PROJECT REPORT

On

DESIGN, SYNTHESIS, AND EVALUATION OF SCHIFF BASE DERIVATIVES OF QUERCETIN AS ANTI-INFLAMMATORY AGENTS

Submitted by

GLENDA SEMANTHY (AB21CHE003)
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PRINCY K.B. (AB21CHE021)

In partial fulfillment for the award of the

Bachelor's Degree in Chemistry

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

2023-2024

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

B.Sc. CHEMISTRY PROJECT REPORT

Name : **GLENDA SEMANTHY, K.A. APARNA, PRINCY K.B.**

Register Number : **AB21CHE003, AB21CHE006, AB21CHE021**

Year of Work : **2023-2024**

This is to certify that the project "**DESIGN, SYNTHESIS, AND EVALUATION OF SCHIFF BASE DERIVATIVES OF QUERCETIN AS ANTI-INFLAMMATORY AGENTS**" is the work done by **GLENDA SEMANTHY, K.A APARNA, PRINCY K.B.**

Dr. Saritha Chandran A.
Head of the Department

Dr. A.A. Shanty
Staff-member in charge

Submitted to the Examination of Bachelor's Degree in Chemistry

Date: …….....

Examiners:……..

:.…….

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

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DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

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Name	**: K.A. APARNA**
Register Number	**: AB21CHE006**
Year of Work	**: 2023-2024**

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Register Number	**: AB21CHE021**
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This is to certify that the project "**DESIGN, SYNTHESIS AND EVALUATION OF SCHIFF BASE DERIVATIVES OF QUERCETIN AS ANTI-INFLAMMATORY AGENTS**" is the work done by **PRINCY K.B.**

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DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

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CERTIFICATE

This is to certify that the project work entitled "**DESIGN, SYNTHESIS, AND EVALUATION OF SCHIFF BASE DERIVATIVES OF QUERCETIN AS ANTI-INFLAMMATORY AGENTS**" is the work done by **GLENDA SEMANTHY, K.A. APARNA, PRINCY K.B.** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

Dr. A.A. SHANTY
Project Guid

DECLARATION

I hereby declare that the project work entitled "**DESIGN, SYNTHESIS, AND EVALUATION OF SCHIFF BASE DERIVATIVES OF QUERCETIN AS ANTI-INFLAMMATORY AGENTS**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is arecord of an original work done by me under the guidance of **Dr. A.A. SHANTY, ASSISTANT PROFESSOR,** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.

GLENDA SEMANTHY
K.A. APARNA
PRINCY K.B.

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GLENDA SEMANTHY
K.A. APARNA
PRINCY

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Chapter 1

Introduction

1.1 SCHIFF BASE

Schiff base or imine is one of the most important organic compounds that has a significant role in organic chemistry. A Schiff base is a nitrogen analogue of an aldehyde or ketone where the carbonyl group is replaced with an imine group. Schiff base is formed when aldehyde or ketone reacts with any primary amine by the elimination of a molecule of water (Figure 1.1).[1]

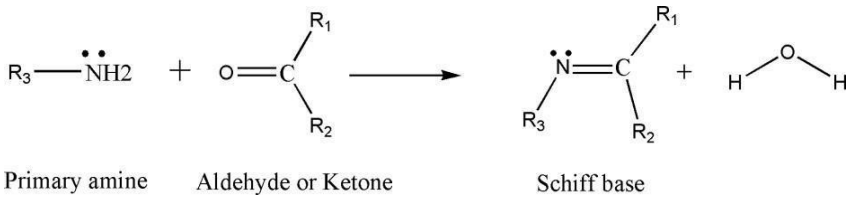


Figure 1.1: General scheme of formation of Schiff base[1]

[Here R_1 may be an aryl or alkyl group and R_2 may be an aryl, alkyl, or hydrogen; if it is hydrogen then the compound is aldehyde otherwise ketone]

The first imine was reported in 1864 by the German chemist Hugo Schiff.In his honour, these groups of compounds, imine are frequently known as Schiff base. Schiff used a classical method for the synthesis of Schiff base i.e., using an azeotropic distillation reaction between primary amine and

aldehyde. [2, 3] Later so many other methods were invented. These Schiff bases are synthesized from various aldehydes and amines under microwave irradiation method, natural acid-catalysed method, ultrasonic method, grinding method, water as a green solvent, miscellaneous method (magnetic nanoparticles, mixing at room temperature, garlic as biocatalyst), etc. In recent years green synthetic methods for Schiff base synthesis have gained significant attention due to the need for more environment-friendly chemical production.[4]

When synthesizing Schiff base, nucleophilic amines are used to attack electrophilic carbonyl compounds via a nucleophilic addition process, forming a hemi-aminal group. Then the hemi aminal group is dehydrated to generate imine compounds. In the first phase of the reaction, the amine reacts with the aldehyde or ketone to generate the unstable addition product carbinolamine. Carbinolamine undergoes acid or base catalyst dehydration. Since carbinolamine is an alcohol, it undergoes a dehydration reaction when subjected to an acid catalyst. Reversible acid or base catalysis or heating often happens during the production of a Schiff base from aldehyde or ketone. When the product is isolated, water evaporated, or both, the formation is pushed to completion (Figure 1.2). [1, 5]

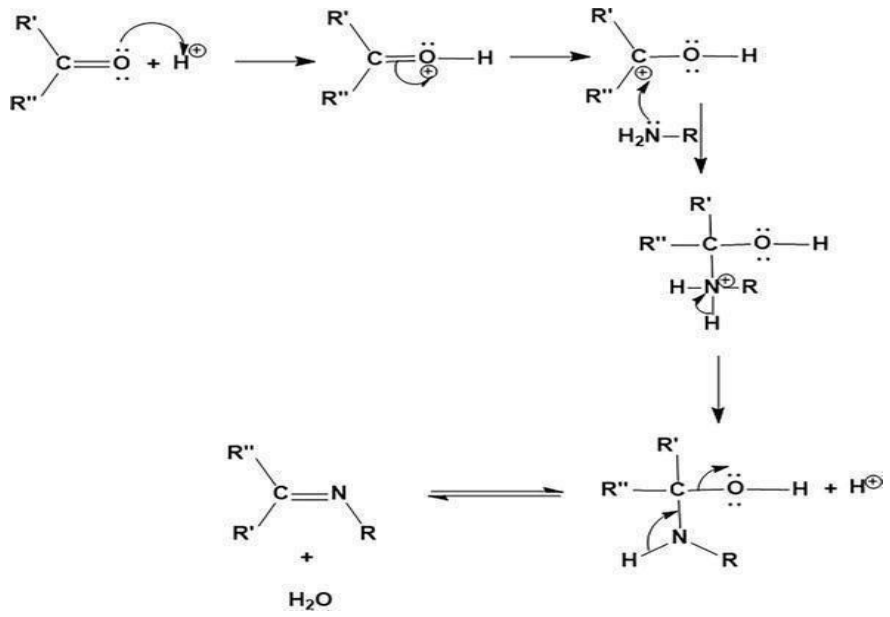


Figure 1.2: **Mechanistic explanation of the formation of Schiff base**[5]

Schiff base with aryl substituents is more stable compared with alkyl substituents. Because aliphatic aldehydes are easily polymerizable, but aromatic aldehydes have efficient conjugation, so they are more stable. Moreover, Schiff bases formed from aldehydes are more stable than from ketones. Due to the less steric hindrance of aldehyde, they react faster. Also, extra carbon in ketone makes it less electrophilic than aldehyde.[6] The simplicity of synthesis and complexation of Schiff base has attracted the interest of researchers because of their excellent thermal, mechanical, electrical, optical, optoelectronic, and fiber forming capabilities.

1.2 APPLICATIONS AND IMPORTANCE OF SCHIFF BASE

Schiff base complexes have a wide range of applications with the help of ongoing research it is expected to uncover even more. Schiff Base has applications in the food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical, and biological activities. These Schiff bases can form metal complexes with almost all transition elements.[7]Therefore, they have a pivotal role in the development of modern coordination chemistry. Now, research shows that these Schiff base complexes have biological interest too. Therefore, these can be found to be key points in the inorganic biochemistry, catalysis, and optical materials

Schiff base complexes show catalytic activity in the decomposition of hydrogen peroxide, isomerization, allylic alkylation, hydrosilylation, annulation, and Carbonylation reactions. For example, Schiff base complexes V(lV), Ni(ll), Mn(ll), and Mo(Vl) are used as catalysts for the epoxidation of various alkenes. Fe(ll) and Cu(ll) were used in the oxidation of catechol.[8] Henry's reaction is catalyzed by Mn(ll) whereas for Michael addition Co(ll) is used and so on.[9, 10] The high thermal stability of these complexes is the reason for their uses as catalysts.

Schiff bases are used as a dye which gives fast colors to leather, wool, and food packages. The phenomena of coordination of Schiff base with metal ion give the Schiff base the good advantages to be in the dye synthesis. Schiff base derived from aniline moiety containing phenyl groups were sometimes called azodyes.[11]

The Schiff base is used in optical computers to measure and control the intensity of the radiation in imaging systems, as well as in molecular memory storage, as organic materials in reversible optical memorize, and

photodetectors in biological systems.[12] Due to photochromic properties, Schiff base could behave as photo stabilizers, dyes for solar collectors, and solar filters.[13] They are also exerted in optical sound technology. Due to its high thermal stabilities, it is used as a stationary phase in gas chromatography.[14, 15] These are the key applications of Schiff base complexes. Due to these multidisciplinary applications, this has been proven to be a highly significant area of research.

