Green synthesis and antioxidant study of coumarin derivatives using lemon juice and oxalic acid.

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Abstract

Coumarins are an important class of heterocyclic compounds possessing a benzopyran-2-one nucleus and are widely recognized for their diverse biological and pharmacological activities. In the present study, coumarin derivatives were synthesized using an eco-friendly approach through Pechmann condensation by employing green catalysts such as oxalic acid and lemon juice extract, and the compounds (CO-1 and CL-1) were obtained in good yield and purified by recrystallization. Structural characterization using FTIR and GC-MS confirmed the successful formation of the coumarin framework, while antioxidant evaluation by the DPPH assay showed that CL-1 exhibited superior activity with a lower IC_{50} value compared to CO-1. Antibacterial studies demonstrated promising inhibitory effects, and molecular docking revealed favorable binding interactions with a maximum binding affinity of –6.409 kcal/mol involving key amino acid residues, indicating strong ligand–protein interactions. Overall, the study highlights the potential of green synthesized coumarin derivatives as effective antioxidant and antibacterial agents and emphasizes their significance in sustainable medicinal chemistry and future drug development.

Keywords:

Coumarin derivatives, Green synthesis, Pechmann condensation, FTIR, GC-MS, Antioxidant activity, DPPH assay, Antibacterial activity, Molecular docking studies.

Introduction

Coumarins are a prominent class of heterocyclic organic compounds possessing a benzopyrone nucleus, specifically 2H-1-benzopyran-2-one, which contributes to their distinctive chemical and biological behavior. These compounds are widely distributed in nature and have been isolated from numerous plant families such as Rutaceae, Apiaceae, and Fabaceae, particularly in species like tonka beans (Dipteryx odorata), sweet clover (Melilotus officinalis), cinnamon, and citrus fruits. Coumarins play essential physiological roles in plants, including functioning as phytoalexins, growth regulators, and natural defense agents. The basic coumarin scaffold is easily modified at various positions, leading to a diverse range of derivatives with enhanced or targeted pharmacological properties. This versatility has attracted significant attention in medicinal chemistry, natural product synthesis, and drug design **[1].**

From a structural standpoint, coumarin derivatives can be classified into several categories: simple coumarins, furanocoumarins, pyranocoumarins, and more complex polycyclic forms. The chemical reactivity of coumarins arises from the conjugated π-system, enabling electrophilic and nucleophilic substitutions that allow a wide spectrum of modifications. The functionalization at positions 3, 4, 6, 7, and 8 is particularly significant, as these substitutions heavily influence the physicochemical properties and bioactivity of the molecule. For instance, the introduction of hydroxyl or methoxy groups enhances solubility and antioxidant potential, while halogenated or alkylated derivatives often show improved antimicrobial or cytotoxic activity. Additionally, the coumarin nucleus possesses inherent fluorescence properties, making these compounds useful not only in pharmaceuticals but also in diagnostic imaging and laser dyes **[2].**

Biosynthetically, coumarins are produced through the phenylpropanoid pathway in plants. The precursor, cinnamic acid, undergoes hydroxylation and lactonization to form coumarins such as umbelliferone, scopoletin, and esculetin. Furanocoumarins like psoralen and xanthotoxin contain a furan ring fused to the coumarin core and are known for their ability to intercalate with DNA upon UV irradiation—a property that has been clinically utilized in PUVA therapy for treating skin disorders like psoriasis and vitiligo. Pyranocoumarins, which contain a fused pyran ring, are less common but show notable biological effects, particularly cytotoxicity against cancer cell lines. The diversity found in natural coumarins has inspired synthetic chemists to develop analogues for therapeutic exploration, often using them as lead scaffolds in rational drug design **[3].**

Various synthetic routes have been developed to obtain coumarin derivatives. Among the most widely used is the Pechmann condensation, which involves the acid-catalyzed reaction of phenols with β-keto esters. This method is simple, cost-effective, and ideal for preparing mono-substituted coumarins. Other methods include the Knoevenagel condensation, Reformatsky reaction, and Perkin reaction. In recent years, environmentally friendly synthetic techniques such as microwave-assisted synthesis, ultrasonic irradiation, and solvent-free reactions have been developed to enhance reaction efficiency and reduce environmental impact. The accessibility of the coumarin core and ease of chemical manipulation make it an ideal scaffold for developing multifunctional molecules. For instance, 3-carboxycoumarins, 4-aminocoumarins, 7-hydroxycoumarins have been widely explored for their diverse applications, including antimicrobial, anticoagulant, and anticancer therapies **[4].**

