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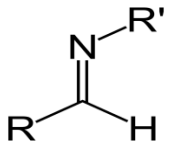
Protease inhibiting and other biological activities of Schiff base derivatives

BY Sathish Kumar

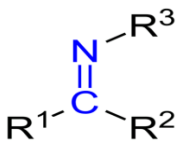
Abstract
Schiff bases exhibit useful biological activities such as anti-inflammatory, analgesic, antimicrobial, antifungal, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic, antiglycation, and antidepressant activities. Schiff bases are also used as catalysts, pigments and dyes, intermediates in organic synthesis, polymer stabilizers, and corrosion inhibitors. Schiff bases, an important group of compounds play a vital role in pharmaceutical as well as in clinical fields. During the last several years, coordination chemistry has been considerably enriched due to the synthesis and characterization of a large number of Schiff base compounds containing sulfur, nitrogen and oxygen as donar sites. Ligands containing N, O or S donars have been found to be useful as potential drugs. Schiff bases are also used as starting material for the synthesis of many bioactive organic compounds such as β-lactams Schiff bases derived from amino compounds have a variety of applications in biological, clinical, analytical and pharmacological areas. Due to its wide application we tested the Schiff base intermediates for its Protease inhibiting activity and is found to inhibit fungal protease and there by restricting its growth. The compounds also showed antioxidant, antimicrobial, antiangiogenic and less Cytotoxicity effects on human peripheral lymphocytes.

Keywords: Cytotoxicity, Schiff bases, antioxidant.

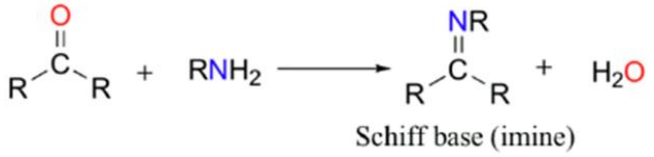
Introduction
A Schiff base, named after Hugo Schiff, is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group. Schiff bases in a broad sense have the general formula $R^1R^2C=NR^3$, where R is an organic side chain [1].



General structure of a Schiff base
Schiff base compounds containing characteristic –C=N- (azomethine) group, so called azomethines, with the general formula $RCH=NR^1$. [1, 2]



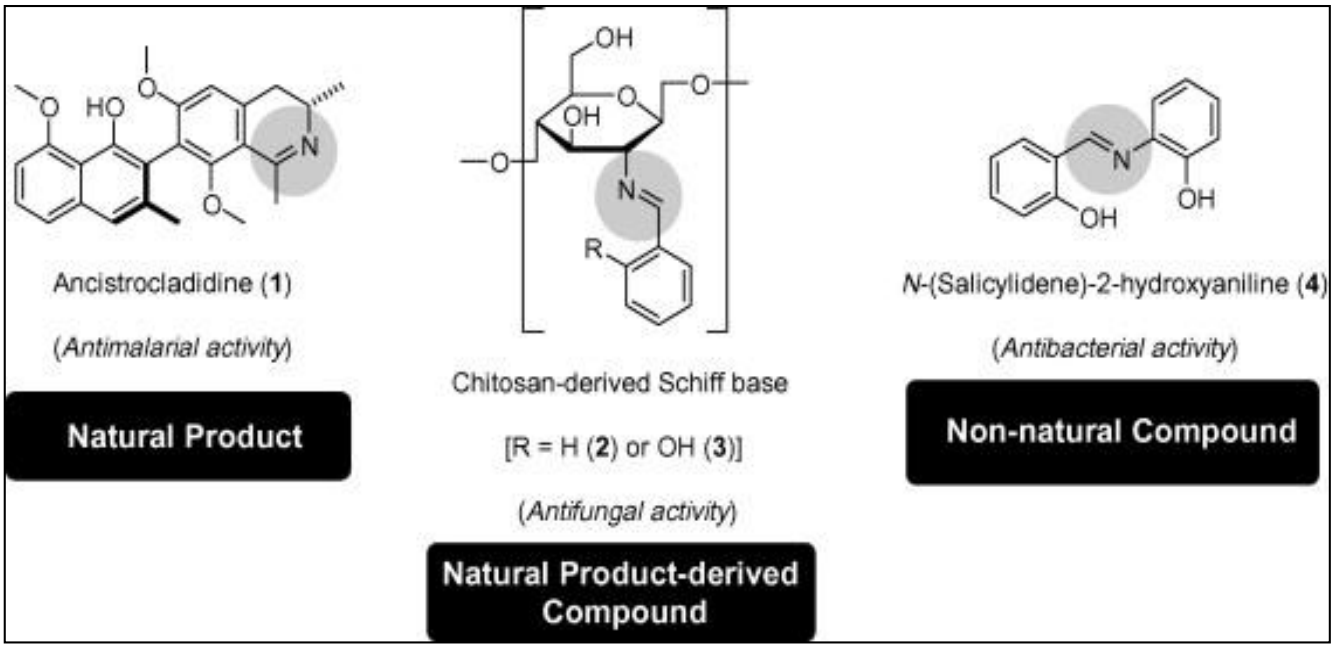
General structure of an azomethine
Schiff bases can also be referred to as imines. The chain on the nitrogen makes the Schiff bases a stable imine or the C=O double bond is replaced by C=N double bond, this type of compound is known as an imine, or Schiff's base [3].



Schiff base (imine)

Schiff bases are important compounds owing to their wide range of industrial applications. Schiff bases are used in the photostabilization of poly (vinyl chloride) polymers against photodegradation by ultraviolet radiation and are also used to improve poly(methyl methacrylate) from degradation and to prevent polystyrene from photodegradation by their addition to polymer films.

Examples of Schiff based derivatives



Biological Importance of Schiff Bases

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. Schiff bases are condensation products of primary amines with carbonyl compounds gaining importance day by day in present scenario. Schiff bases are the compounds carrying imine or azomethine (-C=N-) functional group and are found to be a versatile pharmacophore for design and development of various bioactive lead compounds.

Schiff bases exhibit useful biological activities such as anti-inflammatory, analgesic, antimicrobial, antifungal, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic, antiglycation, and antidepressant activities. Schiff bases are also used as catalysts, pigments and dyes, intermediates in organic synthesis, polymer stabilizers, and corrosion inhibitors [4].

Schiff bases have also used in new kind of chemotherapeutic studies. The literature revealed that a flexible ligand system may be obtained by condensation with a variety of reagents like aldehydes, keones, thiosemicarbazides and carbazides which have much more biological activity.

Antioxidants

A substance that reduces damage due to oxygen such as that caused by free radicals. It is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reaction can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid (vitamin C) or polyphenols.

Well-known antioxidants include enzymes and other substances, such as vitamin C, vitamin E, and beta carotene, which are capable of counteracting the damaging effects of oxidation. These are also commonly added to food products such as vegetable oils and prepared foods to prevent or delay their deterioration from the action of air. They may possibly reduce the risks of cancer. Antioxidants clearly slow the progression of age-related macular degeneration.

Antioxidants are man-made or natural substances that may prevent or delay some type of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidant include: β-carotene, lutein, lycopene, selenium, vitamin-A, vitamin-C, vitamin-E. Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases.

High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin-E may increase risks of prostate cancer and one type of stroke. Antioxidant supplements may also interact with some medicines [5, 6].

Antimicrobial Activity

The word antimicrobial was derived from the Greek words anti (against), mikros (little) and bios (life) and refers to all agents that act against microbial organisms.

This is the destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms. These are the agents that Kills microorganisms, or suppresses their multiplication or growth. Antimicrobial agents are

classified functionally according to the manner in which they adversely affect a microorganism. Some interfere with the synthesis of bacterial cell wall. This results in cell lysis because the contents of the bacterial cell are hypertonic and therefore under high osmotic pressure. A weakening of the cell wall causes the cell to rupture, spill its contents, and be destroyed. The penicillins, cephalosporins and bacitracin are examples of this group of antimicrobials.

A second group of antimicrobial agents interfere with the synthesis of nucleic acids. Without DNA and RNA synthesis a microorganism cannot replicate or translate genetic information. Examples of antimicrobials that exert this kind of action are griseofulvin, fluoroquinolones and *rifampicin.* A third group of antimicrobial agents change the permeability of the cell membrane, causing a leakage of metabolic substrates essential to the life of the microorganism. Their action can be either bacteriostatic or bactericidal. Examples include amphotericin B and polymyxin B.A fourth group of antimicrobial agents interfere with metabolic processes within the microorganisms. They are structurally similar to natural metabolic substrates, but since they do not function normally, they interrupt metabolic processes. Most of these agents are bacteriostatic. Examples include the sulfonamides, aminosalicylic acid and isoniazid. A fifth group interference with translation of protein by the ribosome. This action may be bacteriocidal or bacteriostatic. Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection. Many antimicrobial agents exist, for use against a wide range of infectious diseases [7-10].

Anti Angiogenic Activity

Angiogenesis, the development of new blood vessels from an existing vasculature, is essential in normal development processes and in numerous pathologies, including diabetic retinopathy, psoriasis and tumor growth and metastases. One of the problems faced by angiogenesis researches has been the difficulty of finding suitable methods for assessing the effects of regulators of the angiogenic response. The ideal assay would be reliable, technically straightforward, easily quantifiable and most importantly, physically relevant [11].

