residue [13], m.p (206-208˚C). R_f (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3240 (O-H), 2993 (C-$H_{ali.}$), 2908 (C-$H_{ace.}$), 1751 (C=$O_{lac.}$), 1662 (C=C), 1431 (-CH-$_{asym}$), 1388 (-CH-$_{sym}$), 1141-900 (C-O), 767 δ(O-H) (O.O.P.) [14].

Synthesis of 2,3-*O*-dianisoyl-5,6-*O*-isopropylidene - L-ascorbic acid (III)

To a cold solution of (II) (10g, 46mmol) in pyridine (50ml), anisoyl chloride was added as dropwise (17.5 ml, 129 mmol) with stirring. The resulting mixture was stirred for 2 hours, then kept in dark place at room temperature for 22 hours.

The mixture was poured into ice-water and stirred for 20 minutes, the supernatant was decanted. The oil layer was extracted with chloroform (150 ml), washed with water, dilute hydrochloric acid (5%) (2×100ml.), saturated aqueous sodium hydrogen carbonate (100ml) and water. Dried over anhydrous magnesium sulfate. Chloroform was evaporated to produce a brown syrup and purified from chloroform: petroleum ether (60-80˚C)(1:5) (v/v) to give (III) (76.5%) as a pale yellow solid[15], m.p (102-104˚C). R_f (0.80) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3028 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2939 (C-$H_{ace.}$), 2843 (OC-$H_{ali.}$), 1749 (C=$O_{lac.}$), 1683 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-H) (O.O.P.) [14].

Synthesis of 2,3-*O*-dianisoyl-L-ascorbic acid (IV)

Compound (III) (10g, 23.6mmol) was dissolved in mixture (65%) acetic acid (30ml) and absolute methanol (10ml) and stirred for 48 hours at room temperature. The TLC showed that the reaction was complete (benzene: methanol, 6:4).

To the resulting solution a benzene (40ml) was added and evaporated (repeat this process four times)[15].The residue recrystallized from chloroform and then diethyl ether to yield (IV) (77.7%) as a white crystals, m.p (130-132˚C), R_f (0.42). FTIR (KBr, cm^{-1}): 3444 (O-H), 3008 (C-$H_{ar.}$), 2972 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), 1741 (C=$O_{lac.}$), 1681 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1606 (C=$C_{ar.}$), 1319-1112 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.) [14].

Synthesis of pentulosono-γ-lactone-2,3-enedianisoate (V)

To the stirred solution of sodium periodate (5.6 g, 26 mmol) in distilled waer (60 ml) at (0˚C), a solution of (IV) (10 g, t 26 mmol) in chloroform (60 ml) was added dropwise. After stirring for 15 minutes, ethylene glycol (0.5ml) was added as dropwise, stirring was continued at room temperature for 1 hour [15].

The mixture was filtered and to the filtrate water (40ml) was added then the product was extracted with ethyl acetate (3×50ml), the extracts dried by anhydrous magnesium sulfate, then filtered and the solvent was evaporated and the residue recrystallized from benzene to yield the pure product of compound (V) (45%) as a white crystals, m.p (156-158˚C). R_f (0.70) (benzene: methanol, 6:4) (v/v). FTIR (KBr, cm^{-1}): 3040 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), 2671, 2559 (C-$H_{ald.}$), 1782 (C=$O_{lac.}$), 1749 (C=$O_{ald.}$), 1685 (C=$O_{est.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.). ^{1}HNMR (DMSO δ ppm): 12.5 (s, 1H, CHO), 7.00-7.96 (dd, 8H, aromatic), 3.82-4.10 (s, 6H, 2OCH_3), 3.50-3.57 (s, 1H, H_4). ^{13}CNMR (DMSO δ ppm): 167.50 (C=$O_{lac.}$), 163.32 (C=$O_{est.}$), 131.86 (C-4), 131.83 (C-3), 131.81 (C-2), (123.44, 114.31, 114.28, 114.26) (C$_{ar.}$), 55.90 (OCH_3) [14]. The signal of aldehydic carbonyl was disappeared due to it showed out of the scale [16]. MS, (positive ion) m/z (relative intensity): 413 [M+1, (100)], UV (λ_{max}, nm, $CHCl_3$): 300.

