Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Synthesis, Characterization, *In Vitro* Antioxidant and Anti-diabetic Activity of Schiff Bases Derived from Pyrrole-2-carbaldehyde

A Charles[1], K. Sivaraj[2*]

[1]Research and Development Centre, Bharathiar University, Coimbatore-641 046, Tamil Nadu; email:charlesa2020@gmail.com

[2]Department of Chemistry, Tamilavel Umamaheswaranar Karanthai Arts College, Thanjavur-613002, Tamil Nadu; email: sivaa25@gmail.com

ABSTRACT

A series of new pyrrole-2-carbaldehyde based Schiff base derivatives, have been synthesized and characterized by IR, NMR spectral studies. Synthezied Schiff base ligands were screened for *in vitro* anti-diabetic and antioxidant activity. The compounds were screened for their antidiabetic activity against α-amylase enzyme and compared with standard drug acarbose. *In vitro* antioxidant activity was evaluated by DPPH radical scavenging method and compared with standard ascorbic acid.

KEY WORDS: Schiff base,pyrrole-2-carbaldehyde, antioxidant, antidiabetic, α-amylase

*** Corresponding author:**

Dr. K. Sivaraj

Department of Chemistry,

Tamilavel Umamaheswaranar Karanthai Arts College,

Thanjavur- 613002, Tamil Nadu.

Email id:sivaa25@gmail.com

1. INTRODUCTION

Schiff bases are classified as organic ligands derived from the condensation reactions of primary or secondary amines and corresponding aldehydes or ketones (RCH=NR, where R and R represent alkyl/or aryl substitutes).[1]Schiff bases are considered a very important class of organic ligands possessing diverse applications.[2,3]Free radicals particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) have a greater impact on humans both within the body and from the environment. It is now universally accepted that free radicals have a great impact on humans in the etiology of various diseases like cancer, liver injury, cardiovascular diseases[4], diabetes, neurodegenerative and rheumatism diseases[5] atherosclerosis[6] autoimmune disorders and aging.[7] Although, the body possesses defense mechanisms as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS[8,9], continuous exposure to contaminants and chemicals may increase the amount of free radicals in the body beyond its ability to control and cause irreversible oxidative damages.[10] Therefore, antioxidants with free radical scavenging potential may be relevant in the therapeutic and preventions of diseases where free radicals are implicated.[11] Metal complexes of Schiff bases have been extensively investigated because of their industrial, antifungal, antibacterial, anticancer and herbicidal applications.[12-14] The study of Schiff bases ligands and their metal complexes is by far too large to be fully reviewed.[15] Schiff bases have been used as chelating ligands in coordination chemistry, in anti-oxidative activity, anti-bacterial activity, catalysis, medicine as anti-inflammatory, antibiotics, and in industry for anti-corrosion properties.[16]Oxidative stress is one of the major causes for disease burden in diabetes and related metabolic disorders and antioxidant supplementation is proven to be beneficial. Oxidative stress has also been known to play a pivotal role in the development of diabetes and diabetes-induced complications. Under a diabetic condition, oxidative stress causes a significant reduction in the antioxidant level of the cells, deactivates critical antiatherosclerotic enzymes, alters the structural functions of type IV collagen, and increase proteins glycation.[17] Oxidative stress has a significant effect on glucose transporters and in insulin receptor activity.[18]So, the primary focus of this paper is to analyse the Schiff bases on the *in vitro* inhibitory activity of α-amylase as well as the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging antioxidant properties. Overall, our data will assist researchers in predicting the physicochemical property in order to develop a pharmaceutically active molecule, to combat diabetic-related diseases worldwide.

2. MATERIALS AND METHODS

The chemicals and solvents used in this work were of Analar grade. All the glassware used were washed thoroughly with distilled water and dried in an oven.

Infrared Spectrophotometric Measurement:The infrared spectra of all complexes were recorded in KBr disc on a Shimadzu double beam infrared spectrophotometer and measuring the relative intensity of transmitted light energy versus wave number in the region of 4000-400 cm^{-1}.

^{1}H-NMR Spectrometric Measurement:The proton NMR spectra of the ligand and complexes were recorded using acetone as a solvent in Brucker EXT40178, 400MHz NMR spectrometer.