1.2.1 Schiff base complexes as a Catalyst

Several Schiff bases exhibit excellent catalytic activity in a variety of reactions when moisture is present. In the last few years, many studies on their application in both homogeneous and heterogeneous catalysis have been published. Many Schiff base complexes high heat and moisture stabilities were advantageous for their use as catalysts in high-temperature operations. Understanding the properties of both ligands and metals can aid in the synthesis of compounds with high activity because complexationresults in an increase in activity. For a very long time, Schiff bases were considered essential ligands in coordination chemistry. Because of their remarkable properties and applications in a range of fields, Schiff bases, and their metal complexes have been extensively studied due to their relatively simple synthesis and varied structural makeup. The field of synthetic and structural research finds these compounds to be auspicious.

Carlos Baleizão et al. discovered chiral Mg (III) salen Schiff base catalysts in the asymmetric epoxidation of unfunctionalized olefins in 1990 sparked a great deal of interest in the tetradentate Schiff base, salen-type ligands, and their complexes (Figure 1.3).[16] Salen-Schiff base transition metal complexes have been extensively researched due to their potential use as

catalysts in a range of C-C and C-N bond formation events, including cyclopropanations, aziridination, asymmetric cycloaddition reactions,and A3-coupling.[17, 18]

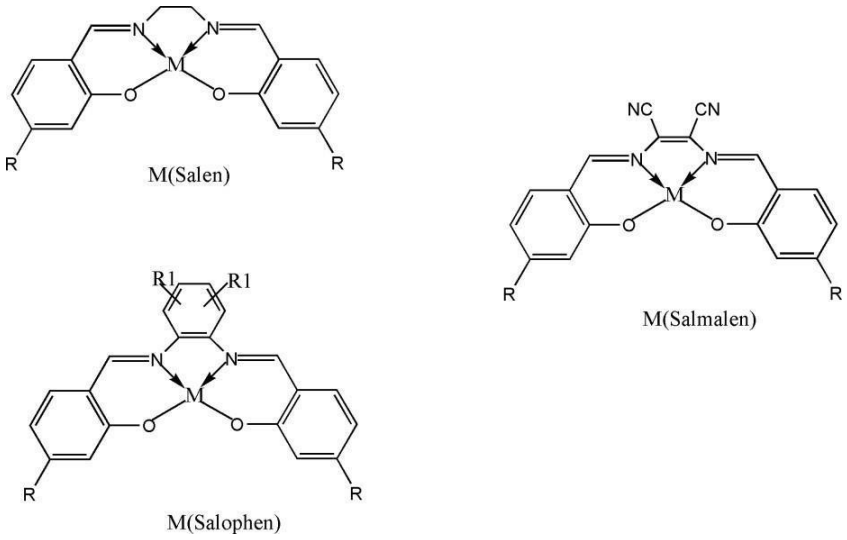


Figure 1.3: Metal Salen-type complexes[16]

The aryl compound's C-N bond design is very important to medicinal chemists. Among the numerous heterocycles that have been reported, tetrazoles represent a noteworthy group. Tetrazoles have been used as ligands in coordination chemistry, stabilizers in the photo industry, linkers to covalently bind synthetic groups to biopolymers in a particular way, etc (Figure 1.4). An interesting example of a multi-component reaction is the A3-coupling reaction by Prof. Chao-Jun Li (Figure 1.4).[19-22]

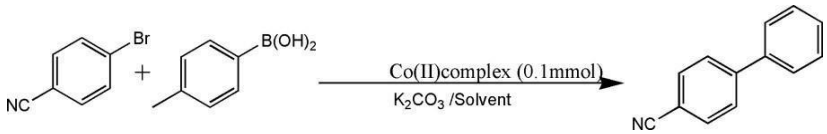


Figure 1.4: 4-bromobenzonitrile with phenylboronic acid[21]

Under suitable commercial conditions, zinc complexes that are dependable for lactide bulk polymerization have been investigated at 150°C. It is possible to produce the anionic Schiff base ligands aerobically, and they are very stable against air, moisture, and other lactide contaminants. Because of this, these compounds have an active, nontoxic zinc core as well as a potent anionic ligand. These compounds' easy aerobic synthesis, high thermal stability under nitrogen, and airtightness make them perfect for lactide polymerization in industrially relevant situations.[23]

One more important catalytic conversion reaction is in oxidation of alcohols into carbonyl compounds. A cobalt (II)-Schiff base with triphenylphosphine was synthesized (Figure 1.5). The catalyst was used for oxidation reactions using different substrates and obtained a good percentage of conversion of the carbonyl compound.[24]

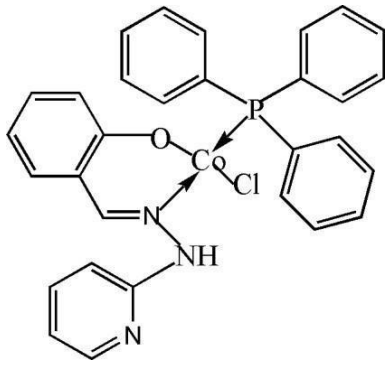


Figure 1.5: Cobalt (II)-Schiff base complexes with triphenylphosphine[24]

Schiff base transition metal complexes are a common oxidation catalyst for a variety of organic substrates due to their low cost, ease of synthesis, and chemical and thermal stability. The oxidation of sulfides to sulfoxides, the

conversion of alkenes to epoxides and diols, the reduction of hydrocarbons, polymerizations, hydroformylation, coupling reactions, and other significant reactions are all catalyzed by Schiff base metal complexes.[25] However, for organic transformations, new Schiff bases and their metal complexes are still desperately needed.

1.2.2 Schiff base as a Corrosion Inhibitor

In moist, humid, and acidic environments, corrosion inhibitors are highly helpful in preventing metal oxidation, and their efficiency on metal surfaces is dependent on the importance of the bonds that are formed. A single pair of electrons can be donated or received by inhibitors that contain nitrogen, oxygen, and sulfur unsaturated bonds, as well as planar conjugated aromatized compounds, which are recommended as effective corrosion inhibitors.[26] Many Schiff base research fields have focused primarily on the application of Schiff bases as corrosion inhibitors in alloys and metals because of the presence of the imine bond. 2-furaldehyde semi-carbazone (FSC) complexes of cobalt (II), zinc (II), and manganese (II) against the corrosion of XC38 carbon steel in 1 M HCl solution (Figure 1.6). Isatin Schiff base is an effective corrosion inhibitor for mild steel in hydrochloric acid solution.[27]

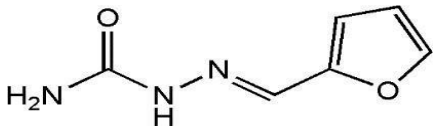


Figure 1.6: 2-Furaldehyde semi-carbazone (FSC) complexes[27]

1.2.3 Schiff Base as a Photometric Sensor

Colorimetric and fluorescent Schiff base sensors are effective instruments for detecting metal ions because of their ease of use, affordability, quick photophysical response, high sensitivity, low detection limit, and use in the environmental and medical domains. Three fundamental ideas underpin the design of these sensors: (a) separation of analytes; (b) separation of a particular analyte from a mixture of analytes; and (c) signal generation as a result of species complexing. This is due to the significant roles that a number of these metal ions including K^{+}, Na^{+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Fe^{2+}, Mg^{2+}, and Co^{2+} play in the biological, medical, and environmental domains. Because of this, among many other domains, their sensing or detection has emerged as one of the most important and difficult research topics.[28]

1.3 BIOLOGICAL ACTIVITIES OF SCHIFF BASE

1.3.1 Antioxidant activity

Many studies are currently being conducted to find out how free radicals interact with biological systems like proteins, DNA, and lipids. Numerous illnesses, including neurodegenerative conditions, atherosclerosis, age-related illnesses, rheumatoid arthritis, cancerous growths, and tumors, are caused by free radicals. Maintaining an appropriate level of naturally occurring antioxidants in the biological system, such as glutathione, vitamin C, and vitamin E, is essential to preventing major health issues. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), also referred to as reactive species (RSs), react to produce all these health issues. There are three ways by which RSs are formed: (i) metal-catalyzed reactions, (ii) the presence of pollutants in the atmosphere, and (iii) irradiation by UV light, X-ray, and gamma rays. Antioxidants are generally hydrogen donors or

electron donors to the reactive site in neutralizing free radicals. One well-studied antioxidant that can be found in grapes is resveratrol. According to reports, C=N and C=C are the only structural differences betweenresveratrol and Schiff base. For biological activity, the Schiff base's C=N linkage is essential. Thymol and carvacrol are well-known antioxidants found in the extract of the plants of thyme species. The Schiff bases of 2- iso-propyl-5-methyl-phenol (thymol/1a) (Figure 1.7), 2-tert-butyl-5- methyl-phenol (1b), and 5-iso-propyl-2-methyl-phenol (carvacrol/1c)(Figure 1.8) exhibited much better antioxidant activity than thymol andcarvacrol in DPPH assay.[29] The Schiff base 3,4-dimethoxybenzenamine shows antioxidant activity. These compounds showed good DPPH scavenging activity ranging from 10.12 to 84.34 μM.[30]

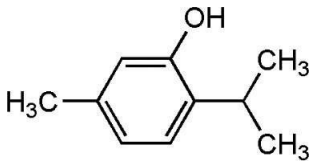


Figure 1.7: 2- Iso-propyl-5-methyl-phenol (Thymol)[30]