Synthesis of Schiff bases (VI-X)

A mixture of primary aromatic amine (0.12mmol), aldehyde (V) (0.05g, 0.12mmol), dry benzene (10ml) and 2 drops of glacial acetic acid were refluxed for (14) hours, the TLC showed that the reaction was complete (benzene: methanol, 8:2). The solvent was evaporated under vacuum and the residue recrystallized from ethanol. The physical data of all Schiff bases are listed in Table (1). The FTIR spectra data are given in Table (2). ^{1}HNMR (DMSO δ ppm) for compound (VII): 10.16 (s, 1H, HC=N), 7.00-7.90 (dd, 12H, aromatic), 3.82 (s, 6H, 2OCH_3), 3.45-3.57 (s, 1H, H_4)[14]. ^{1}HNMR ($CDCl_3$ δ ppm) for compound (VIII): 7.78-8.10 (dd, 8H, aromatic), 7.45 (s, 1H, HC=N), 6.27-7.24 (m, 4H, aromatic), 3.78-3.89 (s, 9H, 3OCH_3), 3.61-3.64 (s, 1H, H_4) [14].

Table (1)
Physical properties of compounds (VI-X).

Comp. No.	Nomenclature	Molecular Formula	M.p˚C or dec.	R_f	Physical state	Yield %
VI	(3-Hydroxyphenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{27}H_{21}O_9N$	110-112	0.54	Brown solid	67
VII	(4-Iodophenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{27}H_{20}O_8NI$	105 (dec)	0.68	Deep-brown solid	70
VIII	(3-Methoxyphenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{28}H_{23}O_9N$	124-126	0.73	Deep-brown solid	78
IX	(4-Methylphenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{28}H_{23}O_8N$	125-127	0.55	Brown solid	75
X	(4-Nitrophenyl)-imine-pentulose-γ-lactone-2,3-enedianisoate	$C_{27}H_{20}O_{10}N_2$	130-133	0.58	Light-brown solid	34

Table (2)
Characteristic FTIR absorption band of compounds (VI-X).

Comp. No.	υ(O-H)	$\upsilon(C-H)_{ar.}$	$\upsilon(C-H)_{ali.}$	$\upsilon(C=O)_{lac.}$	$\upsilon(C=O)_{est.}$	υ(C=N)	$\upsilon(C=C)_{ar.}$
VI	3304	3028	2956 2845	1770	1685	1640	1602
VII	-	3028	2983 2843	1737	1685	1650	1604
VIII	-	3040	2924 2860	1760	1690	1630	1604
IX	-	3028	2983 2872	1760	1685	1650	1604
X	-	3028	2983 2843	1770	1685	1629	1604

Antibacterial testing

The bacterial cultures for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus* and *Sallemonela* were obtained from Center for Market Research & Consumer Protection Laboratory. The bacterial cultures were incubated at 30 ± 0.1ºC for 24 hours by inoculation into nutrient agar. Schiff bases were stored dry at room temperature and dissolved in dimethylsulfoxide (DMSO). Antibacterial activities of each compound were evaluated by the agar disc-diffusion method [17]. Mueller Hinton Agar Media kept at 45ºC was poured in the Petri dishes and allowed to solidify. Disc injected with prepared Schiff bases (50µl) were applied on the solid agar medium by pressing tightly. The Petri plates were placed at 37ºC for 24 hours. At the end of period the inhibition zones formed on media were measured with a zone reader in millimeters.

