

Anti oxidant, Anti Inflammation and Anti Diabetic Activity of Novel Schiff Base Derived from 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide and 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide

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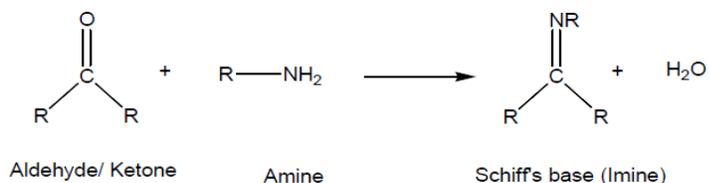
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ABSTRACT: A series of new 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide and 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide based Schiff base derivatives, have been synthesized. Synthesized Schiff base ligands were screened for anti-oxidant, anti-inflammation, and anti-diabetic activity. The compounds were screened for their Antioxidant activity was evaluated by DPPH radical scavenging method and compared with standard ascorbic acid. Anti-inflammation activity were examined by albumin denaturation and compared with standard aspine. Antidiabetic activity against α -amylase enzyme and compared with standard drug acarbose.

Keywords: Schiff base, antioxidant, anti-inflammatory and anti-diabetic agent etc.,

1. INTRODUCTION

A Schiff's base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine according to the following scheme (Scheme 1):



Scheme 1: Formation of Schiff's bases.

Where R, may be an alkyl or an aryl group. Schiff's bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Schiff base of aliphatic aldehyde are relatively unstable and readily polymerizable [1,2] while those of aromatic aldehydes having effective conjugation are more stable [3,4].

Schiff bases appear to be an important intermediate in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base. Schiff bases have been utilized as synthons in the preparation of a number of industrial and biologically active compounds via ring closure, cycloaddition, and replacement reactions.

A considerable number of Schiff-base has potential biological interest, not only have they played a seminal role in the development of modern chemistry, but also they can also be found at key points in the development of inorganic biochemistry, catalysis, optical materials and other field [5]. Anti bacterial activities of substituted Schiff bases like nitro & phenyl derivatives possess more active but activity was lesser than the standard drug. Nitro and halo derivatives of Schiff bases are reported to have antimicrobial and antitumor activities. Schiff bases of gossypol show high antiviral activity [6]. Several Schiff bases possess anti-inflammatory, allergic inhibitors reducing activity radical scavenging, analgesic and anti-oxidative action.

Schiff bases, derived mostly from variety of heterocyclic rings, were reported to possess a broad spectrum of pharmacological activities with a wide variety of biological properties [7]. Development of new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemist. They are known to exhibit a variety of potent activities. The pharmacologically useful activities include antibacterial, anticonvulsant, anti-inflammatory, anticancer, anti-hypertensive, anti-fungal, antipyretic, antimicrobial, anti-HIV, cytotoxic activity, hypnotic and herbicidal activities [8]. Metal complexes of Schiff bases have been reported and these are used as chelating agent in coordination chemistry of transition metals as radiopharmaceuticals for cancer targeting and agrochemicals [9].

Schiff bases are characterized by an imine group $-N=CH-$, which helps to clarify the mechanism of transamination and racemization reaction in biological system [10]. They are also used in the treatment for diabetes and AIDS. As biological models, they help in understanding the structure of bimolecular and biological processes occurring in living organisms. They participate; they are involved in the treatment of cancer drug resistance, and often tested as antimalarials. It could be also used for the immobilization of enzymes [11].

There are numerous publications covering the use of Schiff bases in therapeutic or biological applications either as potential drug candidates or diagnostic probes and analytical tools. The activity of Schiff bases as anticancer compounds [12,13] including radioactive nuclide complexes, antibacterial [14-20], antifungal [21,22], antiviral agents [23] has been extensively studied. Moreover, Schiff bases are present in various natural, semi-synthetic, and synthetic compounds (see Figure 2 for some examples) and have been demonstrated to be essential for their biological activities [24,25].