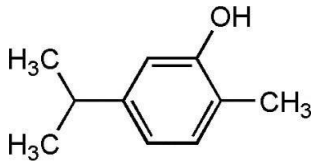


Figure 1.8: 5- Iso-propyl-2-methyl-phenol (Carvacrol)[30]

1.3.2 Anticancer activity

Though their exact mechanism of action remains unknown, Schiff bases have a high potential to inhibit carcinoma cells, an ability that is enhanced upon complexation. Anticancer activity of the already synthesized as well as the novel Schiff bases and their metal complexes such as 3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), PI staining, Sulfo-Rhodamine, Allium cepa, Sulfo- Rhodamine-B-stain (SRB) (Figure 1.9), viability and potato disc against various human and animal cancer cell lines. Quinazolines showed a very high activity against HepG2 and MCF-7 cell lines. Pyrazole-naphthalene derivatives exhibited high activity against numerous carcinoma cells while [Ni(HL1)2(OAc)2] showed the highest. Azosal and its tin(IV) complexes displayed high activity against U- 1242 MG and excellent activity against HCT-116 cell lines. 2-thiouracil sulphonamides displayed high activity against MCF7, CaCo-2 carcinoma cells. Vitamin-B6 and its oxovanadium complex showed good activity against MCF-7, 3T3, and cervical cancer HeLa cancer cell lines in the presence of visible light. Indoles displayed high activity against AMJ13. Porphyrine derivatives exhibited good activity while its binuclear (Y and K) complexes displayed high activity against several carcinoma cells. Chitosan complexes of [Pd(II) and Pt(II)] showed very high anticancer activity against MCF-7 carcinoma cells.[31]

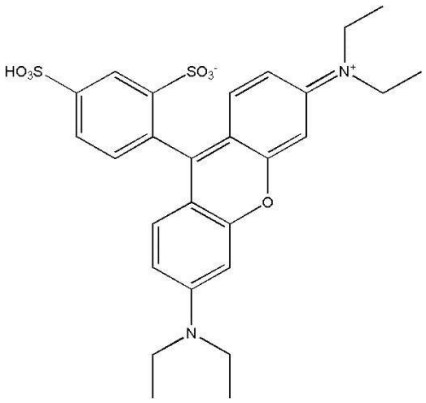


Figure 1.9: Sulfo-Rhodamine-B[31]

1.3.3 Antibacterial activity

ADS1 4-ethyl-6-{(E)-1-[(3-nitrophenyl)imino]ethyl}benzene-1,3-diol and ADS3 4-ethyl-6-[(E)-1-{(2-nitrophenyl)imino]ethyl}benzene-1,3-diol were the two Schiff bases that were created from race acetophenone by S. Baluja et al. Their metal complexes were then developed. The metals copper, nickel, iron, and zinc were chosen to prepare the complexes. To test for antibacterial activity against several clinically significant bacteria, including Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumoniae, and Staphylococcus aureus, a total of eight metal complexes were synthesized. DMF (polar) and 1,4-dioxane (non-polar) were used as solvents in the Agar Ditch technique to measure the in vitro antibacterial activity. The Schiff bases exhibited higher activity than their metal complexes; the metal complexes had different effects on the bacterial strains that were studied and the solvent that was used, indicating that the antibacterial activity depends on the compound's molecular structure, the strain of bacteria that is being studied, and the solvent used. When it came to the examined bacterial strains, the Schiff base ADS3 in the polar solvent DMF exhibited superior antibacterial activity. In 1,4-dioxane, Zn exhibited

the highest antibacterial activity among the four metals, followed by Fe; in DMF, Ni, Zn, and Fe demonstrated the highest antibacterial activity. The most resistant bacterium was P. vulgaris.[32]

1.3.4 Anti-Inflammatory activity

Inflammation is a common localized condition that makes the body reddened and swollen due to some injury or infection. The commonly available drugs i.e. diclofenac, ibuprofen, etc. in the market against inflammation bear acid group which causes severe stomach disturbance in their prolonged use. Thus, such issues required significant attention in terms of introducing acid-free new anti-inflammatory agents with enhanced efficacy. Aminoquinoline Schiff bases have been evaluated as anti-inflammatory agents. B. Bano *et al. discovered* four compounds including (E)-4-((quinoline 5-ylimino)methyl)benzene-1,2,3-triol,(E)-1-(2,4-dimethoxyphenyl)-N-(quinolin-5-ylimino)methanimine,(E)-2,6-dimethoxy-4-((quinolin-5-ylimino)methyl)phenol, and (E)-4-((quinolin-3-ylimino)methyl)benzene-1,2,3-triol were exhibit potent activity with IC50 values between 1.4 ± 0.1 to 2.4 ± 0.1 μg/mL, compared to Ibuprofen (IC50= 2.5 ± 0.6 μg/mL) These active compounds can be served as potential leads for new anti-inflammatory drugs.[33] The p-aminobenzene sulfonamide has been synthesized from acetanilide through the addition of excess chlorosulfonic acid and then concentrated ammonia solution (Figure 1.10); the Schiff base of this derivative exhibited a good level of activity against egg-white induced edema in rat hind paw.[34]

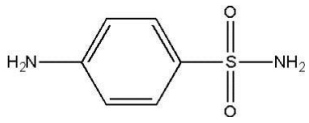


Figure 1.10: p-Aminobenzene sulphonamide[34]

1.4 IMPORTANCE OF THE REACTANTS USED

1.4.1 Quercetin

Flavonoids are a class of compounds commonly found in plants. They all are derivatives of the plant compound, flavone. These compounds together with synthetic analogues, are biologically active in variety of ways.[35] Quercetin is a flavanol occurring in fruits and vegetables with proven beneficial impact on health. It is commonly found in red onions, capers, kale and is also ingested from tea, coffee, cocoa, wine, beer, and vinegar. Infood, quercetin occur in a bounded form with sugar, phenolic acids, alcohols etc.[36] It was discovered by the Nobel prize winner Szent-Gyorgyi. It got its name in 1857 which is derived from quercetum (oak forest). Quercetin is said to be one of the most widely used bioflavonoids for the treatment of metabolic and inflammatory disorders.[37]

Quercetin has a molecular formula $C_{15}H_{10}O_7$ (Figure 1.11) and a molecular mass of 302.236 g/mol. It has a yellow crystalline powdered appearance. Itis poorly soluble in hot water, quite soluble in alcohol and lipids and is in soluble in cold water.[38]

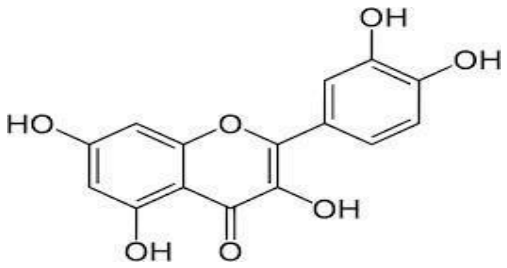


Figure 1.11: Structure of quercetin.

A molecule of quercetin contains five hydroxyl groups whose presence determines the compounds biological activity and the possible number of derivatives. Quercetin has more polar groups than similar flavonoid compounds. Despite the presence of five hydroxyl groups, the quercetin molecule has a lipophilic character and their derivatives can be both lipo- and hydrophilic depending on the type of substituent in the molecule. A lipophilic quercetin molecule can be easily absorbed by the stomach and the secreted in the bile. Quercetin exists in a planar conformation. Among the five-hydroxyl group, two of them are involved in intramolecular hydrogen bonding with the exocyclic oxygen and the other three participate in intermolecular hydrogen bonding. The exocyclic oxygen plays a pivotal role in the molecular structure of quercetin.[39]

1.4.2 Importance

Quercetin has been demonstrated to display the antiviral, antibacterial, anticarcinogenic, anti-inflammatory effects. It is one of the most potent antioxidants among polyphenols. And it shows various biological activities against diseases like diabetes, obesity, etc.[40]

Antioxidant: Quercetin acts as an antioxidant and reduces the damage caused by the free radicles. Antioxidants are capable of neutralizing free radicals which are always present in food as well as in the cells of human body. The antioxidant properties of phenolic compounds are linked withtheir ability to transfer a hydrogen or an electron, as well as with chelationof metal ions and inhibition of the activity of oxidases.[41]

Anti-inflammatory: Quercetin helps in the formation of inflammatory enzymes cyclooxygenase (COX) and lipoxygenase and hence reduces the action of inflammatory mediators such as prostaglandins and leukotrienes. Also, in its role as an anti-inflammatory agent, quercetin inhibits activation of leukocyte respiration through interactions at the membrane at a hydrophobic site on the protein.[42]

Anti-cancer activity: Quercetin has a strong anti-carcinogenic property. The anti-carcinogenic properties of quercetin result from its significant impact on an increase in the apoptosis of mutated cells, inhibition of DNA synthesis, inhibition of cancerous cell growth, decrease and modification of cellular signal transduction pathways.[40, 43]

1.4.3 4-Aminophenol

4-Aminophenol has a molecular formula $H_2NC_6H_4OH$ (Figure 1.12). It appears in white powder. It is very soluble in dimethylsulfoxide and soluble in acetonitrile, ethyl acetate, and acetone. Slightly soluble in toluene, diethyl ether, and ethanol and not soluble in benzene and chloroform. It is applicable in various fields like Testing for DNA Apple scald inhibitor, Stabilizer for smokeless powder, Antioxidant, Redox indicators, Dyes, etc 4-Aminophenol can be prepared from phenol, nitrobenzene, and 4-Nitrophenol.From phenol, it is prepared by nitration followed by reduction

with iron. On the other hand, the partial hydrogenation of nitrobenzene provides phenylhydroxylamine, which rearranges primarily to 4-Aminophenol. This rearrangement is called Bamberger rearrangement. From nitrobenzene, electrolytic conversion of nitrobenzene produces phenylhydroxylamine which spontaneously rearranges to 4-aminophenol.It is applicable in the industrial synthesis of paracetamol, treating 4-aminophenol with acetic anhydride gives paracetamol, which is a parent to amodiaquine, and mesalazine.[44,46]

Figure 1.12: Structure of 4-aminophenol.