Results and Discussion

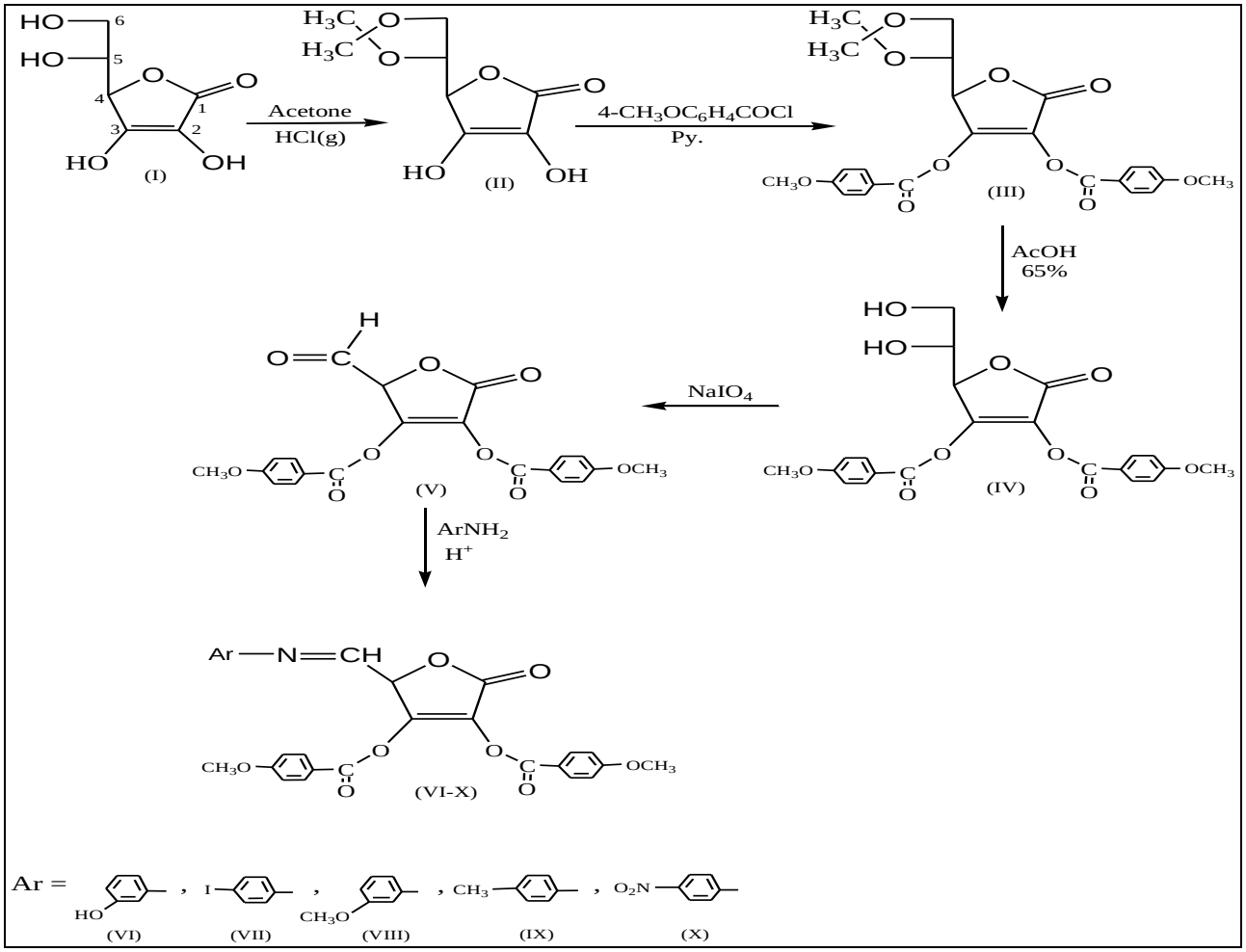
In the present work the synthesis of new Schiff bases were achieved from pentulosono-γ-lactone-2,3-enedianisoate (V), scheme (1). The first step employs the protection of the hydroxyl groups at C-5 and C-6 positions in L-ascorbic acid with acetal formation leading to compound (II) using dry acetone in acidic media, following Salomon[13] method. This is followed by esterification of the hydroxyl groups at C-2 and C-3 positions with excess of anisoyl chloride in dry pyridine.

The FTIR spectra for compound (II), Fig.(1) and (III) were confirmed the formation of compound (III) by disappearance of the bands for (O-H) of compound (II) and

exhibited the band at (1683) cm^{-1} for (C=O) of the ester in compound (III) spectrum.