***Synthesis of N-((1H-pyrrol-2-yl)methylene)aniline Schiff base (Compound 1) (Fig.1)*:** Schiff base was prepared by condensation of pyrrole-2-carbaldehyde(0.01mol) with aniline (0.01 mol)in alcoholic medium and the mixture was refluxed for 4-5 hours at 50-60 ºC.^{1}H-NMR:δ 7.088-7.182 (m, 4H, Ar-H), signal at δ 8.335 ppm assigned for azomethine proton.

Synthesis of N-((1H-pyrrol-2-yl)methylene)-4-methylanilineSchiff base (Compound 2) (Fig.2): Schiff base was prepared by condensation of pyrrole-2-carbaldehyde(0.01mol)with p-toluidine (0.01 mol)in alcoholic medium and the mixture was refluxed for 4-5 hours at 50-60 ºC.^{1}H-NMR:δ 7.065-7.165 (m, 4H, Ar-H).

Synthesis of N-((1H-pyrrol-2-yl)methylene)-4-methoxyaniline Schiff base (Compound 3) (Fig.3): Schiff base was prepared by condensation of pyrrole-2-carbaldehyde(0.01mol) with 4-methoxyaniline (0.01 mol)in alcoholic medium and the mixture was refluxed for 4-5 hours at 50-60 ºC.

Synthesis of N-((1H-pyrrol-2-yl)methylene)-3,5dimethylaniline Schiff base (Compound 4) (Fig.4):Schiff base was prepared by condensation of pyrrole-2-carbaldehyde(0.01mol)with 3,5-dimethylaniline (0.01 mol)in alcoholic medium and the mixture was refluxed for 4-5 hours at 50-60 ºC.^{1}H-NMR:δ 2.505 (s, -CH_3), 2.507(s, -CH_3);6.134- 6.810 (pyrrolyl -CH) 7.009 – 7.369(Ar-H); 8.272 (s, CH=N).

After checking with TLC ,the resultant products were recrystallized with ethanol and taken for further characterization.

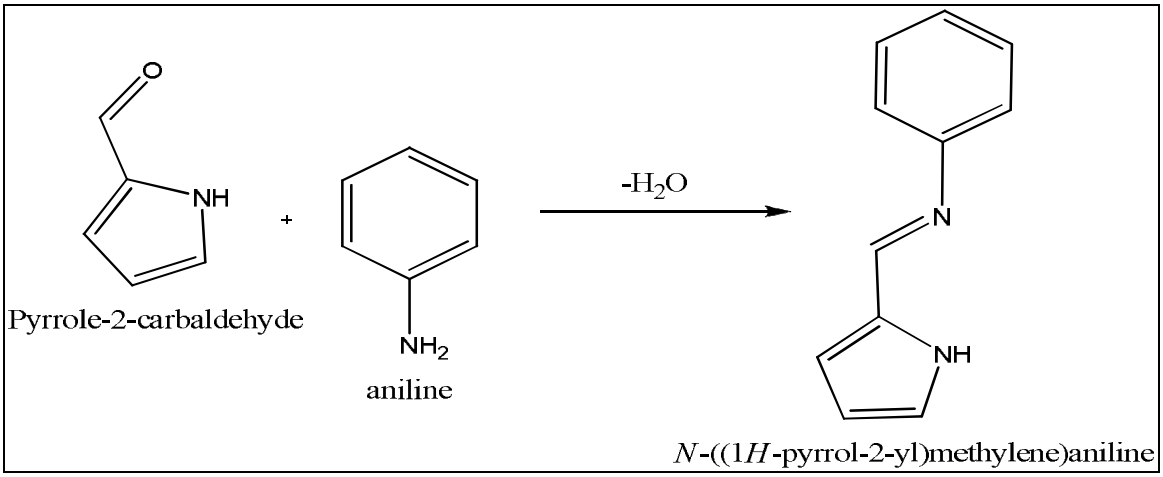

Fig.1: Synthesis of *N-((1H-pyrrol-2-yl)methylene)aniline Schiff base*(Compound 1)