1.4.4 4-Chloro-o-phenylenediamine

4-Chloro-o-phenylenediamine has a molecular formula $C_6H_7ClN_2$ (Figure 1.13). It is a chlorinated aromatic amine. It appears in brown crystalline solids or powder form at room temperature. It is slightly soluble in water, but soluble in benzene and very soluble in ethanol and ether. It is stable in normal temperature and pressure. 4-Chloro-o-phenylenediamine is prepared by nitrating 1,4-Dichlorobenzene to give 2,5- Dichloronitrobenzene whichis then reacted with an aqueous ammonium hydroxide solution. Then hydrogenation of obtained 4-Chloro-2-nitraniline in the presence of a catalyst is done and the desired 4-Chloro-o- phenylenediamine is obtained. It applies in the field to the synthesis of experimental pharmaceuticals, itcan be used as an oxidising base for dye preparation, as a chemical intermediate to produce 5-Chlorobenzotriazole,

as a curing agent for epoxy resins, as a reagent in gas chromatography, etc.[45]

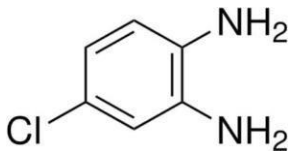


Figure 1.13: Structure of 4-Chloro-o-phenylenediamine.

1.4.5 Diphenylamine

Diphenyl amine has a molecular formula $(C_6H_5)_2NH$ (Figure 1.14). It is a colorless solid, but commercial samples are yellow due to the presence of oxidized impurities in it. Diphenylamine is a derivative of aniline, consisting of an amine bound to two phenyl groups. It is moderately soluble in water and dissolves well in many organic solvents. It is used mainly asan industrial antioxidant, dye mordant, and reagent and is also used inagriculture as an anthelmintic and fungicide. Diphenylamine is produced by the thermal deamination of aniline over oxide catalysts. It is applicable in various fields like Testing for DNA Apple scald inhibitor, Stabilizer for smokeless powder, Antioxidant, Redox indicators, Dyes, etc.[46]

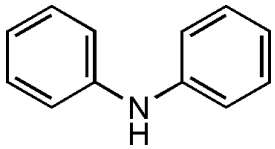


Figure 1.14: Structure of Diphenylamine.

Chapter 2

Materials and Methods

2.1 REAGENTS

1) Quercetin

2) 4- Aminophenol

3) 4- Chloro-o-phenylene diamine

4) Diphenylamine

2.2 SOLVENTS

1) Methanol

2) Ethanol

3) Petroleum Ether

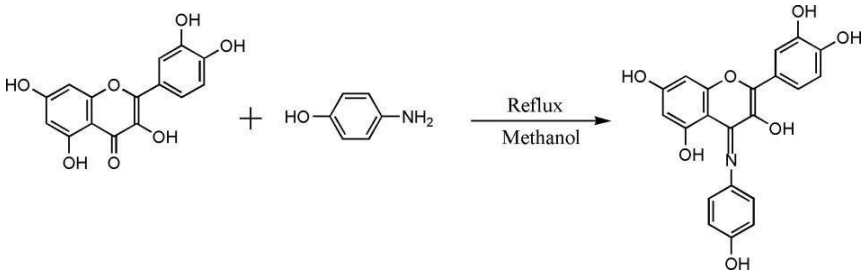
4) DMSO

2.3 PREPARATION OF LIGAND

2.3.1 Synthesis of Schiff base from Quercetin and 4-Amino phenol (QAP)

Quercetin in methanol and 4-Amino phenol in methanol were combined in a 1:1 ratio and heated for 6 hours under reflux in an RB flask (Scheme 1).

After cooling and being concentrated, the resultant solution was given time for slow evaporation. After filtering, collecting, and washing with ethanol, the precipitate that had a dark brown hue was recrystallized and dried. The crystals were collected (Figure 2.1).

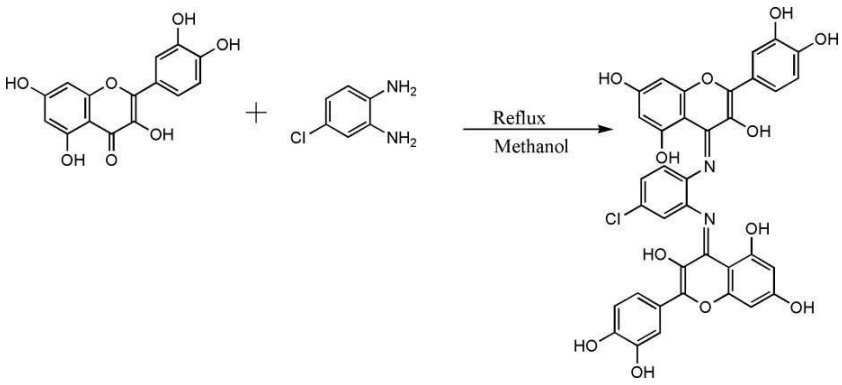


Scheme:1 Preparation of ligand QAP

Figure 2.1: Crystals of QAP

2.3.2 Synthesis of Schiff base from Quercetin and 4-Chloro-o-phenylenediamine (QCP)

Quercetin in methanol and 4-Chloro-o-phenylenediamine in methanol were combined in a 1:1 ratio and heated for 6 hours under reflux in an RB flask (Scheme 2). After cooling and being concentrated, the resultant solution was given time for slow evaporation. After filtering, collecting, and washing with ethanol, the precipitate that had a brown hue was recrystallized and dried. The crystals were collected (Figure 2.2).

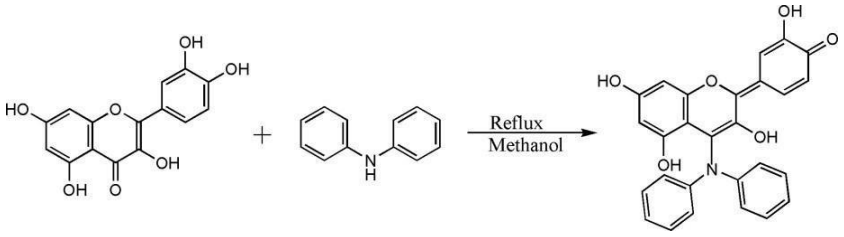


Scheme:2 Preparation of ligand QCP

Figure 2.2: Crystals of QCP

2.3.3 Synthesis of Schiff base from Quercetin and Diphenyl amine (QDP)

Quercetin in methanol and Diphenyl amine in methanol were combined in a 1:1 ratio and heated for 6 hours under reflux in an RB flask (Scheme 3). After cooling and being concentrated, the resultant solution was given time for slow evaporation. After filtering, collecting, and washing with ethanol, the precipitate that had a yellow hue was recrystallized and dried. Thecrystals were collected (Figure 2.3).



Scheme:3 Preparation of ligand QDP

Figure 2.3: Crystals of QDP

2.4 INSTRUMENTAL TECHNIQUES

2.4.1 CHN Elemental Analysis

CHN elemental analysis provides a measure of carbon, hydrogen, and nitrogen elemental content in a sample. It can be used on a wide range of sample types including solids, liquids, and volatile substances. It helps to determine the structure of the sample substance by knowing the composition of the elements. it gives a pure and accurate measurement of the elements present in a sample.[47] CHN elemental analysis is based on the combustion of the sample. Upon combustion, the sample generates uniform compound gases of elements C, H, and N. Then it is measured using gas chromatography.[48]

CHN Elemental Analysis was recorded at the SAIF, Cochin University of Science and Technology, Kochi, India.

2.4.2 Infrared Spectroscopy

Infrared spectroscopy or vibrational spectroscopy is concerned with thestudy of absorption of infrared radiation, which results in vibrational transitions. Infrared spectroscopy is an important analytical technique for determining the structure of both inorganic and organic compounds. It isalso used to study and identify functional groups in solid, liquid, or gaseous form. Infrared radiation refers broadly to that part of the electromagnetic spectrum between visible and microwave regions. The Fourier Transform Infrared Spectrometer is the common laboratory instrument used to determine the infrared spectrum.[49]

Infrared spectra were recorded in the range 4000-400 cm-1 at the Department of Chemistry, Bharata Mata College, Kochi, India.

2.4.3 Ultraviolet-Visible Spectroscopy

Ultraviolet-Visible Spectroscopy (UV-Visible) is the absorption of electromagnetic radiation in the ultraviolet-visible region by a molecule or atom or ion giving electronic transitions in various energy levels hence it is also known as Electromagnetic Spectroscopy. Molecules containing both bonding and non-bonding electrons absorb energy and get excited to higher energy levels. There can be four possible types of transitions (π-π*, n-π*, σ-σ*, and n- σ*), and the following is their order of energy: σ- σ* > n- σ* > π-π* > n-π*.[50]

Ultraviolet-Visible Spectroscopy was recorded in the range 200 to 700 nm at the Department of Zoology, St. Teresa's Collage (Autonomous), Kochi, India.