In order to prepare aldehyde (V), the acetal moiety was cleaved under acidic condition[18] (65% acetic acid) for compound (III) to give (IV) and oxidation of the product with sodium periodate to result (V), which gave a positive Tolen's test by formation a silver mirror[19]. The FTIR spectra for compound (IV) and (V) were confirmed the formation of compound (V) by disappearance of the bands for (O-H) of compound (IV) and exhibited the band at (1749) cm^{-1} for (C=O) in compound (V) spectrum. The structure of (V) was confirmed by ^{1}HNMR, which exhibited a signal at δ(12.5) ppm for (CHO) and was characterized by ^{13}CNMR, Fig. (2) and (U.V-Vis) spectrum which showed one peak at (300) nm (33333 cm^{-1}) assigned to (n ⟶ π*) and (π ⟶ π*) transitions. Finally, the mass spectrum showed a highest mass signal at [M+1] =413 with signal intensity 100%.

In order to obtain compounds (VI-X) by condensation of primary aromatic amines such as (3-amino phenol, (4-iodo, 3-methoxy, 4-methyl, 4-nitro) aniline with aldehyde (V) using dry benzene in acidic media leading to these compounds. All FTIR spectra for these compounds exhibited disappearance of the band at (1749) cm^{-1} for (C=O) of the aldehyde (V) and displayed of the bands at (1629-1650) cm^{-1} due to (C=N) for compounds (VI-X), Figs. (3) and (4) FTIR spectra for compounds (VII) and (X). The structures of compounds (VII) and (VIII) were confirmed by ^{1}HNMR which showed disappearance of the signal at δ(12.5) ppm for (CHO) and exhibited the signals at δ(10.16) ppm and δ(7.45) ppm for (HC=N) in compounds (VII) and (VIII) spectra, Fig. (5) ^{1}HNMR spectrum for compound (VII).



Scheme (1) The scheme of prepared compounds.