More than 18,000 research articles have been published in the last 40years describing the role of angiogenesis (sprouting of new blood vessels from an existing vascular network) in the onset and progression of such diseases as cancer, rheumatoid arthritis, psoriasis and diabetes [12].

Protease Inhibition Activity

A protease (also known as peptidase or proteinase) is any enzyme that performs proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in a polypeptide chain. Proteases have evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms. Protease can be found in animals, plants, bacteria, archaea and viruses.

An up to date classification of protease evolutionary super families is found the MEROPS database [13, 14]. Neutral protease involved in type1 hypersensitivity. Here it is released by mast cells and causes activation of complement and kinins [15].Bacteria secrete proteases to hydrolyse the peptide bonds in proteins and therefore break the proteins down into their constituent monomers. Bacterial and fungal proteases particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated by nutritional signals in these organisms. The net impact of nutritional regulation of protease activity among the thousands of species present in soil can be observed at the overall microbial community level as proteins are broken down in response to carbon, nitrogen, or sulfur limitation [16].

The field of proteases research is enormous. In 2004, approximately 8000 papers related to this field were published each year. Proteases are used in industry, medicine and as a basic biological research tool [17].

Cytotoxicity

Cytotoxicityis the quality of being toxic to cells. Examples of toxic agents are an immune cell or some types of venom. Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, shut down metabolism and release their contents into the environment. Cells that undergo rapid necrosis *in vitro* do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers [18]. Apoptosis is characterized by well-defined cytological and molecular events including a change in the refractive index of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments [19].

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen “hits” from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical. Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as tryphan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components [20]. Alternatively, membrane integrity can be assessed by monitoring the passage of substance that are normally sequestered inside cells to the outside [21].

2. Materials Methods

Antibacterial Activity

Antibacterial are used to treat bacterial infections. The toxicity to humans and other animals from antibacterial is generally considered low. Prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. After prolonged antibacterial use consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment as for recurrent *Clostridium difficile* infections.

The discovery, development and clinical use of antibacterials during the 20th centuary has substantially reduced mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed a golden period of discovery from about 1945 to 1970, when a

number of structurally diverse and highly effective agents were discovered and developed. However since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs.

Antibacterials are among the most commonly used drugs; however antibiotics are also among the drugs commonly misused by physicians, such as usage of antibiotic agents in viral respiratory tract infections. As a consequence of widespread and injudicious use of antibacterials, there has been accelerated emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. The resistance problem demands that renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibacterials.

Antioxidant Activity

DPPH Scavenging Activity Assay

The free radical scavenging activity of the drugs was measured *in vitro* by 2,2'-diphenyl-1-picryldrazyl (DPPH) assay according to the method described [22,23]. The stock solution was prepared by dissolving 24mg DPPH with 100ml methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517nm using the spectrophotometer. A 3ml aliquot of this solution was mixed with 100µl of the sample at various concentrations (10-500µg/ml). The reaction mixture was shaken well and incubated in the dark for 15min at room temperature. Then the absorbance was taken at 517nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Scavenging effect (\%)} = \frac{\text{(control absorbance-sample absorbance)}}{\text{(Control absorbance)}} \times 100$$

Nitric Oxide Radical Scavenging Activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which were measured using the Griess reaction [24].Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. 1ml of sodium nitroprusside (10mM) in phosphate buffered saline (0.2M, pH 7.4) was mixed with 100ml sample solution of various concentration (0,20,40,60,80,100 µg) and incubated at room temperature for 150mins. The same reaction mixture without the sample was used as the control. After the incubation period; 0.5ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediaminedihydrochloride) was added. The absorbance of the chromophore (pink colour) formed was read at 546nm [25].

Ferrous Ion Chelating Assay

Ferrous ion chelating ability was measured according to the method of Suter and Richtes. Three sets of test tubes were taken. One tube was taken as control, to this $FeCl_3$ (200µM) and $K_3Fe(CN)_6$ (400mM) were added and the volume was made up to 1ml by adding distilled water. For the second tube, EDTA (40mM), $FeCl_3$ (200µM) and $K_3Fe(CN)_6$ (400mM) were added and the volume was made up to 1ml by adding distilled water. For the third tube, drugs, $FeCl_3$ (200µM) and $K_3Fe(CN)_6$ (400mM) were added and the volume was made up to 1ml by adding distilled water. The tubes were incubated for 10min at 20°C and read absorbance at 700nm.

$$\text{Chelating activity} = \frac{\text{Absorbance of control- Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity Assay

Isolation of Human Peripheral Lymphocytes

The peripheral lymphocytes were isolated from 10 to 15ml of freshly drawn venous blood from healthy male donors aged between 22-26 years. Blood was collected in anticoagulant Acid Citrate Dextrose (ACD, 85mM Citric acid, 71mM trisodium citrate, 165mMD-Glucose) in the ratio of 5:1. To this four volumes of hemolysing buffer (0.85% NH_4Cl in 10mM Tris buffer, pH 7.4) was added, mixed well and incubated at 4°C for 30min. then the cells were centrifuged at 1200 rpm for 12min, the supernatant was discarded, pellet was washed thrice with 10ml of Hanks Balanced Salt Solution (HBBS, 137mM KCl, 8.5mm phosphate buffer pH 7.4, 0.8mm MgSO4 and 5mM D-Glucose) and suspended in the same buffer solution. Cells were suspended in HBBS and it's stored at 4°C [26].

Cell Viability Test

The cell viability was determined by tryphan dye blue exclusion method [27]. To 10µl of cell suspension an equal volume of 0.4% tryphan blue dye was added. The cells were then charged to haemocytometer and the cell number was counted. The dead cells being permeable to tryphan blue appeared blue against white color of the viable cells. The percentage of viability was calculated as follows;

$$\text{Percentage of Viability} = \frac{\text{number of viable cells}}{\text{Total number of cells}} \times 100$$

Anti Angiogenic Activity

Shell less chorioallantoic membrane assay is an angiogenic assay used for validation of angioinhibitory activity of any compound. The CAM assay to note the antiangiogenic activity was performed using the dendrimers. The antiangiogenic effect was studied according to the method of Auerbach *et al* (Auerbach R *et al*., 1974). Briefly, fertilized hens eggs were surface sterilized using 70% alcohol. The eggs were incubated in fan assisted humidified incubator at 37°C. on the 4th day, the eggs were cracked out into thin films of the hammock within a laminar flow cabinet and were further incubated. On the 5th day when blood vessels were seen proliferating from the centre of the eggs within the hammock, filter paper discs loaded with 100µg of dendrimers were placed over the proliferating blood vessels and the eggs were returned to the incubator. Results for antiangiogenic effect of the compounds were observed after 24hours.

Protease Inhibition Activity

Preparation of enzyme: *Aspergillus niger* was grown on a solid state fermentation. The broth from it is taken in a centrifuge tube and centrifuge at 1200rpm for 10min. Supernatant is discarded. Take the pellet and it is suspended in phosphate buffer. This is used as an enzyme.

Add 0.2ml of enzyme and drug at different concentrations (10, 20, 30, 40, 50, 100, 200, 400, 500) to the respective test tubes.0.5ml of 2% Substrate is added to all the tubes. Incubation at room temperature for 10min. 1.5ml of buffer (tris buffer 0.5M, Ph 8.5) is added. 2ml of 10% TCA and 1ml of supernatant is added. Incubate for time intervals (1hr, 2hr, 3hr). 2.5ml of 0.4M sodium bicarbonate and 1ml of FC reagent is added to all the test tubes. Incubate for 30min. measure the absorbance at 660nm.