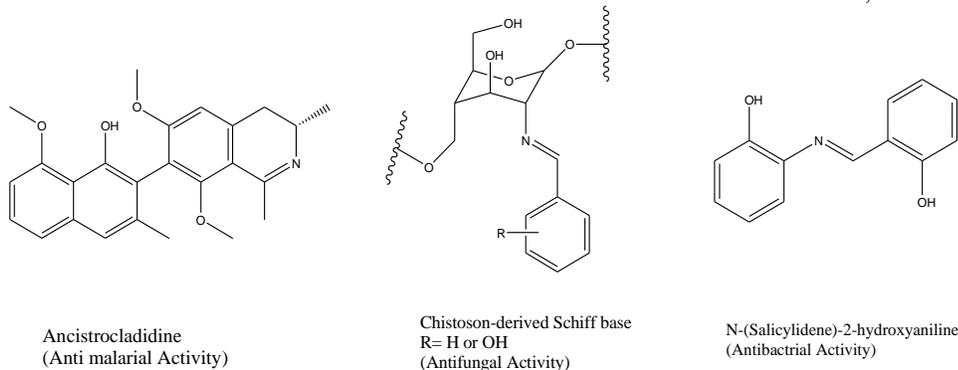


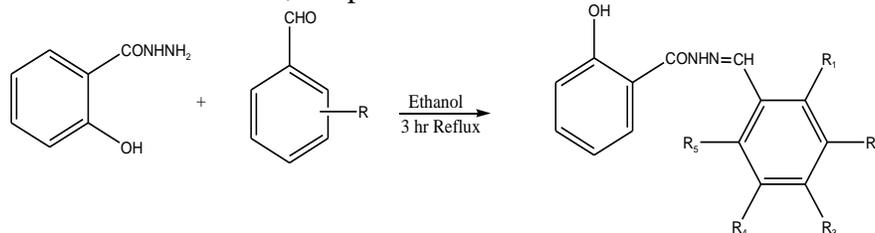
Figure 1. Some examples of biologically active Schiff bases.

2. MATERIALS AND METHODS

Synthesis of the Schiff Base. All reagents and solvents used were purchased from Sigma-Aldrich and were used without further purification. The starting compound was previously reported and was synthesized by us according to methodologies described in the literature

2.1 Schiff Base Preparation

The derivatives of 2-hydroxy benzoic acid benzylidene hydrazide is synthesized by using 2-hydroxy benzohydrazide different substituted aromatic aldehyde in the presence of ethanol. This reaction mixture is refluxed for 3 hours. The completion of reaction is checked by TLC. After completion of these reaction, the reaction mixture is poured into crushed ice, the product is obtained.



Compound	R ₁	R ₂	R ₃	R ₄	R ₅
1	H	OH	H	H	H
2	H	OH	OCH ₃	H	H

Table 1. Different substituted aromatic aldehyde

1.2 Anti-Oxidant Studies

DPPH scavenging assay

The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method of Mensor et al., (2001).

Reagents

2,2-Diphenyl-1-picryl hydrazyl (DPPH)[•] – 90.25mM in methanol in a dark room.

Procedure

To a methanolic solution of DPPH (90.25 mM), an equal volume of ethanolic Rhizome of *Cyperus rotundus* L (250-1500 µg) was added and made up to 1.0 mL with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Systronics UV-

visible Spectrophotometer. Ascorbic acid was used as standard for comparison. The inhibition of free radicals by DPPH in percentage terms (%) was calculated by using the following equation.

$$\% \text{ Scavenging} = \frac{A \text{ Control OD} - A \text{ sample}}{A \text{ blank5}} \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

2.3 Anti-inflammatory activity:

Inhibition of Albumen Denaturation

Method as prescribed (Sakat et al., 2010) was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100.

2.4 Inhibition Of Alpha-Amylase Enzyme

Starch solution (0.1% w/v) was prepared by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α -amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3,5-di nitro salicylic acid solution 96 mM. The starch solution is added to the both control and plants extract tubes and left to react with α -amylase solution, under alkaline conditions at 25°C. The reaction was allowed for 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540 nm (Malik and Singh 1980).

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

3. RESULT AND DISCUSSION

3.1 Anti-Oxidant Studies

DPPH scavenging assay method

There are several methods available to assess the antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant screening of schiff base. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.

In the present study, the derivatives of 2-hydroxy benzoic acid benzylidene hydrazide high DPPH scavenging capacity, which increased with increasing concentration [Table 2 and Figure 3]. It is evident from the data presented in Table, that the sample possesses DPPH assay activity. For the 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide [A1C], the result shows the percentage of cytotoxicity for 0.2 mg/ml as 15%, 0.4 mg/ml as 30% , 0.6 mg/ml as 34% , 0.8 mg/ml as 42% and 1.0 mg/ml as 49.48%. For the 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C], the result shows the percentage of cytotoxicity for 0.2 mg/ml as 26.4%, 0.4 mg/ml as 36.4% , 0.6 mg/ml as 46.2% , 0.8 mg/ml as 56.5% and 1.0 mg/ml as 66.2%. These inhibition values are compared with standard drug of **Ascorbic acid** for 0.2 mg/ml as 35%, 0.4 mg/ml as 40% , 0.6 mg/ml as 65% , 0.8 mg/ml as 80% and 1.0 mg/ml as 94%

The DPPH assay was carried out at different concentrations of the derivatives of 2-hydroxy benzoic acid benzylidene hydrazide namely 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml. As a part of the investigation on the mechanism of the antioxidant activity, ability of the derivatives of 2-hydroxy benzoic acid benzylidene hydrazide to inhibit DPPH scavenging assay was studied. The in-vitro study of anti-oxidant activity indicates that the inhibition percentage of DPPH scavenging assay by the 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C] higher.