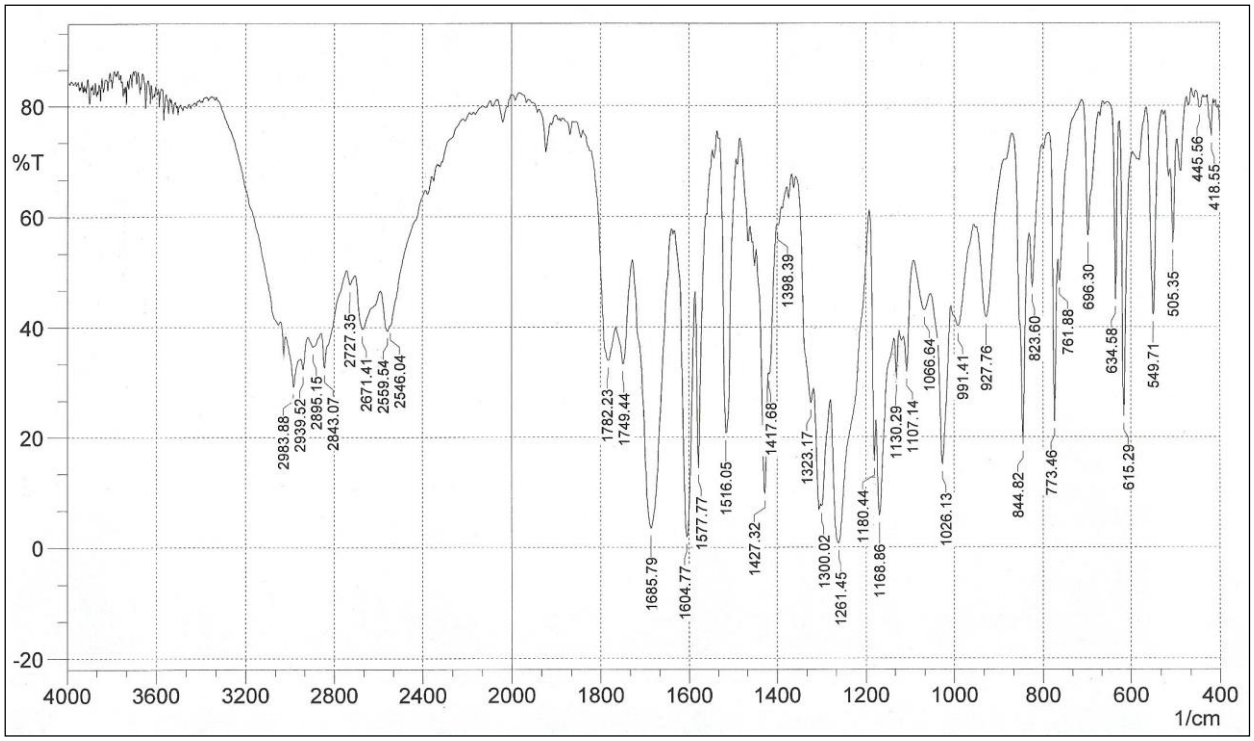


Fig. (1) FTIR- spectrum of compound (V).

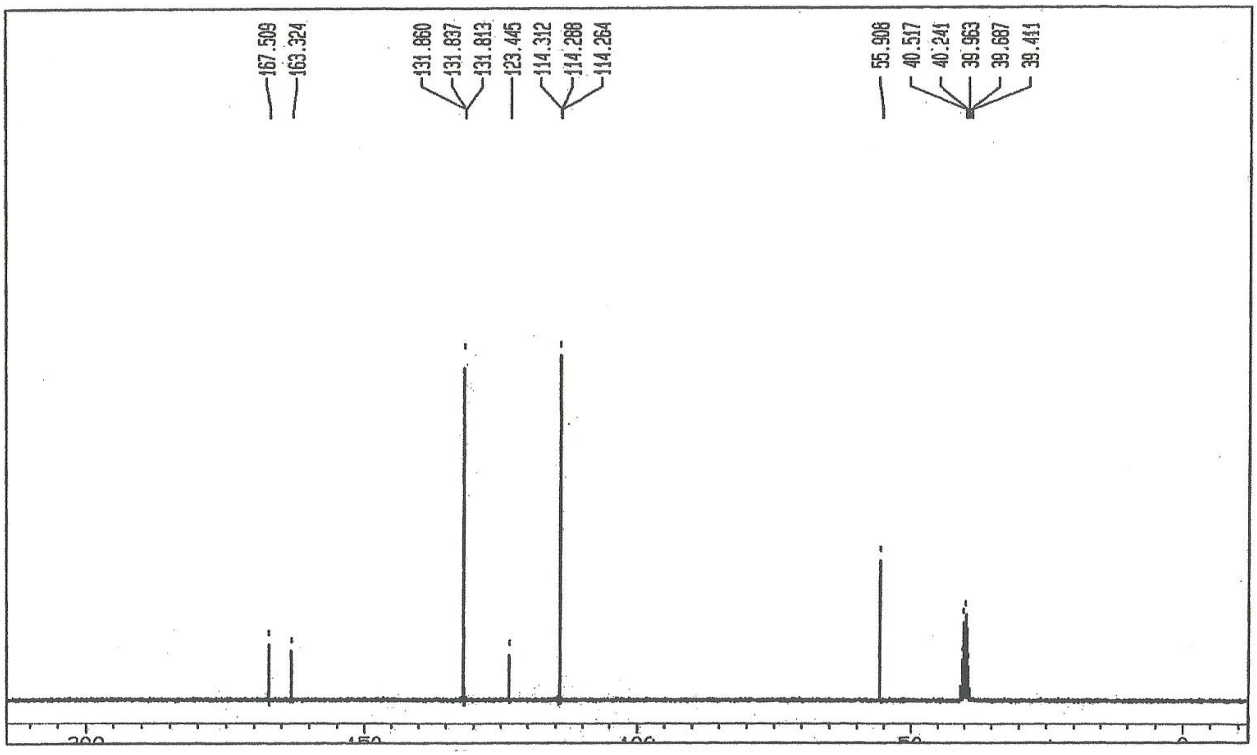


Fig. (2) ^{13}CNMR spectrum of compound (V).