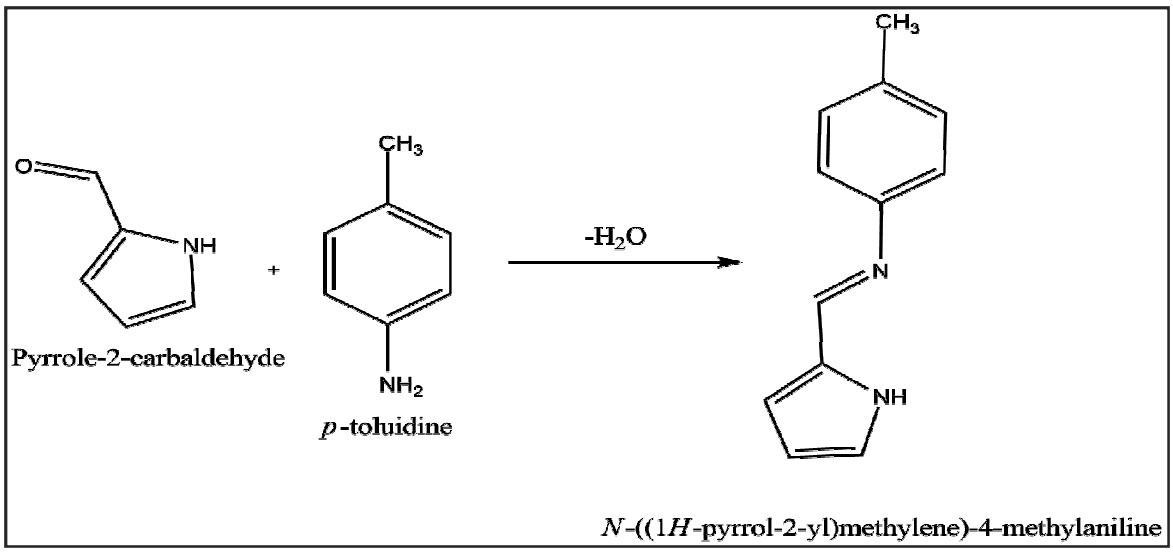

Fig.2: Synthesis of *N-((1H-pyrrol-2-yl)methylene)-4-methylaniline Schiff base (Compound 2)*

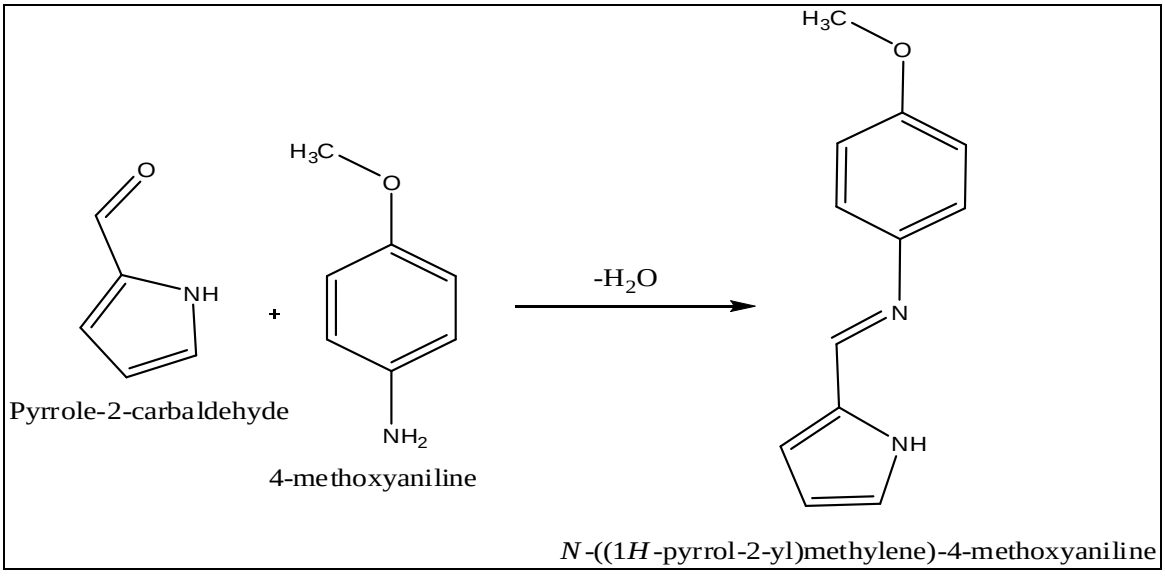

Fig.3: Synthesis of *N-((1H-pyrrol-2-yl)methylene)-4-methoxyaniline Schiff base (Compound 3)*