Pharmacologically, coumarin derivatives exhibit an extensive spectrum of bioactivities. One of the most well-known applications of coumarins is in anticoagulant therapy. Warfarin, a synthetic coumarin derivative, acts as a vitamin K antagonist by

inhibiting the enzyme vitamin K epoxide reductase, which plays a key role in the blood clotting cascade. Warfarin and related derivatives such as acenocoumarol and phenprocoumon are used worldwide to manage thromboembolic disorders. Antimicrobial activity is another major area of interest; several coumarin derivatives have demonstrated potent antibacterial effects against Gram-positive and Gram-negative bacteria and antifungal activity against various pathogenic fungi. This is often attributed to mechanisms such as inhibition of bacterial DNA gyrase or disruption of microbial cell walls. The anti-inflammatory potential of coumarins is attributed to their ability to inhibit pro-inflammatory enzymes like COX-2 and iNOS and reduce the production of cytokines such as TNF-α and IL-6. These effects have been confirmed in both in vitro and in vivo models, making coumarin-based drugs promising candidates for the treatment of inflammatory diseases **[5].**

Antimicrobial-Activity:

Coumarin derivatives inhibit bacterial growth primarily by targeting critical enzymes such as DNA gyrase and topoisomerase IV, essential for DNA replication and transcription. Triazole and pyrazoline-substituted coumarins have shown minimal inhibitory concentrations (MICs) against multi-drug resistant strains comparable to or better than existing antibiotics like chloramphenicol and streptomycin**.** The hydroxyl and halogen groups enhance binding through hydrogen bonding and hydrophobic interactions, overcoming bacterial resistance mechanisms by avoiding efflux pumps and enzymatic degradation**[6].**

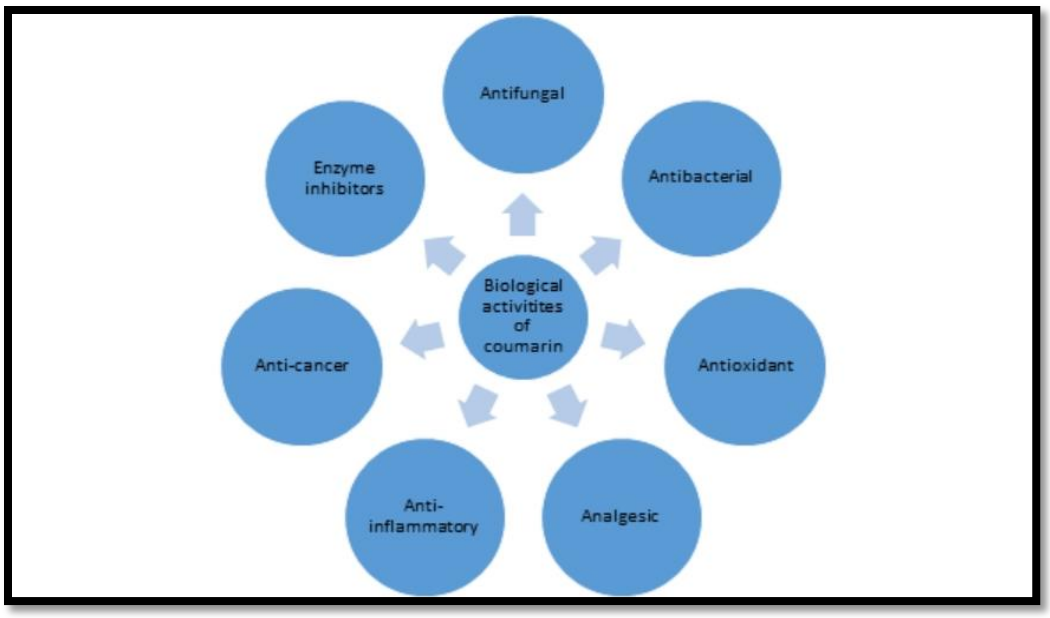

Figure 1: Biological activities of Coumarin derivatives

AnticancerActivity:

The anticancer potential of coumarins involves multiple mechanisms, including DNA intercalation, induction of apoptosis, inhibition of kinases, and modulation of signaling pathways. For instance, coumarin hybrids that inhibit the PI3K/AKT pathway can reduce cancer cell proliferation and metastasis by downregulating survival signals **[7].** Nanomolar IC_{50} values reported for derivatives like scopoletin and esculetin against resistant cancer lines highlight their potency. Chalcone- and pyrazole-linked coumarins have also shown synergistic effects, disrupting cell cycle checkpoints and enhancing reactive oxygen species (ROS) production to trigger cancer cell death **[7,9].**

Anticoagulant and Anti-inflammatory Effects:

Warfarin, a classic anticoagulant coumarin derivative, works by inhibiting vitamin -K epoxide reductase, impairing the synthesis of clotting factors II, VII, IX, and X

[8]. Structural analogues with improved pharmacokinetics and reduced side effects are being developed. Coumarins also downregulate COX-2 and pro-inflammatory cytokines such as TNF-α and IL-6, exhibiting analgesic and anti-inflammatory activities useful in chronic inflammatory diseases **[8,10].**

Neuroprotective Effects:

Neurodegenerative disorders have been targeted by coumarins through inhibition of acetylcholinesterase (AChE) and monoamine oxidase (MAO) enzymes, enhancing neurotransmitter availability and reducing neurotoxicity **[9].** Certain coumarin derivatives act as metal chelators and antioxidants, protecting neurons from oxidative damage and apoptosis, offering multi-target therapeutic potential in Alzheimer's and Parkinson's diseases **[8].**

Antioxidant study:

Coumarin derivatives are a prominent class of naturally occurring and synthetic compounds known for their wide range of biological activities, particularly antioxidant properties. Their antioxidant activity is mainly attributed to their ability to scavenge free radicals and inhibit oxidative stress, which is associated with aging and various diseases such as cancer, diabetes, and neurodegenerative disorders. Structural modifications on the coumarin ring, especially at positions 3, 4, and 7, have shown to enhance their electron-donating ability and radical stabilization. These features make coumarin derivatives attractive candidates in the design of new antioxidant agents **[11].**

Recent Advances in Synthesis

To meet the demand for sustainable and efficient coumarin synthesis, new synthetic methodologies have been developed. Microwave-assisted organic synthesis

(MAOS) dramatically reduces reaction times and enhances yields by rapidly heating reaction mixtures, enabling eco-friendly syntheses of coumarin derivatives [1,10]. Solvent-free syntheses and the use of reusable catalysts like ionic liquids and metal-organic frameworks (MOFs) minimize environmental impact and waste generation **[10].**

metal-organic frameworks (MOFs) and click chemistry have revolutionized coumarin derivatization, allowing rapid assembly of diverse libraries by combining three or more reactants in one pot, thereby accelerating drug discovery pipelines **[1].** For example, the Huisgen 1,3-dipolar cycloaddition efficiently attaches triazole rings to the coumarin scaffold under mild conditions, producing molecules with high bioactivity and target specificity **[6].**

Photocatalytic and enzymatic methods have also emerged, offering regioselective and stereoselective pathways under ambient conditions, expanding the scope of coumarin chemistry **[10].**

Applications and Future Prospects

The intrinsic fluorescence and tunable photophysical properties of coumarin derivatives have broadened their applications beyond therapeutics into bioimaging, fluorescence sensors, and optoelectronics. By manipulating electronic substituents, researchers have developed coumarins with specific excitation/emission wavelengths ideal for cellular imaging and environmental monitoring **[8].**

Hybrid drug design, combining coumarin with other pharmacophores like flavonoids, quinolines, and steroids, enhances pharmacology by targeting multiple pathways simultaneously. This strategy reduces drug resistance and toxicity, particularly in complex diseases like cancer and neurodegeneration**.** Computational

drug design, including molecular docking and dynamics simulations, is increasingly integrated with synthesis to predict binding affinities and guide the rational design of potent coumarin-based drugs. This in silico approach shortens development timelines and improves candidate selection **[7].**

Future research aims to harness the full therapeutic potential of coumarins by exploring novel bioactive hybrids, sustainable synthetic methods, and multifunctional materials with applications spanning medicine, agriculture, and technology.