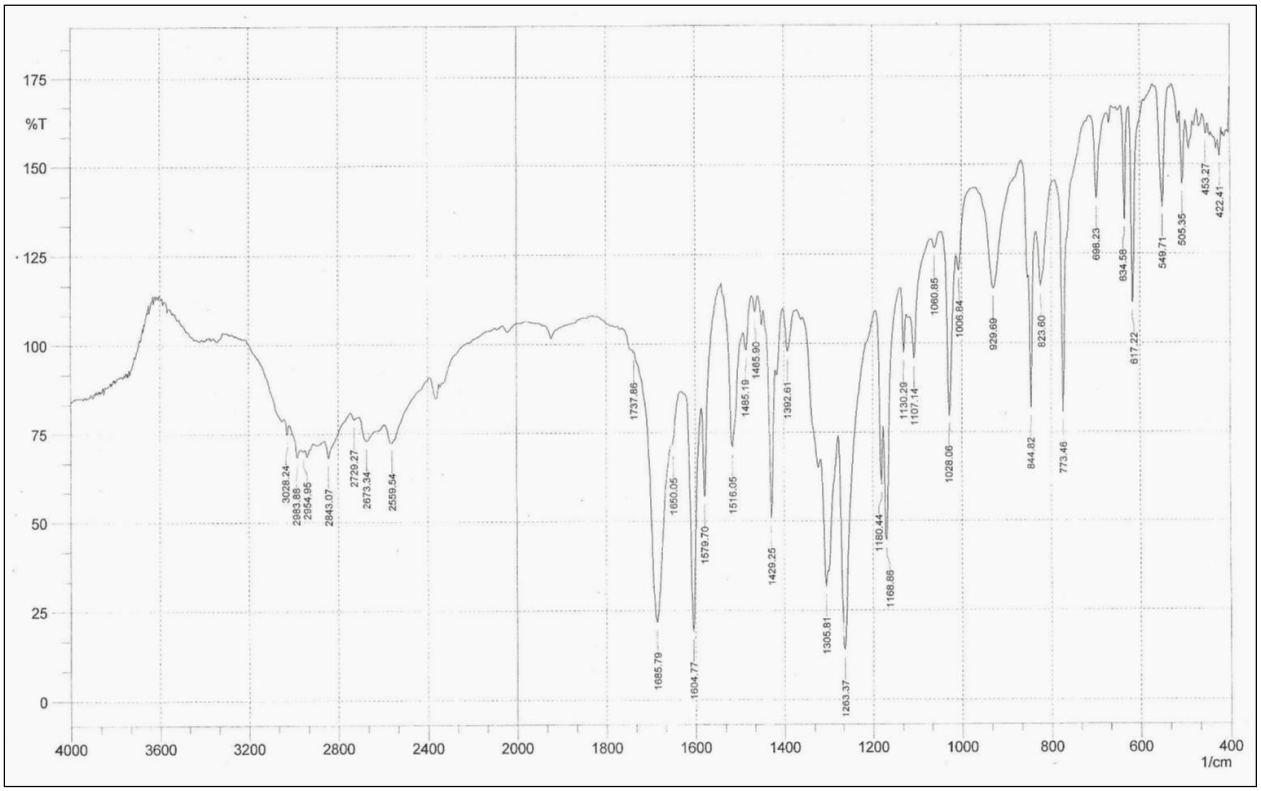


Fig. (3) FTIR- spectrum of compound (VII).