3. Results

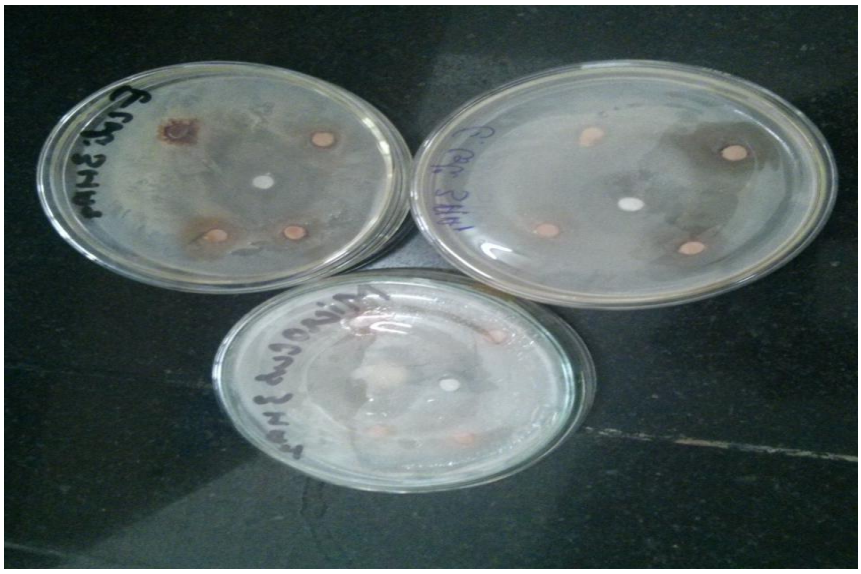


Fig 1: Antibacterial Activity

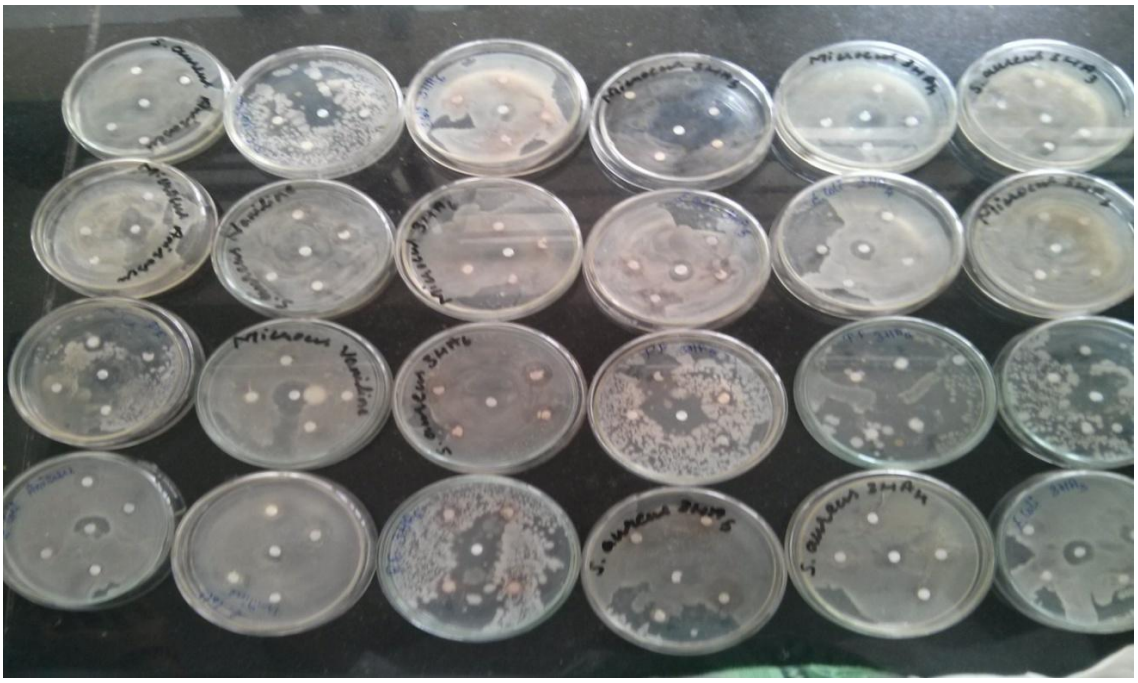
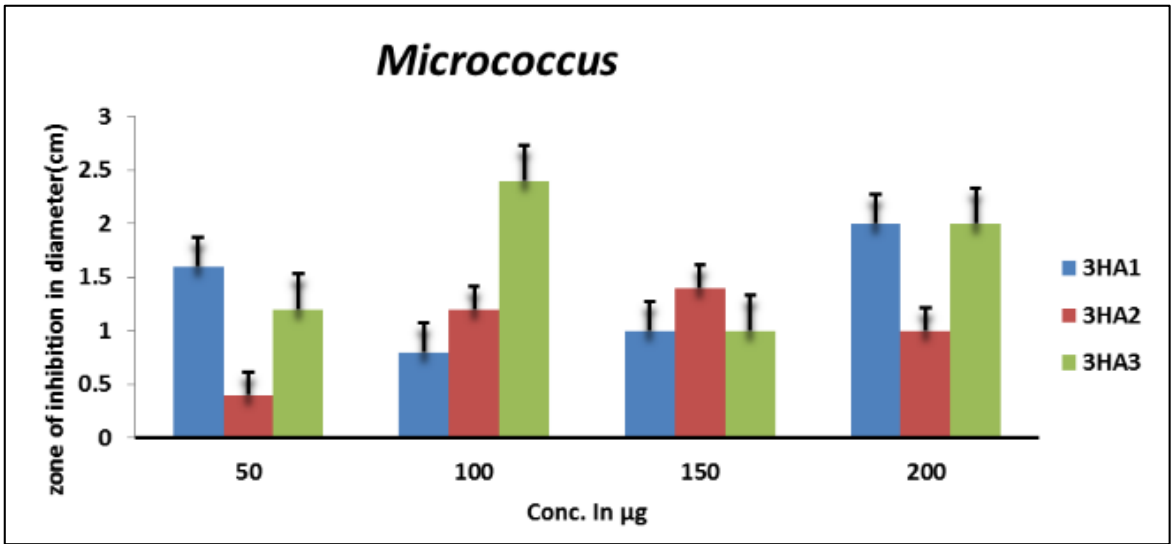
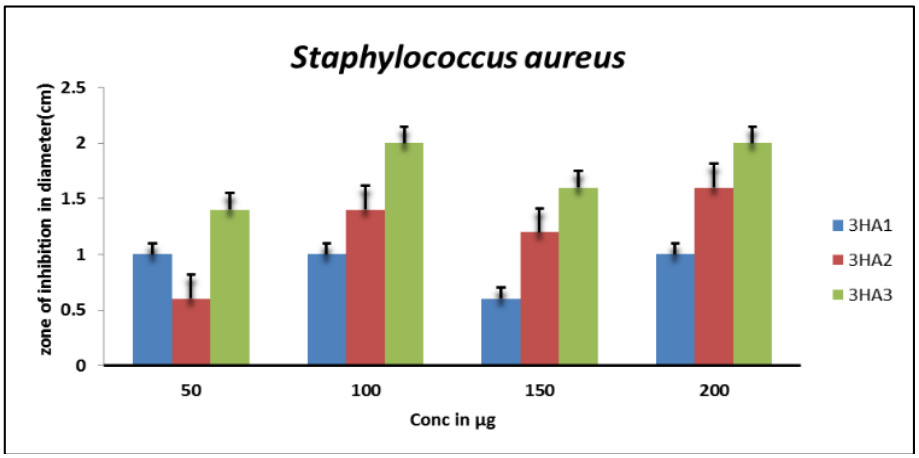