S.No	Test	Concentration of the sample (mg/ml)	% of inhibition of the A1C	% of inhibition of the A2C	Ascorbic acid (Standard)
1	DPPH	0.2	15	26.4	35
2		0.4	30	36.4	40
3		0.6	34	46.2	65
4		0.8	42	56.5	80
5		1.0	49.48	66.2	94

Table.2 Anti Oxidant activity of Schiff base by DPPH Scavenging assay activity.

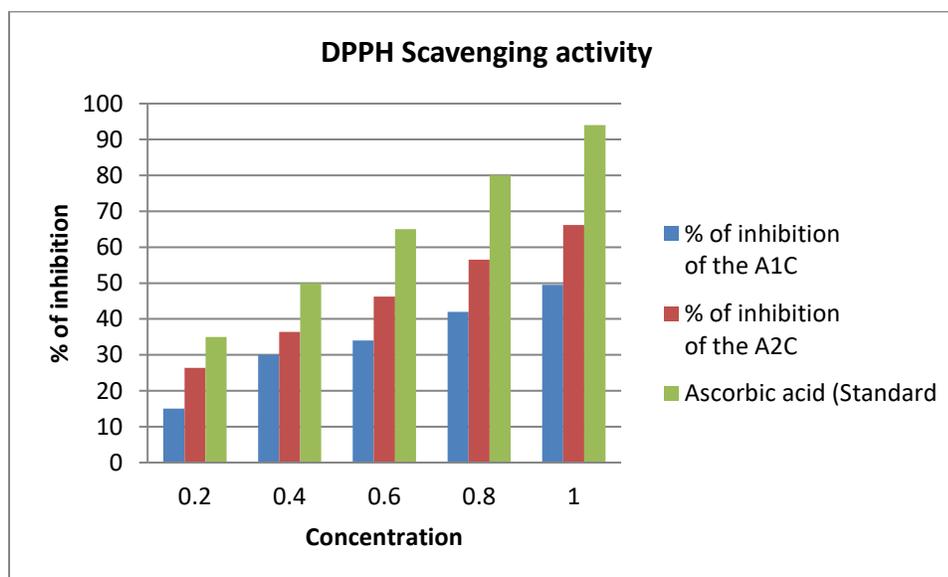


Fig.2 Graphical representation of Anti oxidant activity of Schiff base by DPPH Scavenging assay activity.

3.2 Anti- inflammatory activity

Inhibition of Albumen Denaturation method

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study, the protein denaturation bioassay was selected for in vitro assessment of the anti-inflammatory property

of the derivatives of 2-hydroxy benzoic acid benzylidene hydrazide. The Albumen Denaturation is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein. The mechanism of denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding. In the present study, denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the Schiff base to inhibit protein denaturation was studied. Selected Schiff base were effective in inhibiting heat-induced albumin denaturation. Aspirin was used as a standard antiinflammation drug as shown in Figure [Table 3 and Figure 4]. The albumin denaturation method was carried out at different concentrations of the derivatives of 2-hydroxy benzoic acid benzylidene hydrazide. samples, albumin denaturation, 100µg/ml 200µg/ml 300µg/ml 400µg/ml and 500 µg/ml.

For the 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide [A1C], the result shows the percentage of cytotoxicity for 100 µg/ml as 30%, 200 µg/ml as 35%, 300 µg/ml as 50% , 400 µg/ml as 53% and 500 µg/ml as 60%. For the 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C], the result shows the percentage of cytotoxicity for 100 µg/ml as 35%, 200 µg/ml as 45%, 300 µg/ml as 56% , 400 µg/ml as 64% and 500 µg/ml as 75.5%. These inhibition values are compared with standard drug of Aspirin for 100 µg/ml as 35%, 200 µg/ml as 46%, 300 µg/ml as 56% , 400 µg/ml as 67% and 500 µg/ml as 77%.

As a part of the investigation on the mechanism of the anti oxidant activity, ability of extract to inhibit Inhibition of Albumen Denaturation was studied. The in-vitro study of Anti- nflammatory activity indicates that the inhibition percentage of Albumen Denaturation by *Schiff base* of 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C] activity is higher than 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide [A1C].