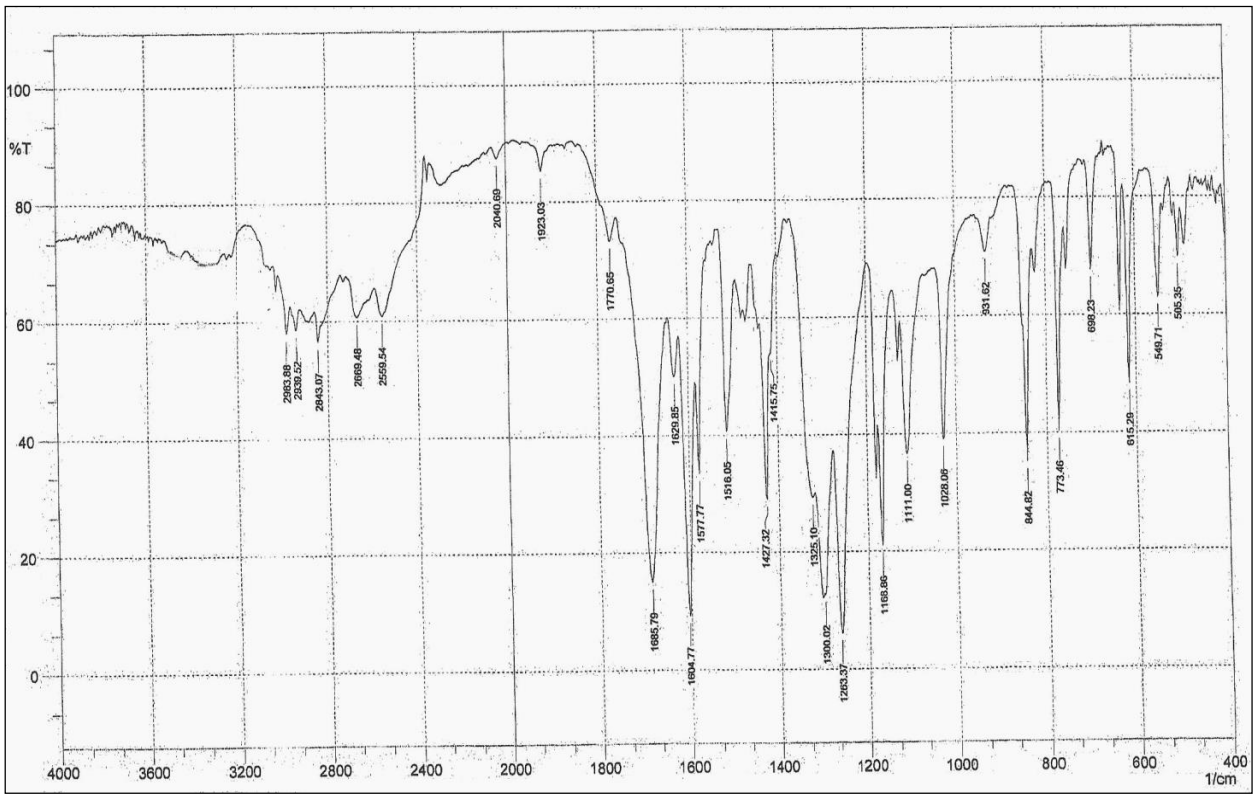


Fig. (4) FTIR- spectrum of compound (X).