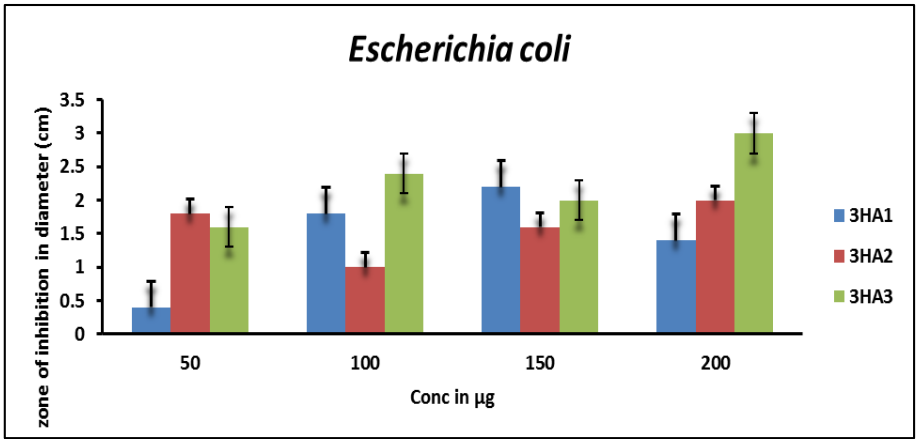
Fig 2



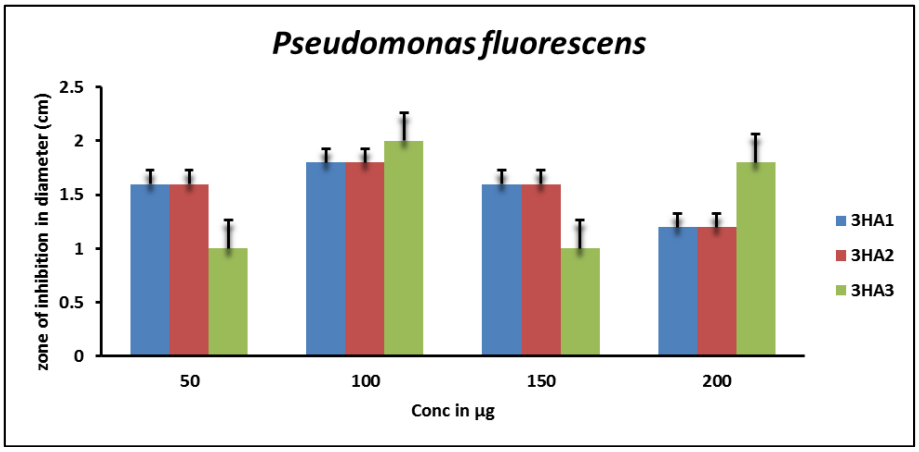
Graph 1



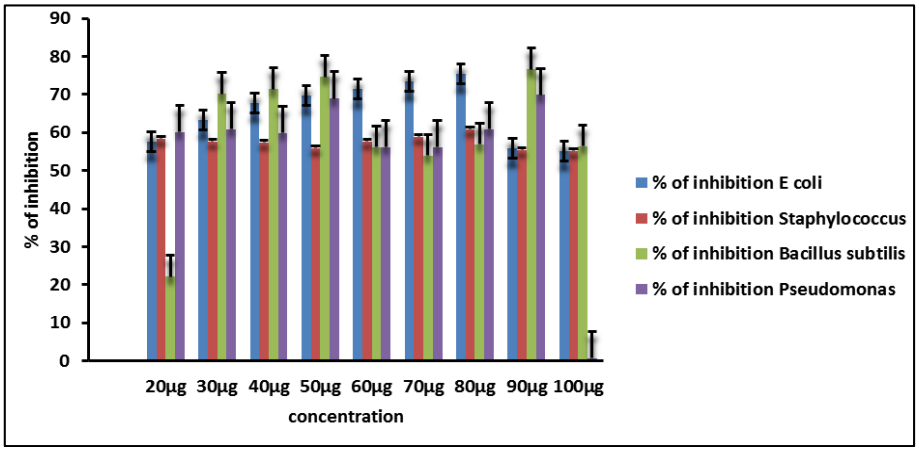
Graph 2



Graph 3



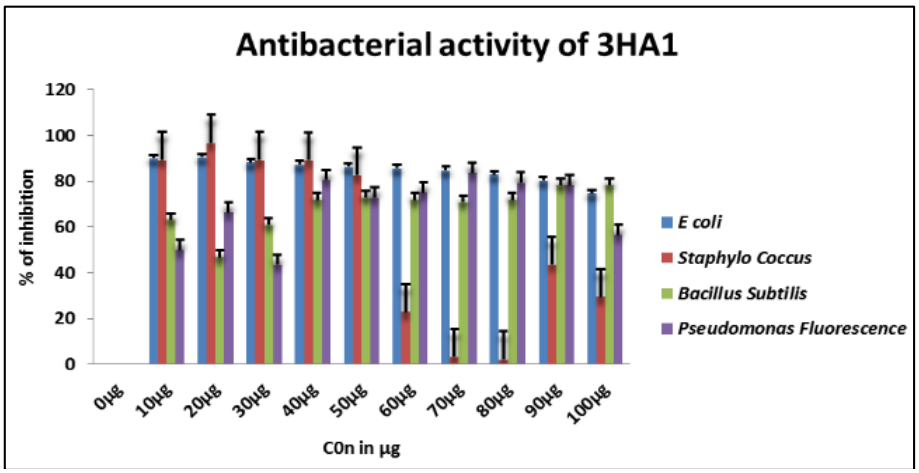
Graph 4



Graph 5

Table 1: Absorbance of microbial growth at 600nm

Column1	*E coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas fuorescens*
10µg	0.406	0.406	0.406	0.406
20µg	0.172	0.169	0.316	0.162
30µg	0.149	0.172	0.121	0.159
40µg	0.131	0.173	0.116	0.163
50µg	0.123	0.179	0.103	0.126
60µg	0.116	0.172	0.178	0.178
70µg	0.108	0.167	0.187	0.178
80µg	0.1	0.159	0.175	0.159
90µg	0.179	0.181	0.095	0.122
100µg	0.182	0.182	0.177	0.403



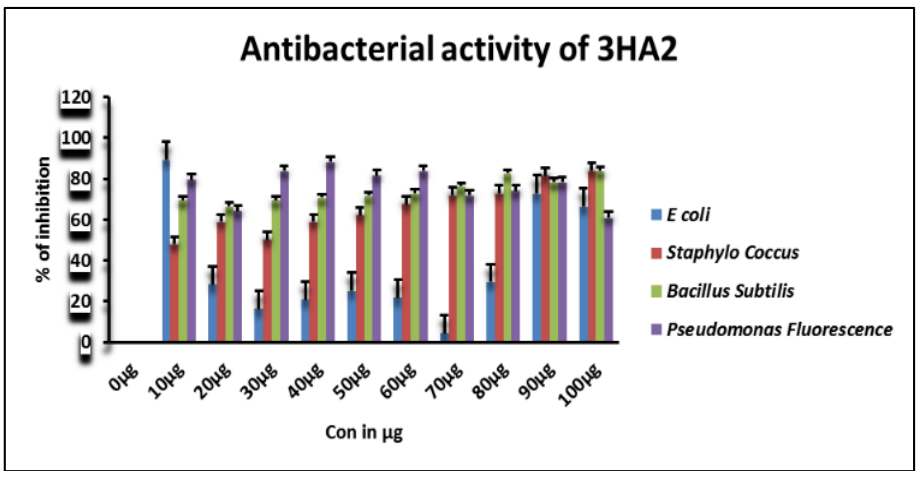
Graph 6

Table 2

Bacterial Strains	Minimum Inhibitory Concentration	% of Microbial growth inhibition by 3HA1 Compare to Control (0µg)
E Coli	100µg	86
P. fluorescence	100µg	88
B.subtilis	100µg	94
Staphylococcus	100µg	80

Table 3: Absorbance of microbial growth at 600nm.

Column1	*E coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas fuorescens*
10µg	0.092	0.099	0.34	0.46
20µg	0.089	0.031	0.49	0.31
30µg	0.11	0.099	0.36	0.52
40µg	0.118	0.1	0.26	0.18
50µg	0.126	0.16	0.25	0.25
60µg	0.133	0.71	0.26	0.23
70µg	0.141	0.89	0.27	0.15
80µg	0.159	0.9	0.26	0.19
90µg	0.182	0.52	0.2	0.2
100µg	0.234	0.65	0.2	0.4



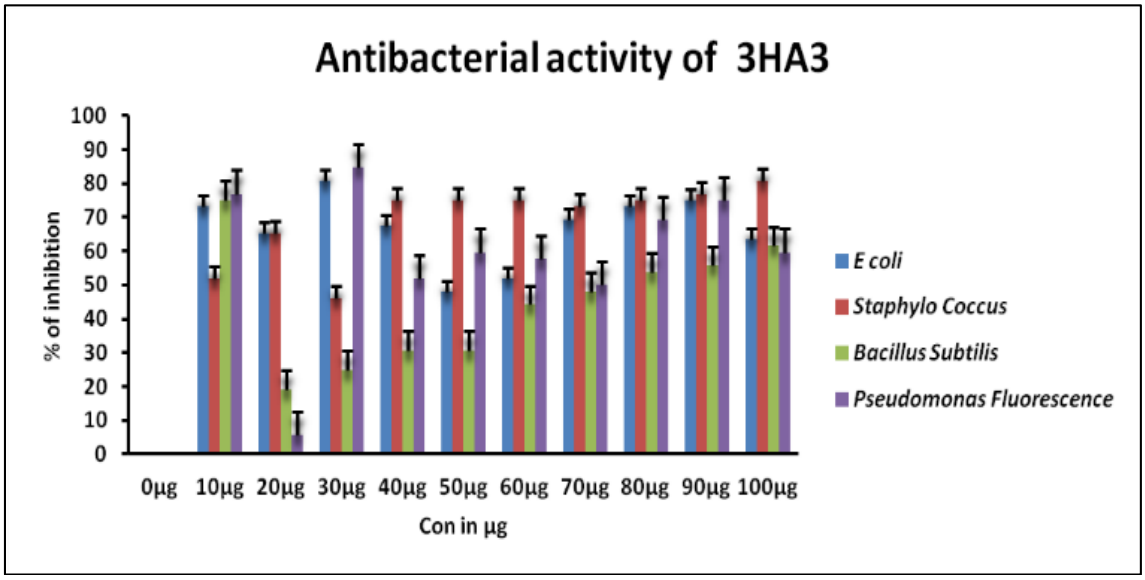
Graph 7

Table 4

Bacterial Strains	Minimum Inhibitory Concentration	% of Microbial growth inhibition by 3HA2 Compare to Control(0µg)
E Coli	20µg	23
P. fluorescence	20µg	47.92
B.subtilis	20µg	52.06
Staphylococcus	20µg	40.49

Table 5: Absorbance of microbial growth at 600nm

Column1	*E coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas fuorescens*
10µg	0.1	0.48	0.28	0.19
20µg	0.66	0.38	0.31	0.33
30µg	0.77	0.46	0.28	0.15
40µg	0.73	0.38	0.27	0.11
50µg	0.69	0.35	0.26	0.17
60µg	0.72	0.3	0.25	0.15
70µg	0.88	0.26	0.22	0.26
80µg	0.65	0.25	0.16	0.24
90µg	0.25	0.17	0.2	0.2
100µg	0.31	0.15	0.15	0.36