2.4.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance spectroscopy is a technique used to study molecules. It involves recording the interactions between radiofrequency electromagnetic radiation and the nuclei of molecules in a strong magnetic field. NMR Spectroscopy is used in research and quality control. It can determine the purity and content of a sample as well as its molecular structures. It also analyses mixtures that contain known compounds. NMR Spectroscopy is based on the reorientation of atomic nuclei with non-zero nuclear spin in an external magnetic field. NMR Spectroscopy provides both quantitative and qualitative data on the composition of a sample.[51]

NMR Spectroscopy was recorded in the range 0-20 ppm for H1 NMR and 0-220 ppm for C13 NMR at the SAIF, Cochin University of Science and Technology, Kochi, India.

Chapter 3

Results and discussion

3.1 ELEMENTAL ANALYSIS

The elemental analysis obtained agrees with the assigned chemical formula of the proposed structure of Schiff bases. The analytical data for the Schiff base QAP, QCP, and QDP are given in Table 3.1.

Compound	Empirical formula	Formula weight	Colour	Calculated (found %)		
				C	H	N
QAP	$C_{22}H_{17}O_7N$	407.37	Brown	64.86 (64.80)	4.21 (4.16)	3.44 (3.38)
QCP	$C_{36}H_{23}O_{12}N_2Cl$	711.03	Light Brown	60.81 (60.74)	3.26 (3.20)	3.94 (3.89)
QDP	$C_{27}H_{19}O_6N$	453.44	Yellow	71.52 (71.46)	4.22 (4.17)	3.09 (3.01)

Table 3.1: Elemental analysis

3.2 INFRARED SPECTROSCOPY

The peaks shown in the IR Absorption spectrum give important information about the different functional groups present in the Schiff base (Figure 3.1 – 3.3). The peak obtained in the range 1690-1640 cm^{-1} indicates thepresence of the imine (C=N) group. The peaks obtained in the 3500-3200 cm-1 range indicate the phenolic (OH) group. The peak obtained in the range 1180-1100 cm^{-1} indicates the aromatic amine (C-N) group. FT-IR spectral bands of Schiff bases and their spectra are given in Table 3.2.

COMPOUND	**υ(OH)**	**υ(C=N)**	**υ(C-N)**
QAP	3279.28	1660.49	-
QCP	3287.71	1660.21	-
QDP	3382.80	-	1142.01

Table 3.2: Infrared spectral data of Schiff bases

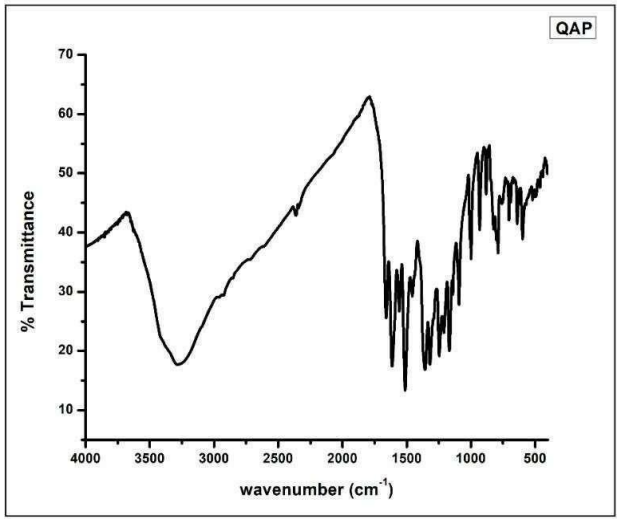


Figure 3.1: IR spectrum of QAP

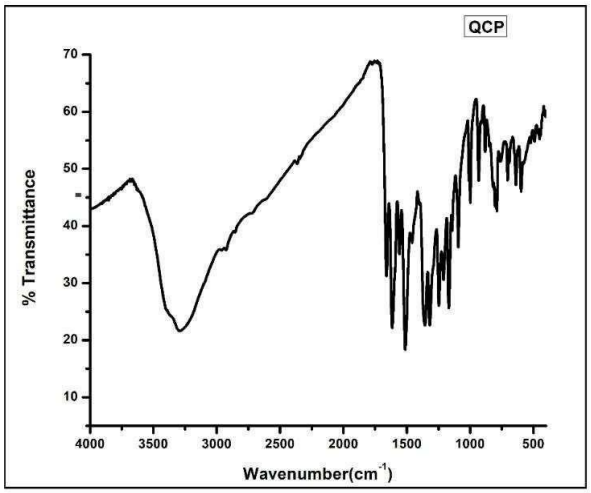


Figure 3.2: IR spectrum of QCP

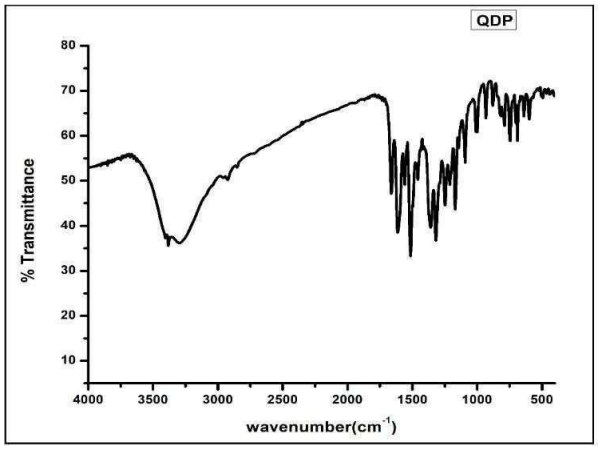


Figure 3.3: IR spectrum of QDP

3.3 UV-VISIBLE SPECTROSCOPY

UV-Visible Spectroscopy is used to study the electronic structure and its dynamics in atoms and molecules. The UV-Visible spectra of compounds QAP, QCP, and QDP were taken in methanol (Figure 3.4 – 3.6). The $\pi - \pi^*$ transitions are assigned to the transitions of the aromatic ring and the $n - \pi^*$ transitions are assigned to the transitions of the CN group. The UV-Visible spectra of the compounds QAP, QCP, and QDP show significant bands at 255.39 nm, 254.60nm, and 256.64 nm which indicate the transitions within the aromatic ring. The bands obtained for QAP, QCP, and QDP at 370.39 nm, 371.96 nm, and 370 nm show characteristic transitions within the C=N group. The spectral data are given in Table 3.3.

COMPOUND	**$n - \pi^*$**	**$\pi - \pi^*$**
QAP	370.39	255.39
QCP	371.96	254.60
QDP	370	256.64

Table 3.3: UV-Visible Spectral data of Schiff bases

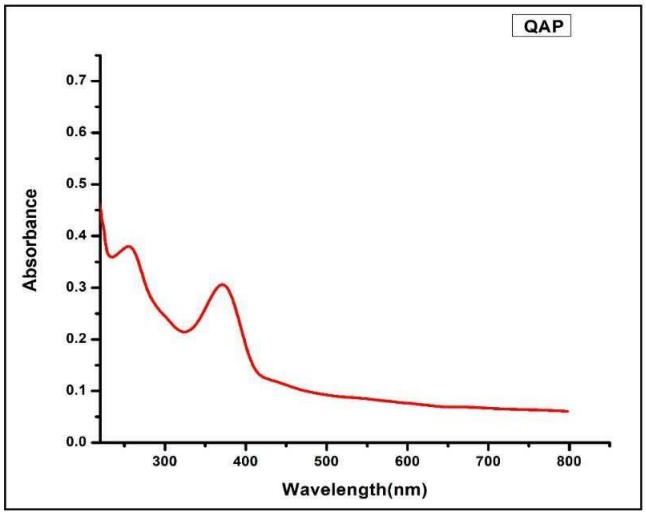


Figure 3.4: UV-Visible Spectrum of QAP

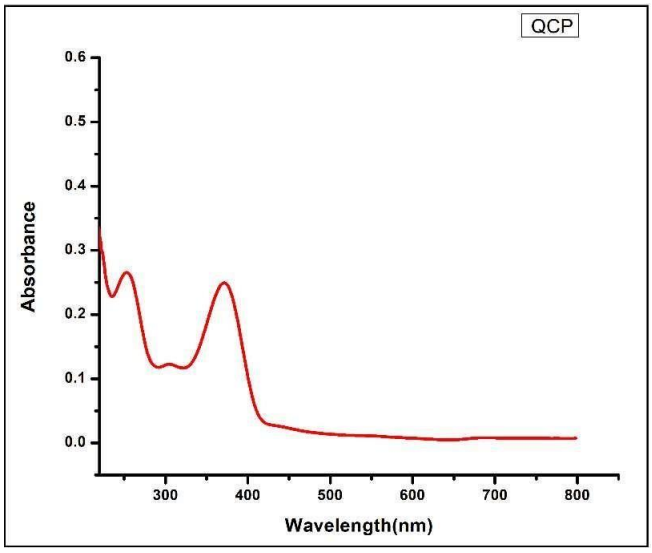


Figure 3.5: UV-Visible Spectrum of QCP

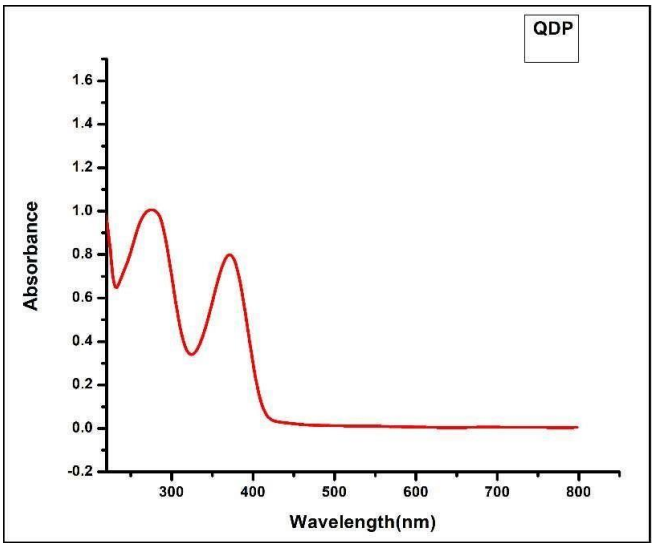


Figure 3.6: UV-Visible Spectrum of QDP

3.4 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

For unknown compounds, NMR can either match against spectral libraries or infer the basic structure directly. Once the basic structure is known, NMRcan be used to determine molecular conformation in solution as wellas study physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. To achieve the desired results, a variety of NMR techniques are available.