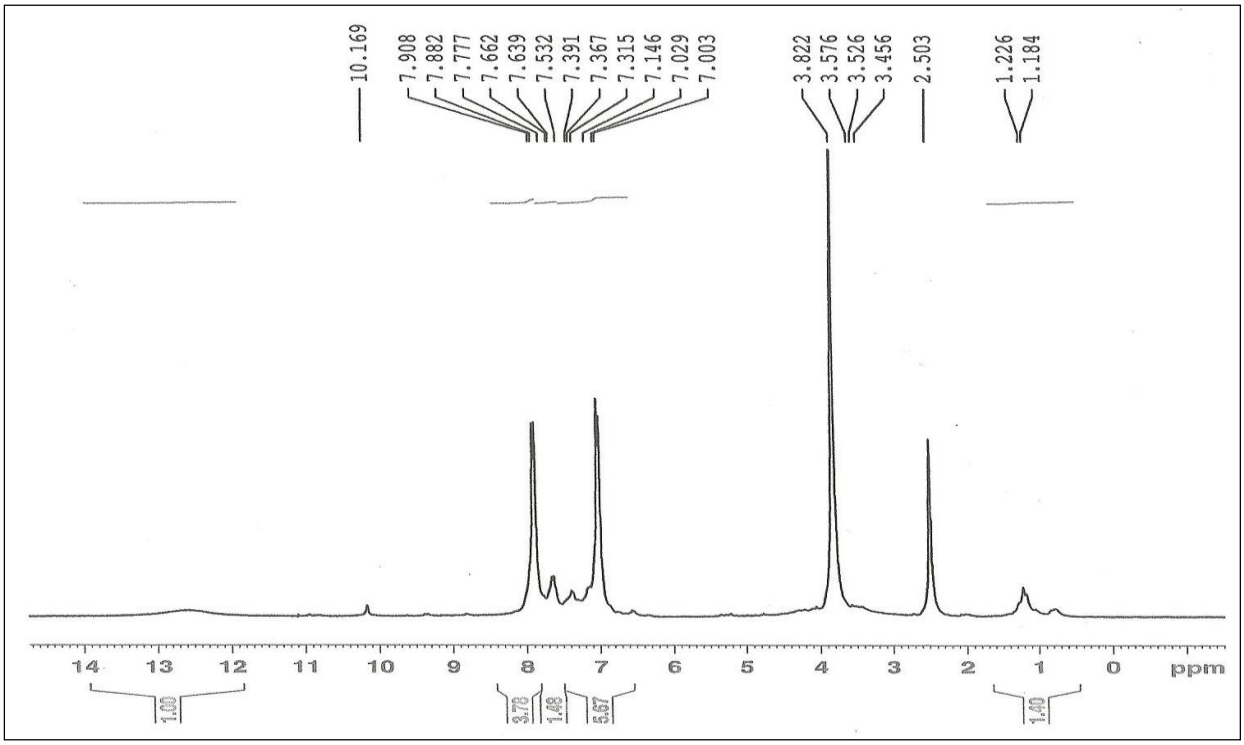


Fig. (5) ^{1}HNMR spectrum of compound (VII).

Biological study

The results of the antibacterial screening of the Schiff bases at a concentration of 20mg/ml against all bacteria have been found. The inhibition zones were measured in mm and results are shown in Table (3). The results of antibacterial screening, indicate that Schiff bases show significant activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus* and *Sallemonela* while compounds VI, VII, and X were found to be more active against all tested bacterial strains because of the presence of hydroxyl group in the compound (VI) which itself is active against microbes.

Table (3)
Antibacterial activity of compounds (V) and (VI-X).

Comp. No.	*S. aureus*	*E. coli*	*P. aeruginosa*	*Streptococcus*	*Sallemonela*
V	-	-	-	-	-
VI	+	++	+	+	+
VII	+	+	+	+	+
VIII	-	-	-	-	-
IX	-	+	-	-	-
X	+	+	+	+	+

- : 0 mm, + : 5 mm, ++ : 10 mm

Conclusion

Schiff bases derived from D-erythroascorbic acid were successfully synthesized from reaction of aldehyde (pentulosono-γ- lactone- 2,3- enedianisoate) with different amines. Schiff bases (VI, VII and X) exhibited activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus* and *Sallemonela* while compounds (V, VIII and

IX) have no effect on these bacteria except compound (IX) showed activity against *Escherichia coli*.

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الخلاصة

تم تحضير عدد من قواعد شف الجديدة المشتقة من حامض الارثرواسكوربيك وذلك من تكثيف مركب (٥) (بنتيولوسونو- كاما -لاكتون - ٢،٣ ين ثنائي انيسويت) مع ٣- امينو فينول، (٤- ايودو، ٣- ميثوكسي، ٤- مثيل، ٤- نايترو) انيلين بوجود حامض الخليك الثلجي والبنزين الجاف. شخصت المركبات المحضرة بالطرق الطيفية طيف الاشعة تحت الحمراء، وبعضها بواسطة طيف الاشعة فوق البنفسجية والمرئية، طيف الرنين النووي المغناطيسي, طيف الكتلة و قياس بعض خصائصها الفيزيائية وتقييم الفعالية الحيوية لها.

الكلمات المفتاحية: قواعد شف، ايمين، الفعالية الحيوية، حامض الارثرواسكوربيك.