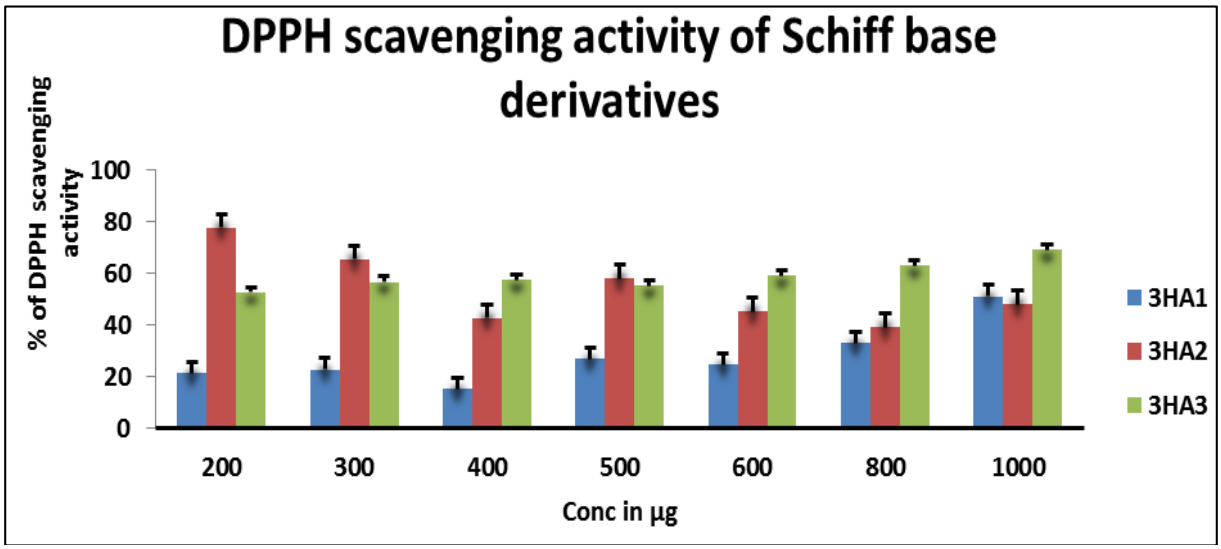
Graph 8

Table 6

Bacterial Strains	Minimum Inhibitory Concentration	% of Microbial growth inhibition by 3HA3 Compare to Control (0µg)
E Coli	20µg	45.9
P. fluorescence	20µg	57.7
B.subtilis	20µg	33.7
Staphylococcus	20µg	25.19

Table 7: Absorbance of microbial growth at 600nm

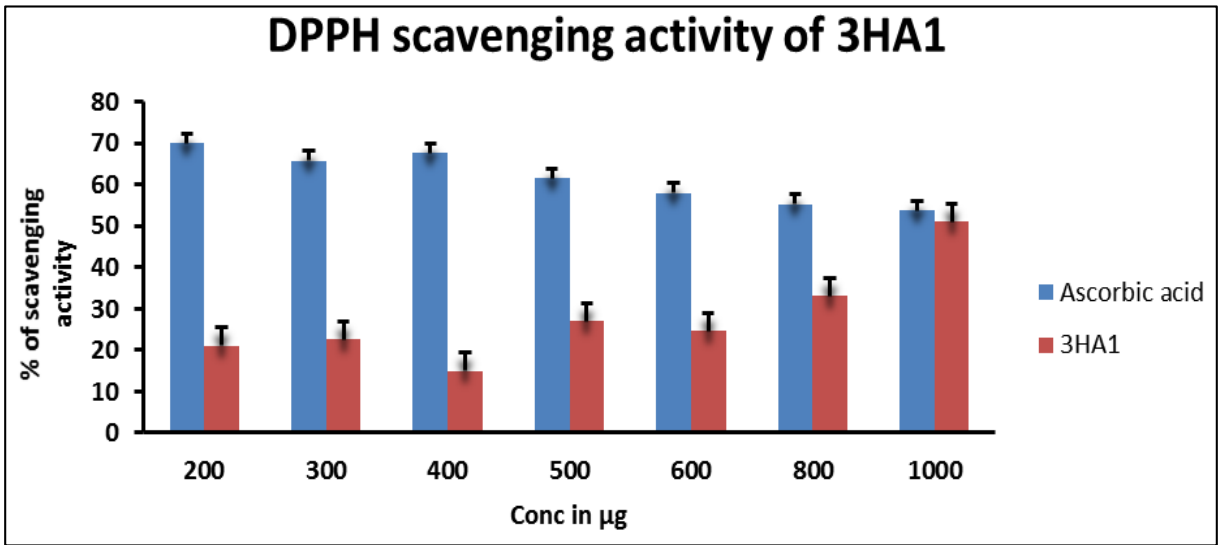
Column1	E coli	Staphylococcus aureus	Bacillus subtilis	Pseudomonas fuorescens
10µg	0.14	0.25	0.13	0.12
20µg	0.18	0.18	0.42	0.49
30µg	0.1	0.28	0.39	0.08
40µg	0.17	0.13	0.36	0.25
50µg	0.27	0.13	0.36	0.21
60µg	0.25	0.13	0.29	0.22
70µg	0.16	0.14	0.27	0.26
80µg	0.14	0.13	0.24	0.16
90µg	0.13	0.12	0.23	0.13
100µg	0.19	0.1	0.2	0.21



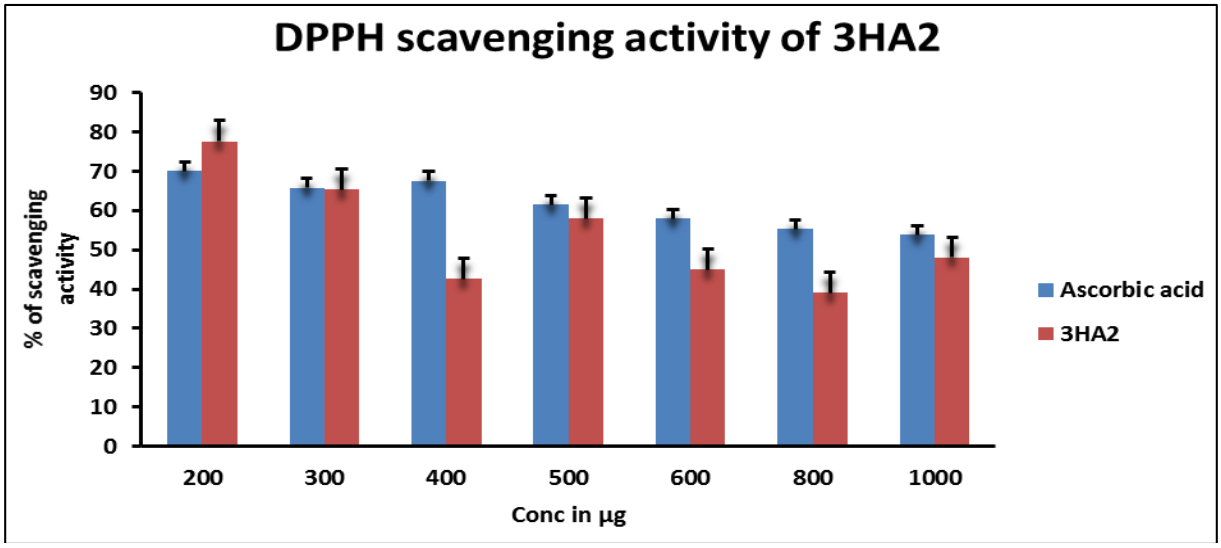
Graph 9: Antioxidant Assay Dpph Scavenging Activity

compounds	Ascorbic acid	3HA1	3HA2	3HA3
IC 50 in µg	1000	1000	600	200

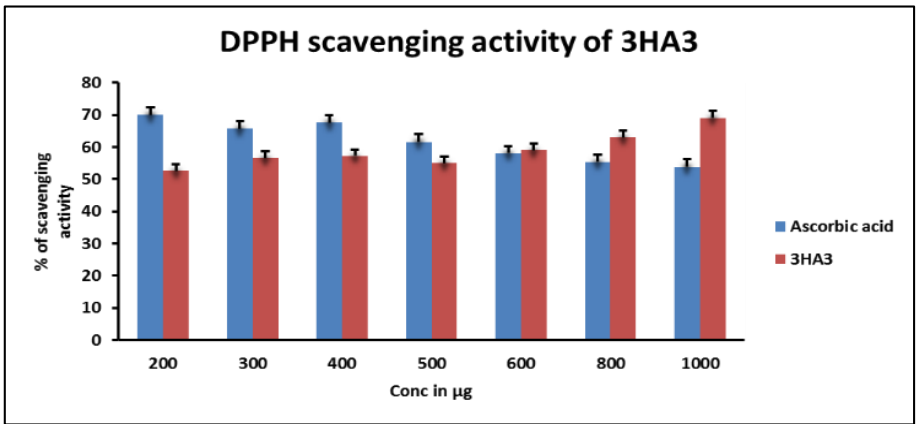
The maximum % of scavenging activity was seen in 3HA1.