Docking studies: Molecular docking serves as a powerful computational tool to predict the binding orientation and affinity of coumarin analogs within the active sites of biological macromolecules. This approach aids in elucidating structure-activity relationships (SARs), guiding the rational design of novel therapeutics with improved efficacy and selectivity **[12].** Furthermore, docking studies on coumarin derivatives have been instrumental in identifying potential inhibitors of targets such as acetylcholinesterase, tyrosinase, topoisomerase, and various kinases, which are relevant in neurodegenerative, dermatological, and oncological disorders **[13].**

Coumarin derivatives have emerged as a highly significant class of heterocyclic compounds due to their wide-ranging biological and pharmacological activities. Structurally defined as benzopyran-2-ones, coumarins occur naturally in various plants and microorganisms and are recognized for their favorable pharmacokinetic profiles and diverse bioactivities including antimicrobial, anti-inflammatory, anticoagulant, antioxidant, anticancer, antiviral, and neuroprotective effects.

emphasized the antimicrobial potential of various synthetic coumarin analogues, especially hybrid structures such as β-lactam-triazole and coumarin-sulphonamide

compounds. These analogues have demonstrated activity against drug-resistant bacterial strains, highlighting the importance of structure-activity relationship (SAR) studies in optimizing biological performance **[14].**

Similarly,provided a comprehensive review of the synthesis of coumarin derivatives, particularly those incorporating heterocyclic frameworks. They discussed the application of classical reactions like Perkin, Pechmann, and Knoevenagel condensations, and explored the growing use of nanocatalysts and green methodologies. The review confirmed the versatility of coumarins in drug development, especially for designing agents targeting cancer, Alzheimer's disease, and infectious diseases **[15].**

The anti-inflammatory activity of coumarin Schiff base derivatives was explored by, who synthesized four compounds and assessed them using both *in silico* docking (against COX-2) and in vitro protein denaturation assays. Compound 7 demonstrated the most potent activity, showing promising therapeutic potential as a safer alternative to NSAIDs **[16].**

In another study, synthesized coumarin–nicotinonitrile hybrids and evaluated their acetylcholinesterase inhibitory activity, relevant to Alzheimer’s disease treatment. Certain derivatives exhibited greater inhibitory activity than donepezil, a standard therapeutic agent, while maintaining low cytotoxicity across cancer cell lines, thereby reinforcing the multifunctional potential of coumarins **[17].**

highlighted the role of coumarin derivatives such as warfarin and acenocoumarol as vitamin K antagonists (VKAs) used in anticoagulation therapy. The review also covered the structure-based design of novel VKAs with enhanced safety and

pharmacological profiles, stressing the clinical relevance of coumarin scaffolds in cardiovascular disease management **[18].**

Objectives of the project:

- To design and synthesize coumarin derivatives using simple, eco-friendly methods.
- To characterize the coumarin derivatives using spectral techniques (FTIR and Mass Spectrometry).
- To study the antioxidant activity of the coumarin derivatives.
- To make the docking study of the prepared coumarin derivatives.

4.1 INSTRUMENTATION

4.1.1 Fourier Transform Infrared Spectroscopy (FTIR):

An infrared spectrum is a sample's fingerprint, with absorption peaks corresponding to the frequency of vibrations between the bonds of the atoms that make up the material. Infrared spectroscopy has been an effective technique for Because each material is a unique atom combination, no two compounds create the same infrared spectrum.

As a result, infrared spectroscopy can provide a positive identification (qualitative analysis) of any type of material. Furthermore, the magnitude of the peaks in the spectrum indicates the amount of material present. Infrared is a useful tool for quantitative analysis when combined with modern software algorithms.

Fourier Transform Infrared (FTIR) spectrometry is designed to overcome the limitations of dispersive equipment. The lengthy scanning procedure was a significant constraint. It is preferable to measure all IR frequencies at the same time. The approach employed a basic optical device known as an interferometer to generate a signal that contained all infrared frequencies "embedded" inside it. The signal is normally recorded in a few seconds.

The measured interferogram signal cannot be analyzed directly because the analyst requires a frequency spectrum for identification. It is necessary to have a method of "decoding" the individual frequencies. This is possible because of a well-known mathematical technique known as the Fourier transformation.

Figure 2:Fourier Transform Infrared Spectrometer (FTIR)

For numerous reasons, Fourier transforms infrared spectroscopy is favored over dispersive or filter methods of infrared spectral analysis:

- It is a non-destructive procedure.
- It provides a precise measurement method that does not require external calibration.
- It can increase speed by collecting a scan every second.
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- It can increase sensitivity by combining scans taken at intervals of one second to cancel out random noise.
- It offers higher optical throughput.
- It has a simplified mechanical design with only one moving part.