S.No	Test	Concentration of the sample (µg/ml)	% of Protein Denaturation of the A1C	% of Protein Denaturation of the A2C	Aspirin (Standard)
1	Albumin denaturation	100	30	35	35
2		200	35	45	46
3		300	50	56	56
4		400	53	64	67
5		500	60	75.5	77

Table.3 Anti-inflammatory activity of Schiff base by albumin denaturation

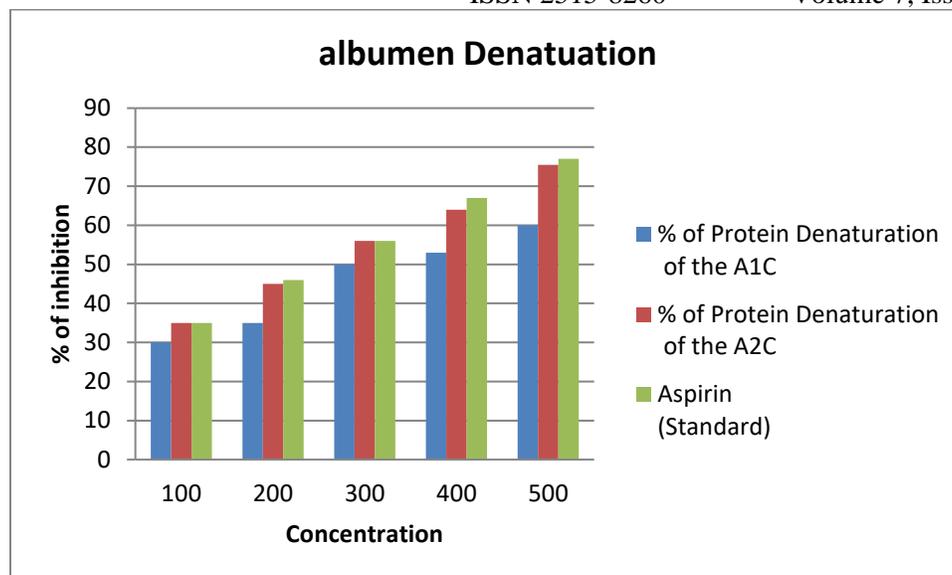


Fig.3 Graphical representation of Anti-inflammatory activity of Schiff base by albumin denaturation

3.3 Anti diabetic activity

Inhibition of Alpha-Amylase Enzyme

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. A therapeutic approach to decrease the hyperglycaemia is to inhibit the carbohydrate digesting enzymes (α -glucosidase and α -amylase), thereby preventing the breakdown of carbohydrates into monosaccharides which is a main cause of increasing blood glucose level. Therefore, developing compounds having inhibitory activities towards carbohydrate hydrolysing enzymes may be a useful way to manage diabetes. As shown in Figure 4 and Table 4, α -amylase and α -glucosidase were significantly inhibited in a dose-dependent manner by the -Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide [A1C] and 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C]. The results suggest that with the increased 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C] and 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide [A1C], concentration, the activity levels of enzyme were remarkably reduced, Hence, the biomolecules likely enhanced the antidiabetic potential of the synthesized compounds. α -Amylase inhibitory actions were observed in increasing order, as Acarbose (Figure 5). Comparable results were observed. However, the foregoing results suggest that the synthesized 2-hydroxy benzoic acid benzylidene hydrazide based Schiff base derivative are potentially better antidiabetic particles at inhibiting carbohydrate digesting enzymes, and could prove an effective approach in the diabetes care.

For the 2-Hydroxy benzoic acid (3-Hydroxy benzylidene)-Hydrazide [A1C], the result shows the percentage of cytotoxicity for 20 $\mu\text{g/ml}$ as 14%, 40 $\mu\text{g/ml}$ as 23%, 60 $\mu\text{g/ml}$ as 30% , 80 $\mu\text{g/ml}$ as 48% and 100 $\mu\text{g/ml}$ as 59%. For the 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C], the result shows the percentage of cytotoxicity for 20 $\mu\text{g/ml}$ as 20.%, 40 $\mu\text{g/ml}$ as 30.5%, 60 $\mu\text{g/ml}$ as 41.5% , 80 $\mu\text{g/ml}$ as 50.5% and 100 $\mu\text{g/ml}$ as 60.5%.

These inhibition values are compared with standard drug of Acarbose for 20 $\mu\text{g/ml}$ as 44%, 40 $\mu\text{g/ml}$ as 54%, 60 $\mu\text{g/ml}$ as 68% , 80 $\mu\text{g/ml}$ as 78% and 100 $\mu\text{g/ml}$ as 90%. The Alpha-Amylase Enzyme was carried out at different concentrations of derivatives of 2-hydroxy benzoic acid benzylidene hydrazide namely Alpha-Amylase Enzyme 20 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$, 60 $\mu\text{g/ml}$, 80 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$.