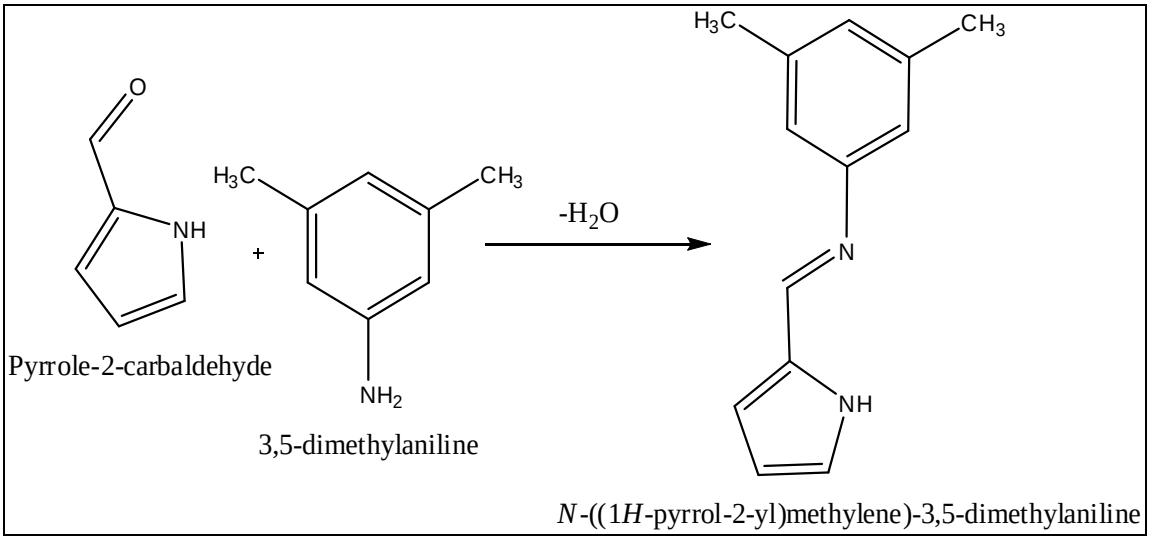

Fig.4: Synthesis of *N-((1H-pyrrol-2-yl)methylene)-3,5dimethylaniline Schiff base (Compound 4)*

DPPH Radical Scavenging:

The antioxidant assay of synthesized compounds against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was measured by UV spectrometer at λ 517 nm as described Shimada, et al., (1992).[19]A 2 ml aliquot of DPPH solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations (20, 40, 60 and 80 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The inhibition percentage (%) of radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = (Ac - As) / Ac \times 100$$

where Ac is the absorbance of the control reaction (containing all reagents except the test compound), and As is the absorbance of the test compound.

InvitroAnti-diabetic activity:

The effect of on α-amylase activity was determined according to the method described by Mellem*et al*., (2015) with some modification.[20] Preliminary experiments were conducted to establish optimal assay conditions such as temperature, substrate enzyme and inhibitor concentration. The anti-diabetic activity was investigated through the inhibition of α-amylase, an enzyme active in the digestion of starch, which thus reduces the absorption of glucose. Briefly 1 mL of each complex (dissolved in DMSO) was taken in pre-labelled test tubes. A volume of 20 µL of α-amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation 2 mL acetate buffer was added to each test tube, thereafter, 200 µL of 1% starch solution was added to each test tube and the mixture was reincubated for 1 h at 37 °C. Then 200 µL of 1% iodine solution was added to each test tube. Absorbance of the mixture was taken at 540 nm. Sample, substrate and α-amylase blank were undertaken under the same conditions. Each experiment was done in triplicate. Percent α-amylase inhibition was determined by the following equation:

$$\textbf{Percent Inhibition} = [(Ac - As)/ Ac] \times 100$$

where Ac is the absorbance of the control reaction (containing all reagents except the test compound)and As is the absorbance of the test compound. Acarbose was used for the standard reference.

Statistical analysis

Data were presented as mean ± SD. Microsoft Excel 2007 were used for the graphical and statistical evaluations.

3. RESULTS AND DISCUSSION

FT-IR spectra

The IR spectra [Table 1] of the synthesized Schiff base ligands show bands in the region 1616-1618 cm^{-1} which are assigned to υ(C=N) stretching vibration, which indicate the presence of azomethine group.[21,22] The band in the region 1409 - 1417 cm^{-1} corresponds to υ(C=C) stretching vibration due to phenyl group. IR spectrum of compound 3 shown in Fig.5 and ^{1}H NMR spectrum of compound 4 shown in Fig.6.