The Schiff base's H^1 NMR and C^{13} NMR spectrum is recorded in DMSO using TMS as an internal standard shown in (Figures 3.7 - 3.12). The chemical shift values of different protons obtained are as in Table 3.4.

COMPOUND	ASSIGNMENTS
QAP	**H^1 NMR (DMSO δppm);** 6.185 (1H, Ar-H), 6.190 (1H, Ar-H), 6.407 (1H, Ar-H), 6.412 (1H, Ar-H), 6.876 (1H, Ar-H), 6.897 (1H, Ar-H), 7.526 (1H, Ar-H), 7.547 (1H, Ar-H), 7.673 (1H, Ar-H), 7.679 (1H, -OH), 9.454 (3H, -OH), 12.481 (2H, -OH). **C^{13} NMR (DMSO δppm);** 93.815 (Ar-C), 98.649 (Ar-C), 103.458 (Ar-C), 115.517 (Ar-C), 116.064 (Ar-C), 120.437 (Ar-C), 122.413 (Ar-C), 123.348 (Ar-C), 124.698 (Ar-C), 126.338 (Ar-C), 136.179 (Ar-C-OH), 145.513 (Ar-C-OH), 147.252 (Ar-C-OH), 148.157 (Ar-C-O), 156.591 (Ar-C-O), 161.168 (Ar-C-OH), 164.362 (Ar-C-OH), 176.287 (Ar-C=N).
QCP	**H^1 NMR (DMSO δppm);** 6.192 (2H, Ar-H), 6.418 (4H, Ar-H), 6.889 (3H, Ar-H), 7.544 (1H, Ar-H), 9.343 (6H, -OH), 10.836 (2H, -OH), 12.490 (2H, -OH). **C^{13} NMR (DMSO δppm);** 93.829 (Ar-C), 98.664 (Ar-C), 103.472 (Ar-C), 114.394 (Ar-C), 115.522 (Ar-C), 116.092 (Ar-C), 116.958 (Ar-C), 120.457 (Ar-C), 122.422 (Ar-C), 132.182 (Ar-C), 136.199 (Ar-C-Cl), 137.450 (Ar-C-OH), 145.527 (Ar-C-OH), 147.247 (Ar-C-O), 148.165 (Ar-C-OH), 156.595 (Ar-C-O), 161.182 (Ar-C-OH), 164.386 (Ar-C-OH), 176.298 (Ar-C=N),
QDP	**H^1 NMR (DMSO δppm);** 6.194 (1H, Ar-H), 6.200 (1H, Ar-H), 6.414 (1H, Ar-H), 6.419 (1H, Ar-H), 6.884 (1H, Ar-H), 6.905 (1H, Ar-H), 7.537 (1H, Ar-H), 7.564 (1H, Ar-H), 7.689 (1H, Ar-H), 7.695 (1H, Ar-H), 8.129

	(2H, Ar-H), 9.396 (3H, Ar-H), 9.396 (2H, -OH), 12.504 (2H, -OH). **C^{13} NMR (DMSO δppm);** 93.817 (Ar-C), 98.654 (Ar-C), 103.472 (Ar-C), 115.528(Ar-C), 116.068 (Ar-C), 117.163 (Ar-C), 120.098 (Ar-C), 122.427 (Ar-C), 129.608 (Ar-C), 136.201 (Ar-C-OH), 143.862 (Ar-C), 145.522 (Ar-C), 147.255 (Ar-C-OH), 148.165 (Ar-C-O), 156.602 (Ar-C-O), 161.190 (Ar-C-OH), 164.371 (Ar-C-OH), 176.301 (Ar-C-N).