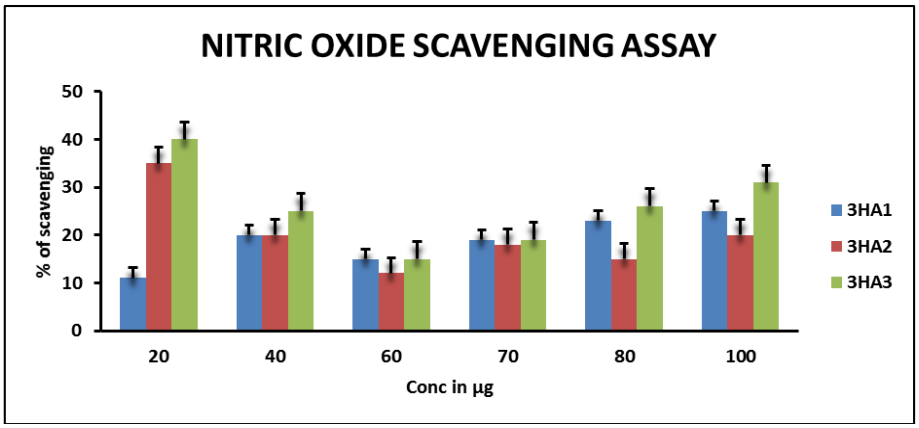
Graph 10



Graph 11



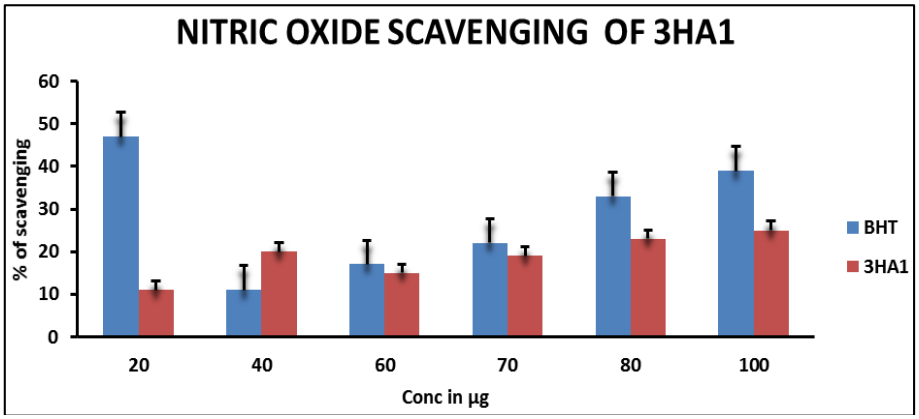
Graph 12



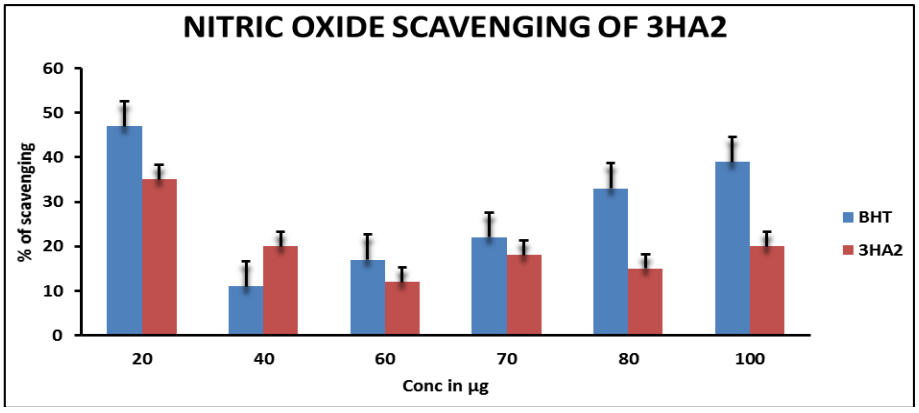
Graph 13. Nitric Oxide Scavenging

The maximum % of scavenging activity was seen in 3HA1.

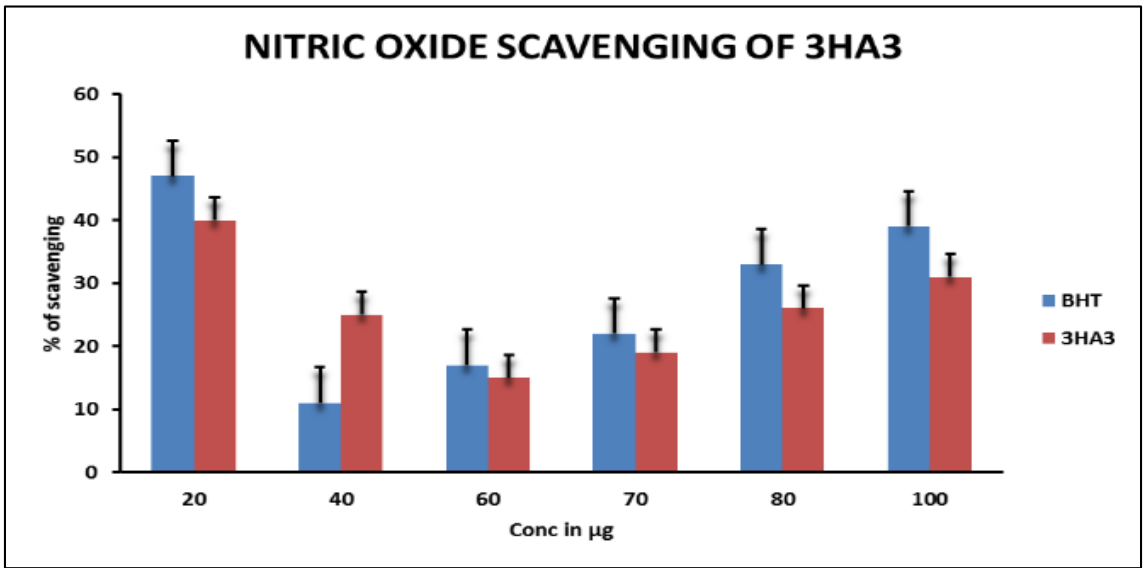
compounds	BHT	3HA1	3HA2	3HA3
IC 50 in µg	275	50	33	50