Table.1: IR Spectral data (cm^{-1})of ligand and their metal complexes

S.No	Schiff base	Frequency(cm^{-1})	
		υ(C=N)	**υ(C=C)**
1	*N-((1H-pyrrol-2-yl)methylene)aniline Schiff base* (Compound 1)	1618	1415
2	*N-((1H-pyrrol-2-yl)methylene)-4-methylaniline Schiff base (Compound 2)*	1618	1417
3	*N-((1H-pyrrol-2-yl)methylene)-4-methoxyaniline Schiff base (Compound 3)*	1616	1409
4	*N-((1H-pyrrol-2-yl)methylene)-3,5dimethylaniline Schiff base (Compound 4)*	1617	1411

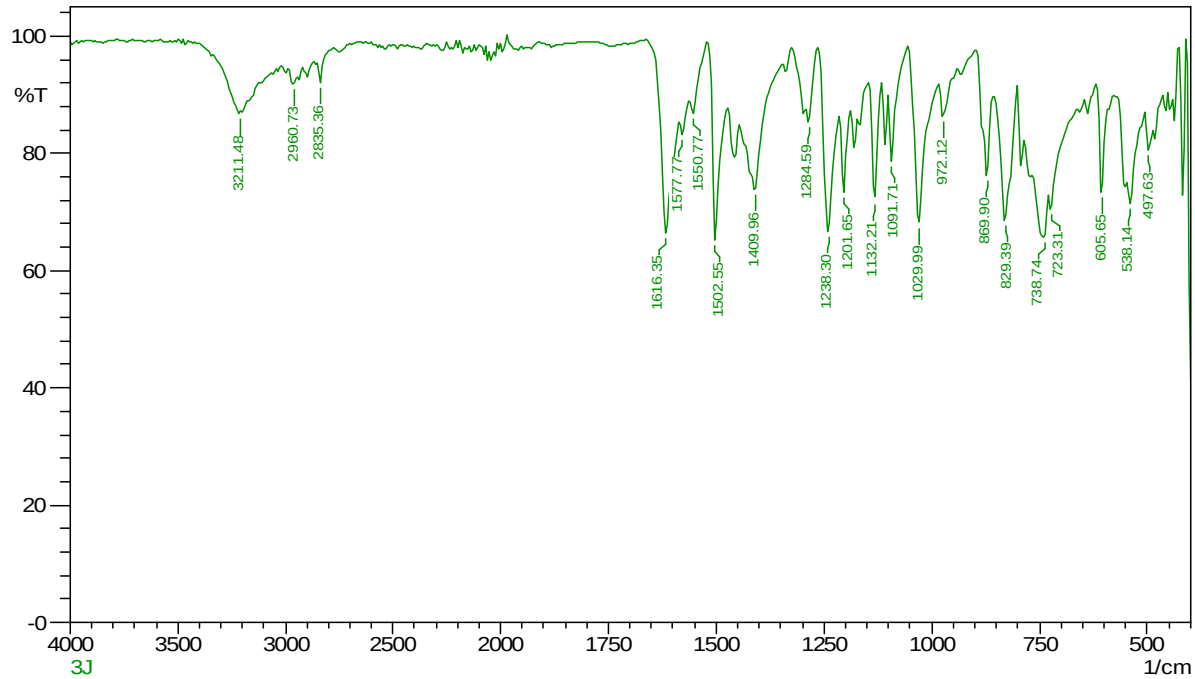

Fig.5 : IR spectrum of *N-((1H-pyrrol-2-yl)methylene)-4-methoxyaniline Schiff base (Compound 3)*