Albumen Denaturation did not show any significant difference at 20 µg/ml and 40µg/ml, Schiff base, however, it was significant for 0.15µg/ml, 0.20µg/ml and 0.25 µg/ml for the nanoparticles, all the values are compared with standard drug of Acarbose (Figure 5). Antidiabetic activity of synthesized A2C based on inhibition of α-amylase and activity.

As a part of the investigation on the mechanism of the Anti diabetic activity, ability of Schiff base to Inhibition of Alpha-Amylase Enzyme was studied. The in-vitro study of Anti diabetic activity indicates that the inhibition percentage of Alpha-Amylase Enzyme by activity is 2-Hydroxy benzoic acid (3-Hydroxy -4-methoxy benzylidene)-Hydrazide [A2C], higher.

S.No	Test	Concentration of the sample (µg/ml)	% of Protein Denaturation of the A1C	% of Protein Denaturation of the A2C	Acarbose (Standard)
1	Alpha amylase inhibitory activity	20	14	20	44
2		40	23	30.5	54
3		60	30	41.5	68
4		80	48	50.5	78
5		100	59	60.5	90

Table.4 Anti diabetic activity of Schiff base by Alpha amylase inhibitory activity

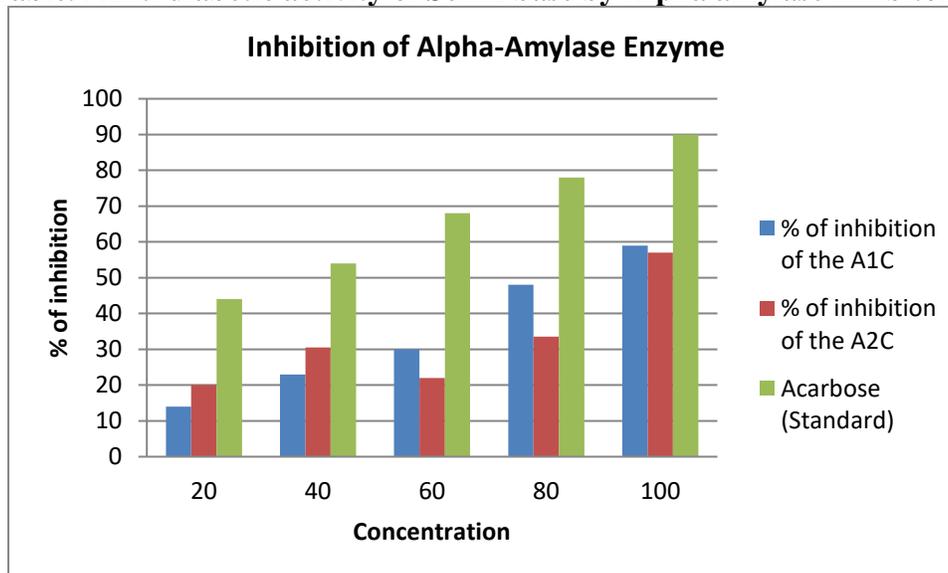


Fig.4 Graphical representation of Anti diabetic activity of Schiff base by Alpha amylase inhibitory activity

4. CONCLUSION

The condensation of 2-hydroxy benzohydrazide different substituted aromatic aldehyde (3-Hydroxy benzaldehyde, 3-Hydroxy-4-methoxy benzaldehyde). The Compound is synthesized by simple condensation in ethanol. Schiff bases were synthesized and characterized. The synthesized compounds studied for their *in vitro*

antioxidant, anti-inflammatory and anti-diabetic activity. The DPPH assay is the most acceptable, fastest and simplest method for the calculation of the free radical scavenging activity. As shown in the Table 2 and Figure 3. The A2C shows better antioxidant property than the standard ascorbic acid with an IC₅₀ values. 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.

Denaturation of proteins is a well-documented cause of inflammation. Phenylbutazones, salicylic acid, flufenamic acid (anti-inflammatory drugs), have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. The in-vitro study of a anti-inflammatory activity indicates that the inhibition percentage of derivatives of 2-hydroxy benzoic acid benzylidene hydrazide α -amylase is a key enzyme in carbohydrate metabolism. Inhibition of α -amylase is one of the strategies for treating diabetes. Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. The anti-diabetic study of these compounds may reduce 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 clear that these compounds can be used as antioxidants, antidiabetic drug in the field of medicinal and food industry.

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