Table 3.4: H^1 NMR and C^{13} NMR spectral data of the Schiff bases

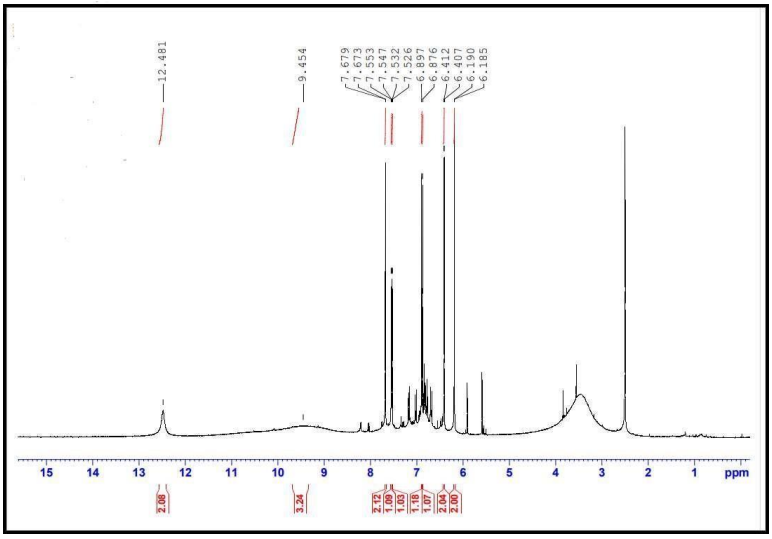


Figure 3.7: H^1 NMR Spectrum of QAP

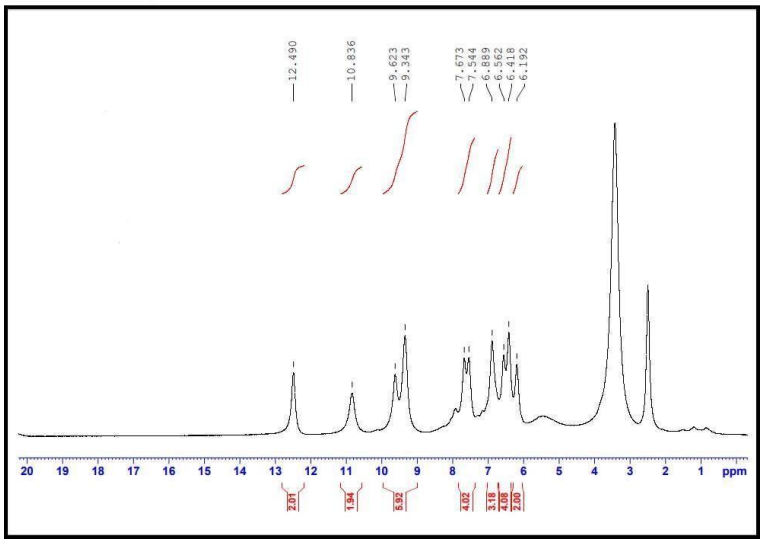


Figure 3.8: H^1 NMR Spectrum of QCP

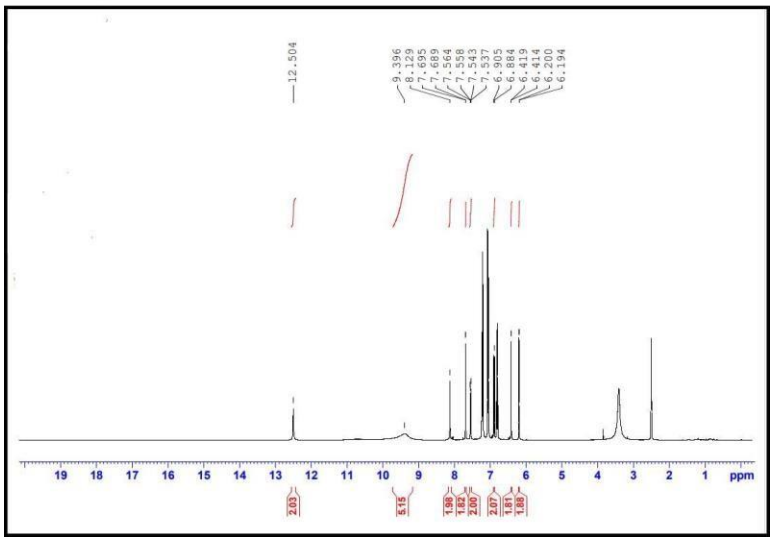


Figure 3.9: H^1 NMR Spectrum of QDP

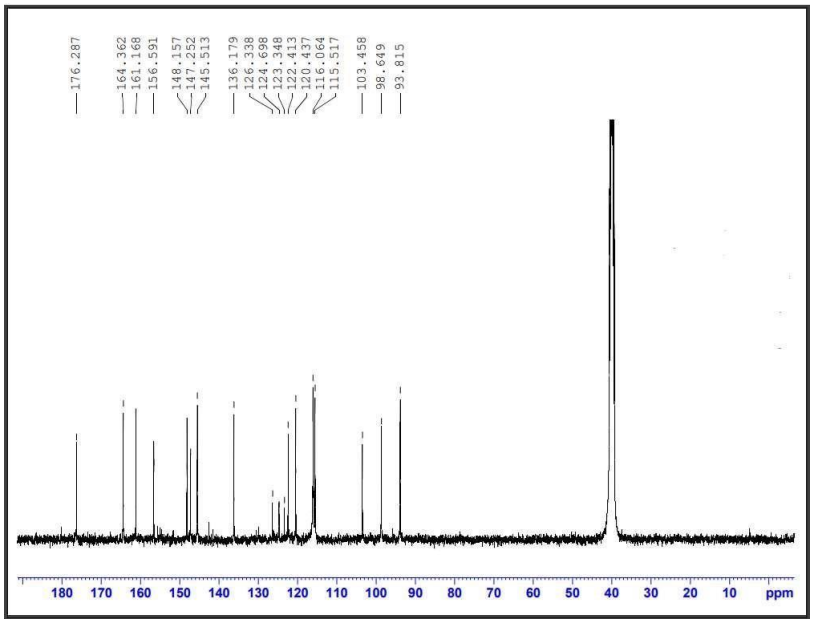


Figure 3.10: C^{13} spectrum of QAP

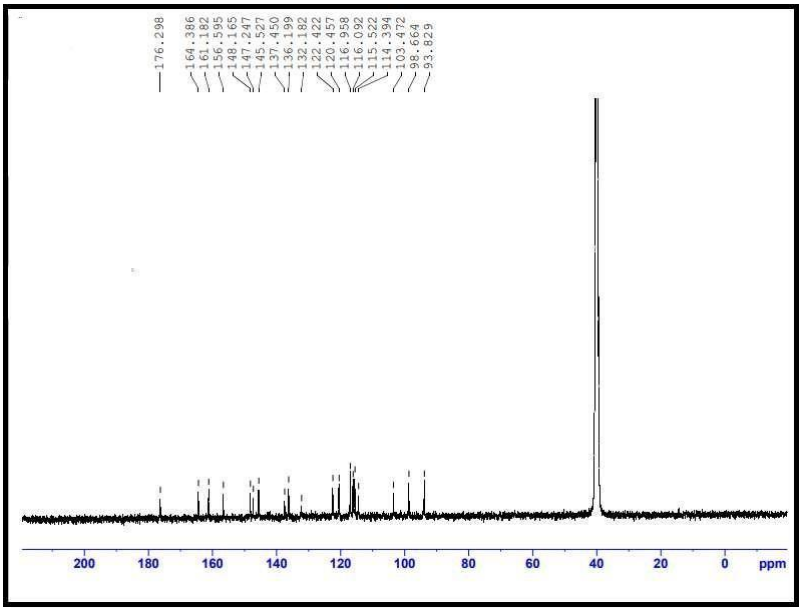


Figure 3.11: C^{13} spectrum of QCP

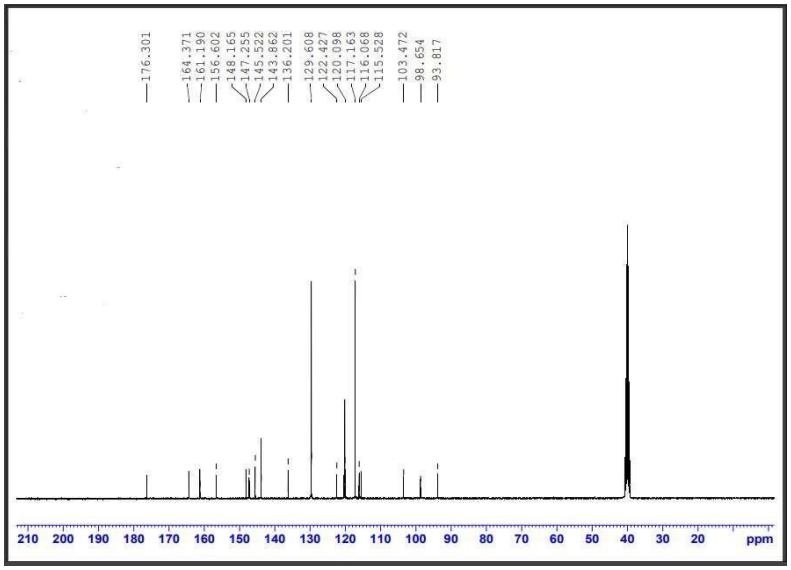


Figure 3.12: C^{13} spectrum of QDP

3.5 ANTI-INFLAMMATORY ACTIVITY OF SCHIFF BASES

3.5.1 INFLAMMATION

Inflammation is the response of our body's immune system towards an irritant. This irritant might be pathogens like bacteria or viruses, or it could also be foreign objects such as a throne on our finger.[52] Inflammation starts immediately when an irritant is identified in our body. The response may be in the form of redness or swelling of the wound.[53]

The steps involved in inflammation are as follows:

- When injured chemical signals are released by activated macrophages and mast cells at the injury site causes nearby capillaries to widen and become more permeable (Figure 3.13).
- Fluid, antimicrobial proteins, and clotting elements move from the blood to the site. Hence clotting begins.
- Chemokines attract more phagocytic cells from the blood to the injury site.
- Phagocytosis takes place and the wound is healed.[54]

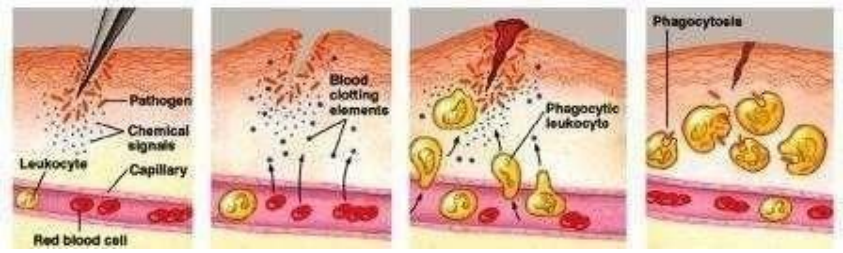


Figure 3.13: Pictorial representation of the steps in inflammation

Acute and chronic are the two different types of inflammation. Acute inflammations are usually short-term inflammation that last for only a few

days whereas chronic inflammations are slow and long-term inflammation that may last for months or even years.[55, 56]

3.5.2 CAUSES OF INFLAMMATION

According to the type of inflammation, the causes can differ. Acute inflammation and chronic inflammation have different causes.[57, 58]

Causes of acute inflammation are:

- Injuries
- Acute infection
- Exposure to a substance (dust, bee sting)
- Ingestion of foreign particles

Causes of chronic inflammation are:

- Organ transplant rejection
- Chronic diseases (cancer, diabetes, asthma)
- Untreated infections
- Lifestyle factors (smoking, alcohol consumption)

3.5.3 EFFECTS OF INFLAMMATION

Inflammation is a natural response by the body to injuries, infection, or harmful stimuli. While acute inflammation is a protective and localized response, chronic inflammation can have various effects on the body (Figure 3.14).

1. Pain and Swelling: Inflammation often leads to pain and swelling in the affected area, which are protective mechanisms to limit movement and promote healing.

2. Redness and Heat: Increased blood flow to the inflamed area causes redness and warmth, aiding in delivering immune cells to the site of injuryor infection.

3. Loss of Function: Inflammation can impair the function of the affected tissues or organs, especially if it becomes chronic.

4. Chronic Diseases: Prolonged or excessive inflammation is associatedwith the development of various chronic diseases, including cardiovascular diseases, autoimmune disorders, and certain cancers.

5. Systematic Effects: In some cases, inflammation can affect the entire body, leading to symptoms like fatigue, fever, and malaise.

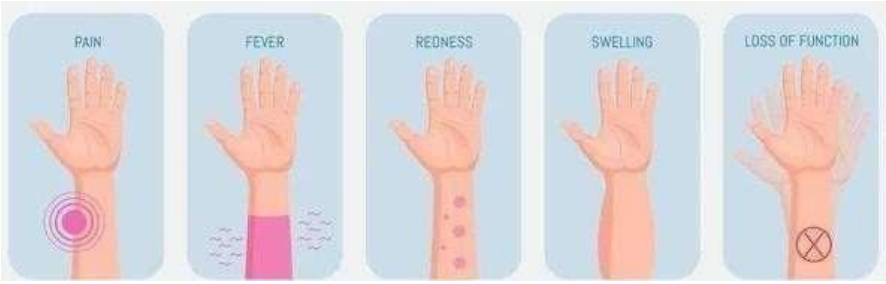


Figure 3.14: Effects of inflammation

Balancing inflammation is crucial, as both insufficient and excessive inflammation can be detrimental. Chronic inflammation, in particular, is implicated in the pathogenesis of numerous health issues.[53, 59]

3.5.4 PREVENTION OF INFLAMMATION

To prevent inflammation, consider maintaining a healthy lifestyle with regular exercise, a balanced diet rich in anti-inflammatory foods like fruits and vegetables, omega-3 fatty acids, and avoiding excessive alcohol and processed foods. Adequate sleep and stress management also play crucial roles in inflammation prevention. Always consult a healthcare professional for personalized advice.[60]

Now there are many drugs to prevent inflammations. Especially some chronic inflammatory diseases are autoimmune, whereas others are ‘‘autoinflammatory.’’ In autoimmune diseases, the T cell dominates as the

primary dysfunctional cell or initiator of the disease process. A cluster of cytokines such as TNF-a, IFN-g, IL-2, IL-12, IL-23, and IL-17 participate in maintaining autoreactive T cells. Rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, psoriasis, lupus, and multiple sclerosis are examples of autoimmune diseases in which the inflammation is secondaryto a disease process that is driven by autoreactive T cells. Most autoimmune diseases can be treated with any one of a number of ‘‘biologicals’’ (Table 1). The major side effect of biologicals is a reduction in host defense against infection. When detected early, these infections can be treated with antibiotics.[61] Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage the pain and inflammation (Table 3.5).[62]