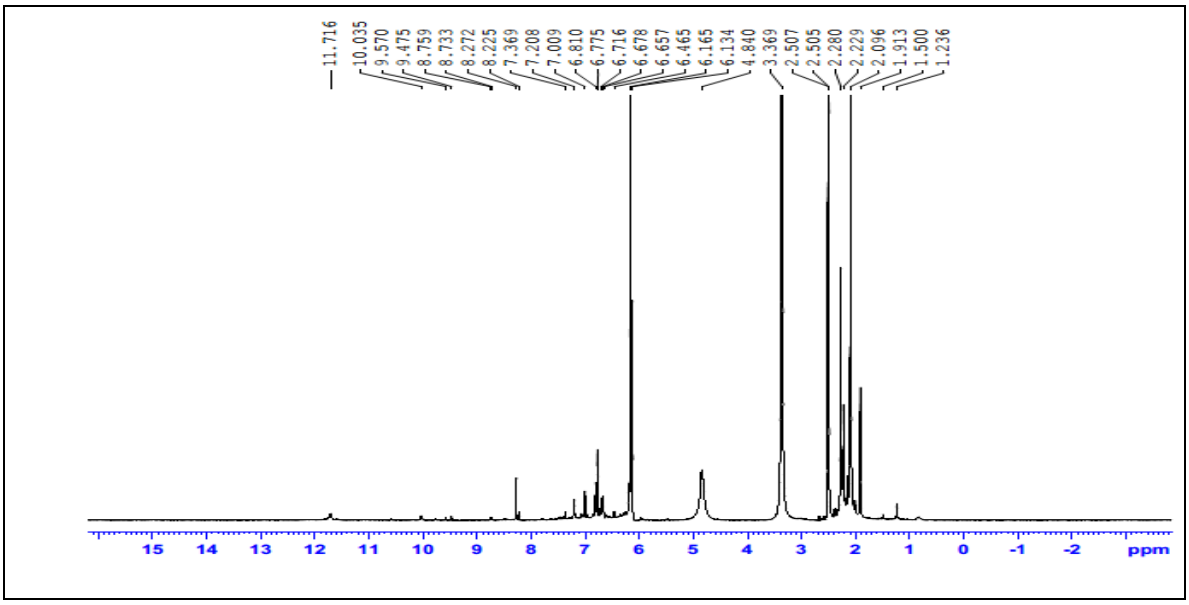

Fig.6: 1H NMR spectrum of *N-((1H-pyrrol-2-yl)methylene)-3,5dimethylaniline Schiff base (Compound 4)*

DPPH Radical scavenging activity

The free radical scavenging activity of the various concentrations of Schiff bases ligands were measured in vitro by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH• (2,2- diphenyl-1-picrylhydrazyl) with purple colour is a stable free radical. On scavenging, with antioxidants (AH) the purple colour of DPPH• reduce to yellow (DPPHH) is the basic principle utilized in this assay.[23] In the present study, the Schiff base ligands were investigated in comparison with the known antioxidant ascorbic acid. From the investigation it was clearly observed that Schiff base ligand and their metals complexes scavenge DPPH. A considerable increase in the percent of scavenging activity is found with increase in concentration of the compounds. The IC_{50} values for DPPH radicals of compounds 1-4 were found to be 113.62, 99.00, 134.82, 116.08 µg/ml respectively (Fig.7 and Table.2). The free ligand is found less efficient in decolorizing the pink color of the DPPH solution than with standard (26.84 µg/ml).

Table 2 : DPPH Radical scavenging activity of synthesized compounds at different concentrations

Parameters	20(µg/ml)	40(µg/ml)	60(µg/ml)	80(µg/ml)	IC_{50} (µg/ml)
Standard (Ascorbic acid)	41.08 ±0.12	65.32 ±0.94	84.30±0.90	98.30±0.20	26.84
N-((1H-pyrrol-2-yl)methylene)aniline Schiff base (Compound 1)	10.65±0.10	17.11±0.32	27.28±0.75	35.9±0.32	113.62
N-((1H-pyrrol-2-yl)methylene)-4-methylaniline Schiff base (Compound 2)	13.89±0.52	20.56 ±0.82	31.76±0.24	41.6±0.58	99.00
N-((1H-pyrrol-2-yl)methylene)-4-methoxyaniline Schiff base (Compound 3)	10.08±0.23	19.18±0.26	26.44±0.61	30.2±0.98	134.82
N-((1H-pyrrol-2-yl)methylene)-3,5dimethylaniline Schiff base (Compound 4)	10.34±0.11	21.16±0.78	29.08±0.82	34.24±0.14	116.08