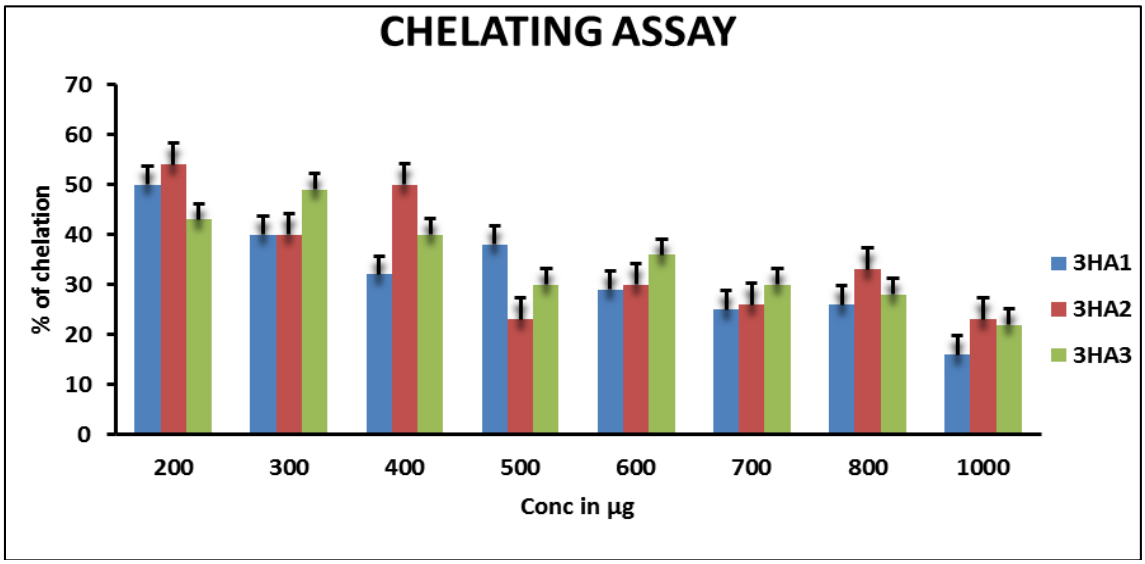
Graph 14



Graph 15



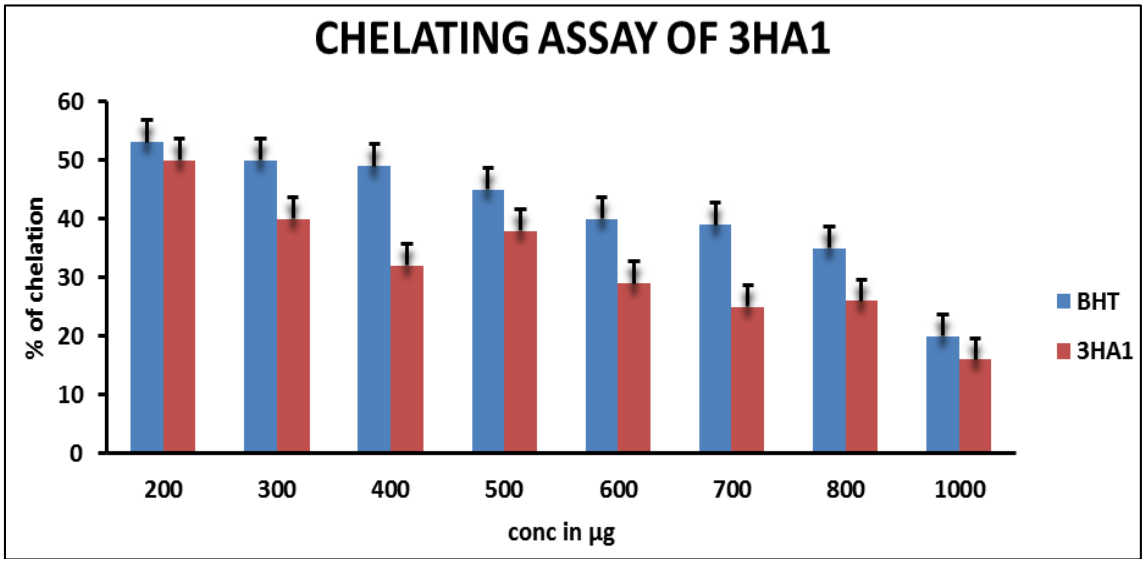
Graph 16



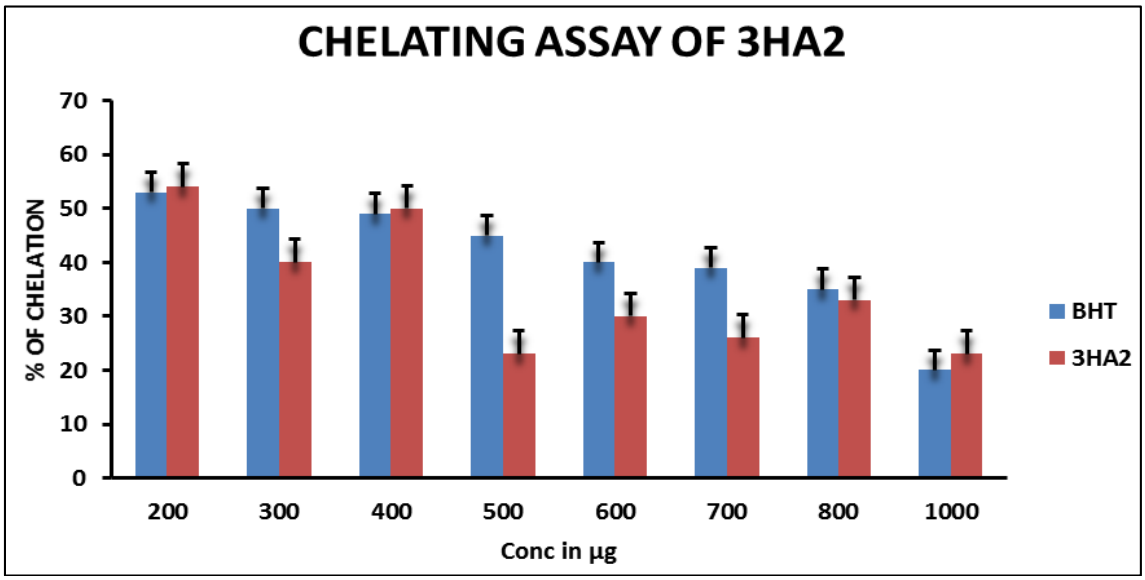
Graph 17: Ferrous Ion Chelating Assay

The maximum % of chelation was seen in 3HA1.

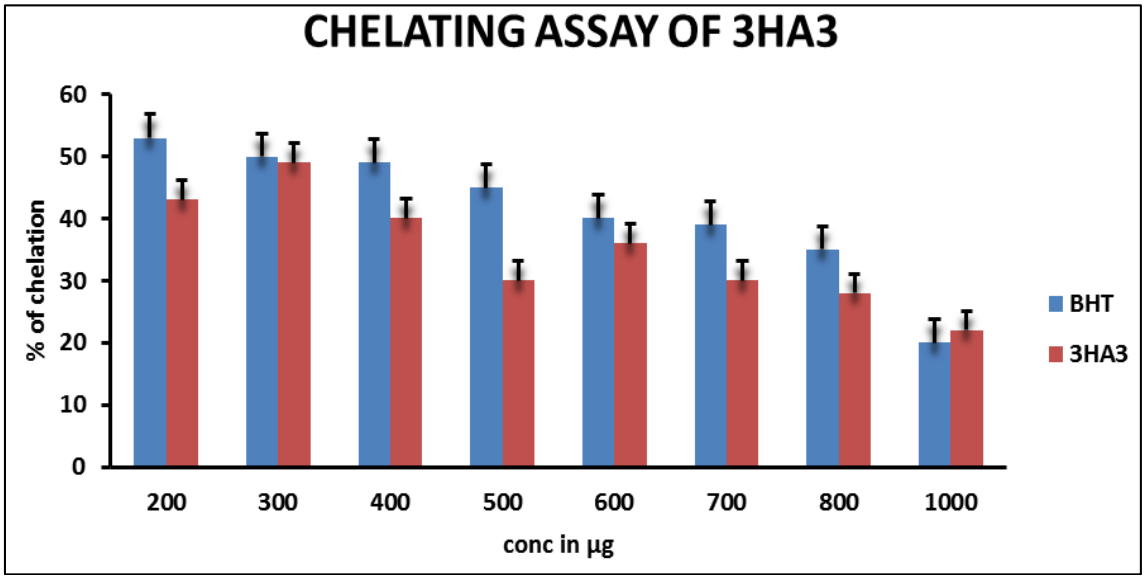
compounds	BHT	3HA1	3HA2	3HA3
IC 50 in µg	300	200	400	300