DRUGS	FUNCTION	DISEASES TREATED
Anti-CD3 (Teplizumab): anti-IL-2 receptor MoAb (daclizumab)	Targeting T cells	Transplant rejection: Type 1 diabetics
Anti-CD20 (rituximab, ocrelizumab, ofatumab): anti-CD22 (epratuzumab): anti-Blys MoAb IgG 1 (belimumab)	Targeting B cells	Type 1 diabetes: rheumatoid arthritis: multiple sclerosis
Anti-TNF-a MoAb (infliximab, adalimumab,	Reducing TNF a activities	Rheumatoid arthritis: Crohn’s disease: psoriasis

golimumab): anti-TNF-a pegylated Fab* (certolizumab): soluble TNF p75 receptor Fc fusion (etanercept)		
Anti-IL-6 MoAb (MEDI5117): Anti-IL-6 receptor (tocilizumab)	Reducing IL-6 activities	Rheumatoid arthritis: juvenile arthritis: juvenile arthritis
Anti-IL-12/23 (Ustekinumab): Anti-IL-17 MoAb (AIN457/LY24398) IL-1 receptor antagonist (anakinral): soluble IL-1 receptor (rilonacept): anti-IL-1B (IgG1) (canakinumab): anti-IL-1b (IgG2) (Xoma 052): anti-IL-1R MoAb IgG1 (AMG 108)	Neutralization of IL-12, IL-23, and IL-17 Reducing IL-1b activities	Rheumatoid arthritis: Crohn's Disease: psoriasis Autoinflammatory diseases
Anti-a4 integrins MoAb (natalizumab): anti-LFA-1 MoAb (efalizumab)	Blocking cell adhesion and migration	Multiple sclerosis: Crohn's Disease: psoriasis

Table 3.5: Anti-Inflammatory drugs

3.5.5 ANTI-INFLAMMATORY STUDY OF SCHIFF BASES

3.5.5.1 MATERIALS

1. Human blood
2. Alsever solution
3. Isosaline
4. Schiff bases

3.5.5.2 PROCEDURE

The anti-inflammatory activity of Schiff bases was assessed by in vitro HRBC membrane stabilization method.[63] Fresh whole human blood (10ml) was collected and transferred to the heparin-zed centrifuged tubes. The collected blood was mixed with an equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline (0.85 %, dissolve 8.5g NaCl in water. Autoclave 15 min at 121°C. Cool to room temperature). To 1mL of HRBC suspension, an equal volume of extracts in three different concentrations 10 mg/ml, 5 mg/ml, and 2.5 mg/ml) was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using a spectrophotometer at 560 nm. The percentage of protection can be hence calculated from the equation given below:

Percent of protection = 100-OD of test/OD of control ×100

Control OD560 nm = 1.179

3.5.6 RESULTS AND DISCUSSION

The anti-inflammatory activity of the Quercetin Schiff bases was studied using the HRBC membrane stabilization method. The % of inhibition of the three compounds at different concentrations was calculated. The % of inhibition values of QAP, QCP, and QDP were tabulated in Tables 3.6-3.8. To find the IC_{50} value of the Quercetin Schiff bases a graph was plotted with values % of inhibition against the concentration of the sample whichis shown in (Figures 3.15-3.17). IC_{50} represents the concentration at whicha substance exerts half of its maximal inhibitory effect.[64] This value is typically used to characterize the effectiveness of an antagonist in inhibiting a specific biological or biochemical process. Low IC_{50} value means that the drug is potent at low concentrations, and thus will show lower systemic toxicity when administered to the patient. The IC_{50} values of the compounds QAP, QCP, and QDP were obtained from the graph andtabulated. The IC_{50} values of QAP, QCP, and QDP are given in the Table 3.9.

Compound	**Concentration of sample (mg/ml)**	**% of Inhibition**
QAP	10	65.7
	5	55.7
	2.5	48.5

Table 3.6: Anti-Inflammatory activity of QAP

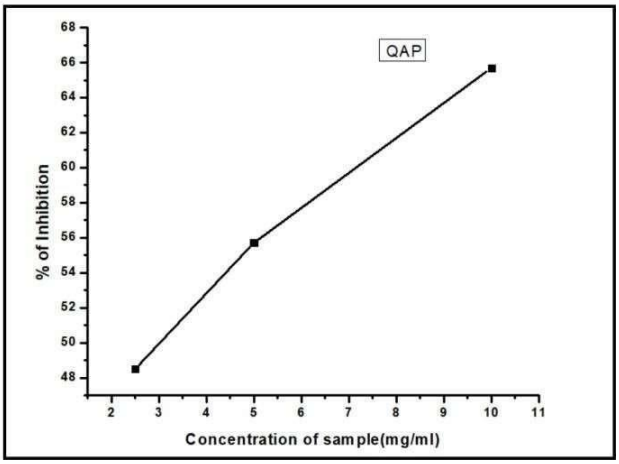


Figure 3.15: Anti-Inflammatory of QAP

Compound	**Concentration of sample (mg/ml)**	**% of Inhibition**
QCP	10	51.7
	5	38.9
	2.5	27.7

Table 3.7: Anti-Inflammatory activity of QCP

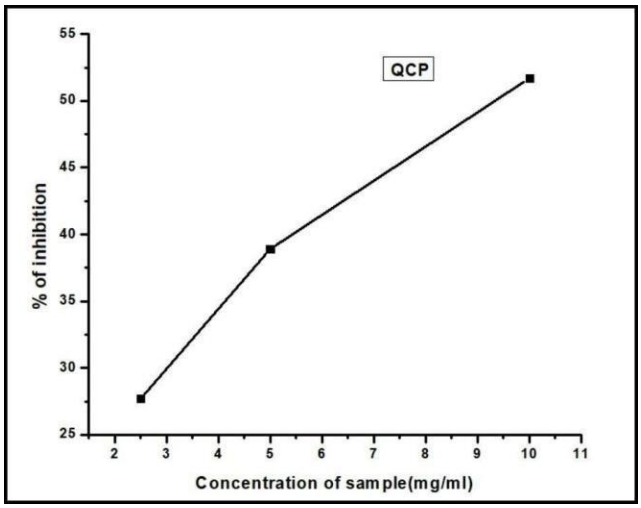


Figure 3.16: Anti-Inflammatory of QCP

Compound	**Concentration of sample (mg/ml)**	**% of Inhibition**
QDP	10	52.9
	5	42.3
	2.5	33.0

Table 3.8: Anti-Inflammatory activity of QDP

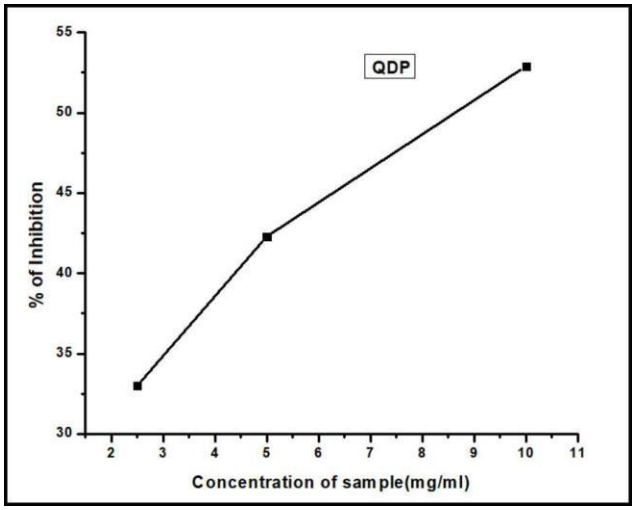


Figure 3.17: Anti-Inflammatory of QDP

COMPOUND	**IC_{50} VALUES (mg/ml)**
QAP	3.0069
QCP	9.3400
QDP	8.6593
Ibuprofen	76.3273

Table 3.9: IC_{50} values

These IC_{50} values of Schiff bases were compared with ibuprofen a widely used non-steroidal anti-inflammatory drug. The IC_{50} value of ibuprofen is 76.3273 mg/ml. The IC_{50} value of QAP, QCP and QDP are much lower than that of ibuprofen. The synthesized Quercetin Schiff bases were good anti-inflammatory agents.

Chapter 4

Conclusions

In the present work three new Quercetin Schiff bases were synthesised and characterized. Quercetin Schiff bases has been synthesized by the condensation of 4-aminophenol (QAP), 4-chloro-o-phenylenediamine (QCP) and diphenylamine (QDP) with Quercetin. All the synthesized Quercetin Schiff bases were characterized by IR, UV-Visible, Elemental analysis, 1H and 13C NMR spectroscopic methods. All these studies give good evidence for the formation the Quercetin Schiff bases. Beside these, this work also evaluates and studied the anti-inflammatory activity of the synthesized Quercetin Schiff bases which is briefly discussed in the work. From the anti-inflammatory studies the IC_{50} values of the Quercetin Schiff bases were obtained. The IC_{50} value of QAP, QCP and QDP are 3.0069 (mg/ml), 9.3400 (mg/ml) and 8.6593 (mg/ml) respectively. From the comparative studies of Schiff bases with ibuprofen, QAP, QCP and QDP is found to be more effective than ibuprofen.

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