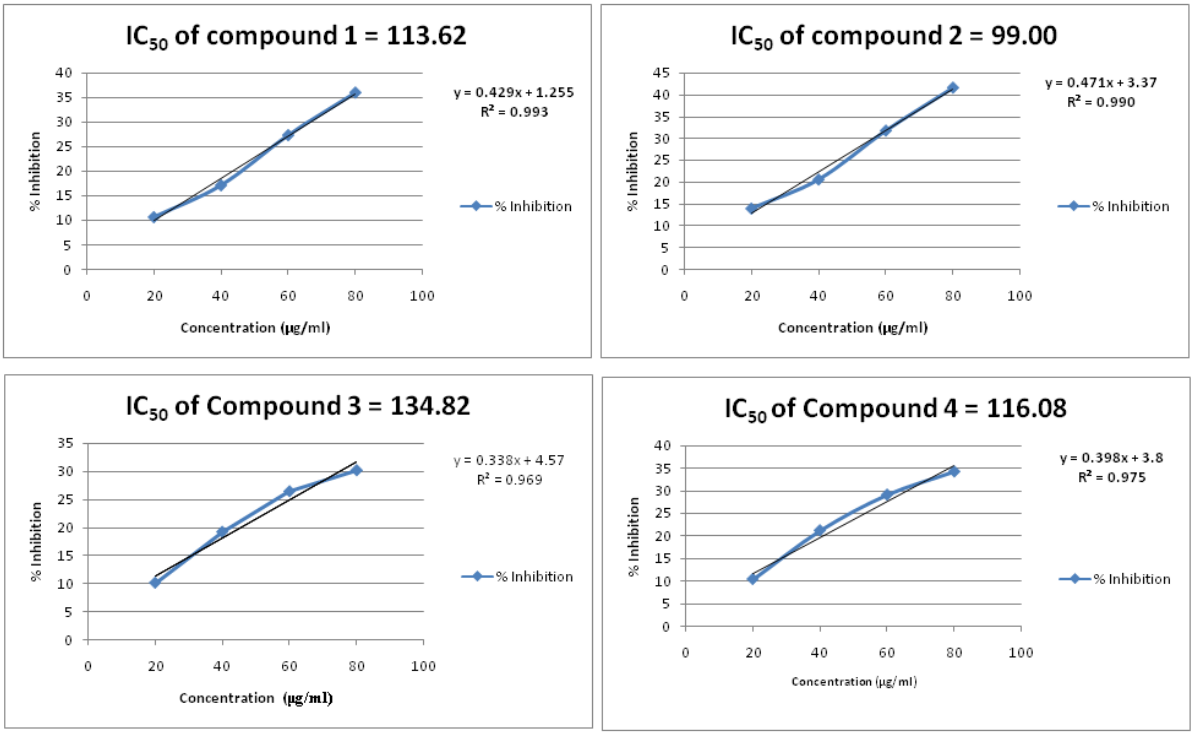

Fig.7: DPPH Radical scavenging activity of compounds 1-4

In vitroanti-diabetic activity :

The anti-diabetic activities were examined by the standard amylase inhibition assay. Inhibitory activity of synthesized compounds against alpha amylase is as shown in Table 3. A close observation of data reveals that all the compounds exhibit minimum inhibition efficiency compared to standard Acarbose.The results of *in vitro* antibacterial activity of the Schiff base ligands compounds 1-4were presented in Table 3. Acarbose was used as standard for this antidiabetic activity.

Table 3. *In vitro* anti-diabetic activity of the ligands and complexes

Sl.No.	Schiff base	% α-amylase inhibition
1	Standarad (Acarbose)	90.90
2	*N-((1H-pyrrol-2-yl)methylene)aniline Schiff base* (Compound 1)	45.12
3	N-((1H-pyrrol-2-yl)methylene)-4-methylaniline Schiff base (Compound 2)	39.90
4	*N-((1H-pyrrol-2-yl)methylene)-4-methoxyaniline Schiff base (Compound 3)*	48.06
5	*N-((1H-pyrrol-2-yl)methylene)-3,5dimethylaniline Schiff base* (Compound 4)	34.28

CONCLUSION

Schiff bases were synthesized and characterized by spectroscopictechniques**.** The synthesized compounds studied for their *in vitro* anti-diabetic antioxidant activity. The results from DPPH

method revealed that compounds are capable of donating electron or hydrogen atom and subsequently react with free radicals or terminate chain reactions in a dose-dependent pattern.The anti-diabetic study of these compounds may reduces the postprandial glucose level in blood by the inhibition of alpha-amylase enzymes, which can be an important strategy in management of blood glucose. Based on the result, it is clearthat these compounds can be used as antioxidants, antidiabetic drug in the field of medicinal and food industry.