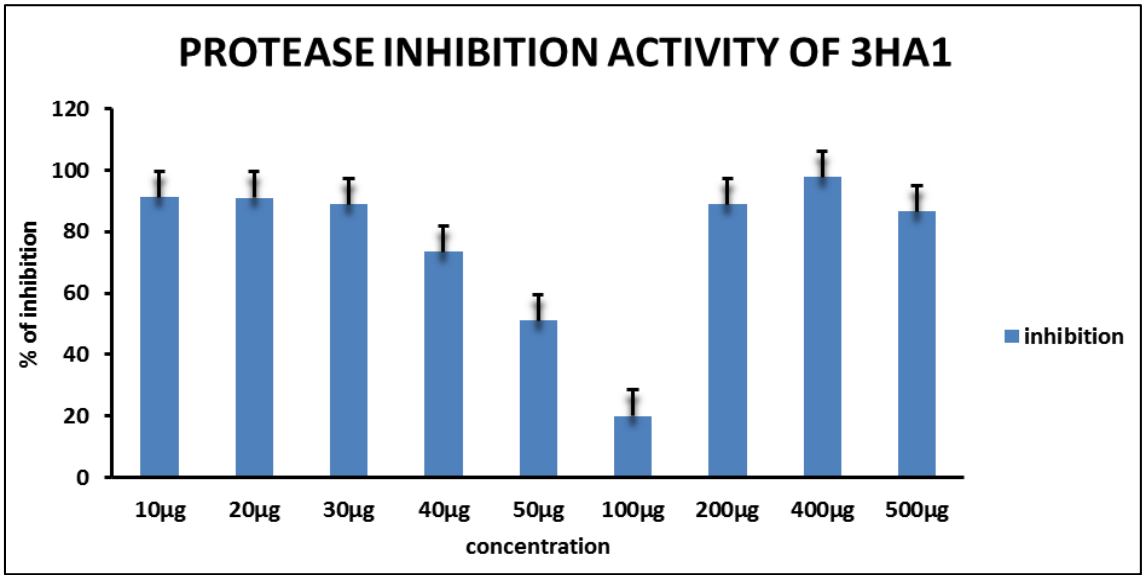
Graph 18



Graph 19



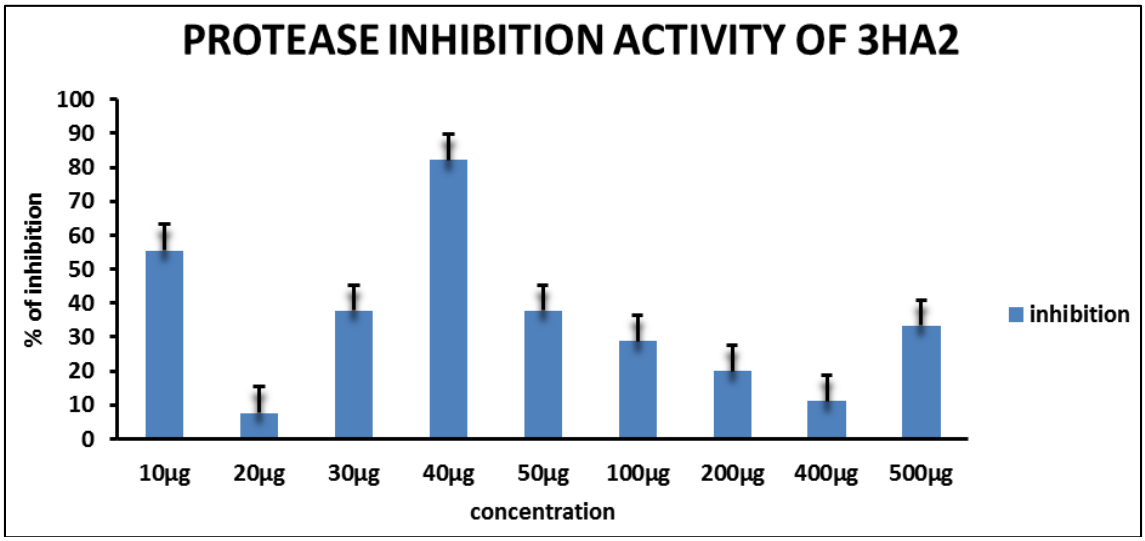
Graph 20



Graph 21: Protease Inhibition Activity

Table 8

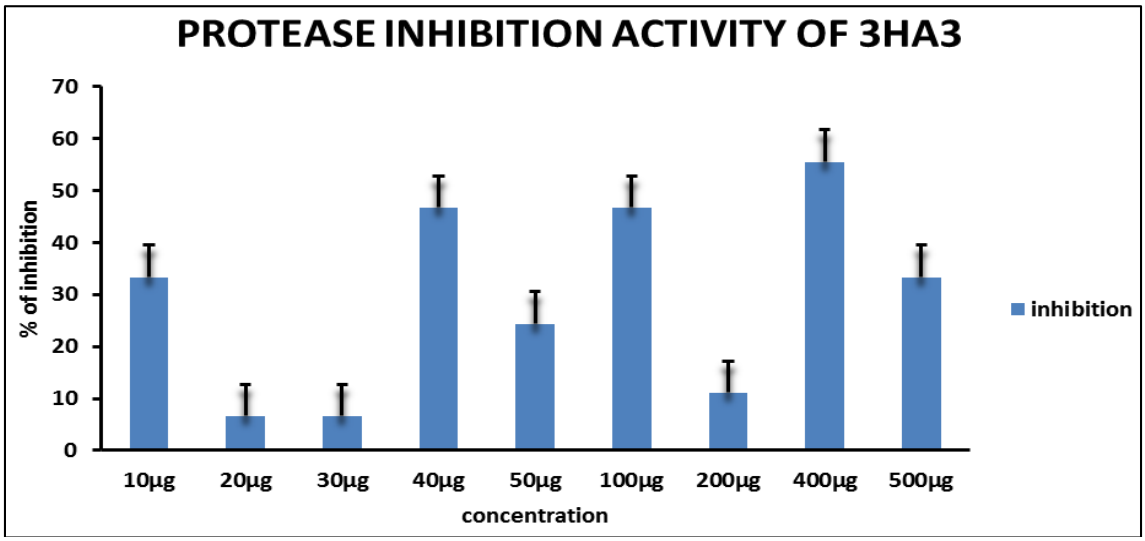
Concentration(Inμg)	ActivityIn Units
10	0.02
20	0.04
30	0.025
40	0.06
50	0.11
100	0.65
200	0.025
400	0.010
500	0.03



Graph 22

Table 9

Concentration(inμg)	Activity in units
10	0.1
20	0.15
30	0.14
40	0.04
50	0.14
100	0.11
200	0.09
400	0.04
500	0.03



Graph 23

Table 10

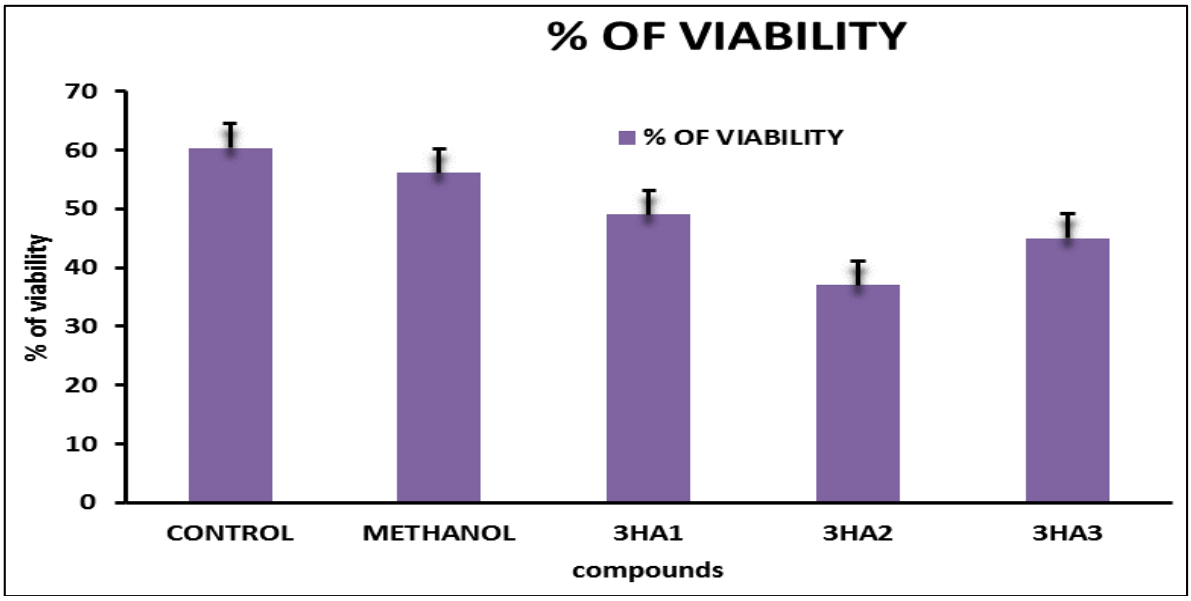
Concentration(inµg)	Activity in units
10	0.15
20	0.21
30	0.21
40	0.18
50	0.17
100	0.12
200	0.20
400	**0.15**
500	**0.1**

compounds	3HA1	3HA2	3HA3
IC 50 in µg	50	20	100

Cytotoxicity Assay

Table 11

Compound	% Of Viability
Control	60.38
Methanol	56.7
3HA1	49
3HA2	37
3HA3	45



Graph 24

Antiangiogenic Activity

Fig3: 3HA1 is positive to antiangiogenic properties. It inhibits the growth of blood vessels.

3ha2-Positive

Fig4: 3HA2 is positive to antiangiogenic properties. It inhibits the growth of blood vessels.

3ha3-Positive

Fig5: 3HA3 shows positive to antiangiogenic properties. It inhibits the growth of blood vessels.

4. Discussion

All the three Schiff bases (3HA1, 3HA2 and 3HA3) showed very good antimicrobial activity. Maximum growth inhibition was shown in *Bacillus subtilis* (Table.2) when compared to *E.coli, Pseudomonas fluorescens* and *Staphylococcus aureus* in 3HA1 compound. Maximum inhibition was shown in *Bacillus subtilis* (Table.3) when compared to *E.coli, Pseudomonas fluorescens* and *Staphylococcus aureus* in 3HA2 compound. Maximum inhibition was shown in *Pseudomonas fluorescens* (Table.4) when compared to *E.coli, Staphylococcus aureus* and *Bacillus subtilis* in 3HA3 compound.

The 3HA3 shows very good inhibition in the organisms *E.coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas fluorescens.* (Graph.1.2.3.4) The absorbance of microbial growth was measured at 600nm (Table.1.3.5.7)

Antioxidantion terminates the chain reaction by removing free radical intermediates and inhibit other oxidation reactivity being oxidized themselves. Low levels of antioxidants or inhibition of the antioxidant enzyme causes oxidative stress and may damage or kill the cells. In the new Schiff base derivatives 3HA1 (Graph.9) showed very good antioxidant activity. Compounds 3HA2 and 3HA3 (Graph.17, Graph.13) showed less antioxidant activity when compared with ascorbic acid. Hence the compounds can also be used for reducing oxidative stress. Compounds 3HA1, 3HA2 and 3HA3 (Graph.13) showed good nitric oxide scavenging activity.

Ferrous ion chelating assay has been done with the compounds. The maximum % of chelation was seen in 3HA1 (Graph18) Along with the compounds, BHT was also checked for the chelating activity. Compound 3HA1 showed the maximum % of chelation, which reveals that the compound 3HA1 is a good antioxidant agent.

Aloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9(10H) anthracenone), a bioactive compound in Aloe Vera, although known to have an anticancer effect, has not been used in current drug research. Optimization of the lead structure could enhance the utility of this compound. Hence aloin was modified using natural amino acids to produce Schiff base, a potential pharmacophore, and its corresponding a glycones. The synthetic derivatives exhibited significance enhancement in their efficacy towards antioxidant (DPPH radical scavenging) and cytotoxic activities than those of the parent compound, aloin showing promise for application in cancer treatment [28].

Compared to toxic level of toluene, chloroform and copper sulphate, all the new Schiff base compounds toxic level is less. Therefore aloin can be modified using these compounds and it can be used for cancer treatment.

High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin-E may increase risks of prostate cancer and one type of stroke. Antioxidant supplements may also interact with some medicines [5, 6].

Bacterial and fungal proteases particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated by nutritional signals in these organisms.

The compounds are subjected to protease inhibition activity. In protease inhibition activity 3HA1 showed maximum

activity (Graph.21) The compounds Activity (in units) has been calculated (Table.8.9.10) Fungi secretes protease to hydrolyze the peptide bonds in proteins. Therefore breakdown into constituent monomers.

Cytotoxicityis the quality of being toxic to cells. Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). The assay has been done with the compounds. When they are compared each other, 3HA1 showed more % of viability and 3HA2 showed less % of viability (Graph.24). The % of viability of compounds are noted in the Table.11

Angiogenesis involves the activation, proliferation and direct migration of endothelial cells to form new capillaries from exciting blood vessels. All the three Schiff base compounds 3HA1, 3HA2 and 3HA3 were also subjected to antiangiogenic activity. The assay has been done. The fig.3.4.5 shows the fertilized eggs are treated with the Schiff base compounds. All these compounds showed positive results. They inhibit the growth of the blood vessels.

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