REFERENCES

1. Kumar S, Dhar DN, Saxena PN. Applications of metal complexes of Schiff bases—a review. J. Sci. Ind. Res.2009; 68(3):181–187.
2. Gaballa AS, Asker MS, Barakat AS, Teleb SM. Synthesis, characterization and biological activity of some platinum(II) complexes with Schiff bases derived from salicylaldehyde, 2-furaldehyde and phenylenediamine. Spectrochimica Acta Part A: Mol. Biomol. Spect. 2007; 67(1):114–121.
3. Genin MJ, Biles C, Keiser BJ.Novel 1,5-diphenylpyrazole nonnucleoside HIV-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: lead identification and SAR of 3- and 4-substituted derivatives. J.Med. Chem. 2000; 43(5):1034–1040.
4. Liao KL, Yin MC. Individual and combined antioxidant effects of seven phenolic agents in humanerythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. J. Agri. Food Chem. 2000;48(6):2266-2270.
5. Halliwell B. Establishing the significance and optimal intake of dietary antioxidants: The biomarker concept. Nut. Review. 1999;57(4):104-113.
6. Wu Y, Hong C, Lin S, Wu P, Shiao M. Increase of vitamin Econtent in LDL and reduction of atherosclerosis in cholesterolfedrabbits by a water-soluble antioxidant rich fraction of Salviamiltiorrhiza. Arteriosclerosis Thomb Vas. Biol.1998;18(3):481–486.
7. Bandyopadhyay U, Das A, Bannerjee RK. Reactive oxygen species, Oxygen damage and pathogenesis. Curr. Sci. 1999; 77(5):658-666.
8. Halliwell B, Aeschbach R, Löliger J, Aruoma OI. The characterization of antioxidants. Food Chem.Toxicol. 1995;33(7):601-617.
9. Sies H. Strategies of antioxidant defense. European J. Biochem. 1993; 215(2):213–219.
10. Tseng TH, Kao ES, Chu CY, Chou FP, Wu Lin HW, Wang CJ. Protective effects of dried flower extracts of Hibiscus sabdariffa L. against oxidative stress in rat primary hepatocytes. Food Chem Toxicol.1997;35(12):1159-1164.

11. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis. Free Radical Res. 1997;26(5):469-478.
12. Prakash A, Adhikari D. Application of schiff bases and their metal complexes-A Review. Int.J.Chem.Tech.Res. 2011;3(4):1891-1896.
13. Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem.Toxicol.1995;33(12):1061-1080.
14. Arora M, Saravanan J, Shivaji M, Bhattacharjee S. Synthesis, characterization and antimicrobial activity of some schiff bases of 2-amino-n-(pacetamidophenylcarboxam)-4,5,6,7-tetramethylene thiophenes. Int. J. Pharm.Pharm Sci. 2013;5(1):315-319.
15. Inada Y, Mochizuki M, Tsuchiya T, Tsuji H. Funahashi S. Equilibrium and kinetics of the dinuclear complex formation between N, N'-ethylenebis (salicylideneiminato)copper(II) and metal(II,I) ions in acetonitrile. Inorganic Chimica Acta. 2005;358(11):3009–3014.
16. Yildiz M, Dulger B, Koyuncu SY, Yapici BM. Synthesis and antimicrobial activity of bis(imido) Schiff bases derived from thiosemicarbazide with some 2- hydroxyaldehydes and metal complexes. J. Ind.Chem. Soc. 2004;81(1):7–12.
17. LeBleu VS, MacDonald B, KalluriR. Structure and Function of Basement Membranes. Exp. Biol. Med. 2007;232(9):1121–1129.
18. Giacco F, Brownlee M. Oxidative stress, and diabetic complications. Circ. Res. 2010; 107(9):1058–1070.
19. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agri. Food Chem.1992; 40(6):945–948
20. Mellem J, Baijnath H and Odhav B. Antidiabetic potential of Brachylaena discolour. Afr J Tradit Complement Altern Med., 2015;12(1):38-44.
21. Boucher LJ, Day WV. Electrochemical Method, 2nd Edition, Willey New York; 1977: 6-13.
22. Silverstein RM, ClaytonBassler G, Terence Morrill C. Spectrometric identification of organic compounds. 5th Edition, John Wiley & Sons, Singapore, 1991; 1:124-127.
23. Ibrahim M, Khan A, Ikram M , Rehman S , Shah M , Nabi1 HU,Ahuchaogu AA. In Vitro Antioxidant Properties of Novel Schiff Base Complexes. Asian J Chem. Sci.2017; 2(2): 1-12.