Advantages of FTIR

It has a higher speed. Because all the frequencies are detected simulate

1. Wide Range of Applications and Versatility

One of the most significant advantages of FTIR is its versatility. It can be used to analyze solids,liquids, and gases. FTIR is applicable in identifying both organic and inorganic compounds and is widely used in industries ranging from pharmaceuticals to polymers, food, cosmetics, and environmental testing.

- **Pharmaceuticals:** Identification of active ingredients, detection of counterfeit drugs, and monitoring of crystallinity in compounds.
- **Environmental Monitoring**: Detection of pollutants in air, water, and soil samples.
- **Forensics**: Identification of unknown substances like fibers, drugs, and explosives.

2. High Sensitivity and Accuracy

FTIR offers high sensitivity and accuracy in detecting even minute amounts of sample materials. Because of the multiplex (Fellgett) advantage, FTIR collects all wavelengths of the infrared spectrum simultaneously, leading to improved signal-to-noise ratio and more accurate spectral data.

- **Quantitative Analysis:** Can be used for quantitative determination of components in mixtures with high precision.

- **Trace Analysis:** Effective in detecting very low concentrations of a substance.

3. Rapid and Non-Destructive Analysis

FTIR is a fast technique that provides results within minutes. Moreover, it is a non-destructive method, meaning the sample remains intact after analysis, which is particularly valuable when working with rare or precious samples.

- **Non-Invasive Testing**: Especially useful for analysing art, historical documents, or delicate biological samples.
- **Minimal Sample Preparation:** Often, no special sample preparation is needed, saving time and reducing the chance of sample contamination.

4. Fingerprint Identification of Compounds

Every chemical compound has a unique infrared absorption spectrum often referred to as a "fingerprint" which allows for precise identification. FTIR can match the obtained spectrum against extensive spectral libraries to identify unknown substances.

- **Reliability:** Because of the unique IR spectra, FTIR can be used reliably for substance verification.
- **Qualitative Analysis:** Excellent for confirming the presence or absence of specific functional groups or chemical bonds.

5. Simultaneous Multi-Component Analysis

FTIR can analyze complex mixtures and identify multiple components in a single scan without separating them. This makes it ideal for real-time monitoring of industrial processes and quality control.

- **Efficiency:** Eliminates the need for separate analysis steps for different components.
- **Process Monitoring**: Used in real-time monitoring of chemical reactions or manufacturing processes.

6. Automation and Computer Integration

Modern FTIR instruments are highly automated and can be controlled via computer software,allowing for easy data acquisition, storage, processing, and comparison.

- **Ease of Use:** User-friendly interfaces and automated data interpretation tools make the technique accessible even to non-specialists.
- **Data Management:** Digital storage of spectra and results simplifies documentation and traceability in regulated environments.

7. Cost-Effective in the Long Run

Although the initial cost of purchasing an FTIR instrument may be high, the overall running costs are relatively low. There are no costly reagents or consumables involved, and the instruments have long service lives.

- **Low Operating Costs:** No need for solvents, chemicals, or complex sample preparation materials.

- **Durability**: Instruments can operate reliably for many years with minimal maintenance.

Applications of FTIR

- ❖ FTIR spectroscopy equipment is commonly used to examine industrially manufactured materials in various quality control processes.
- ❖ FTIR spectroscopy is a common first step in material analysis. A change in the spectrometer's absorption band characteristic patterns suggests a change in the material composition or possible contamination.
- ❖ It is used to dry polymers, photo-resist materials, and polyimides.
- ❖ FTIR spectroscopy investigates the interactions between matter and electromagnetic radiation,which appear as a spectrum.
- ❖ FTIR spectrum analysis has enabled the diagnosis of various organ diseases as well as the quantification of various biomolecules such as proteins, nucleic acids, and lipids.
- ❖ FTIR is a unique approach for characterizing the variation in fuel stability of several bio-diesel/antioxidant mixtures.
- ❖ In most failure analysis investigations, it is used to determine breakdown, oxidation, and uncured monomers.

4.1.2 GC-MS Mass Spectrometry

Gas Chromatography Mass Spectrometry (GC-MS) is a powerful hyphenated analytical technique that combines the separation capabilities of gas chromatography (GC) with the identification and structural elucidation abilities of mass spectrometry

(MS). It is widely used in analytical chemistry, forensic science, environmental analysis, food safety, pharmaceuticals, and biomedical applications. GC-MS is particularly effective for analyzing volatile and semi-volatile organic compounds, offering both qualitative and quantitative data with high sensitivity and specificity. The combination of these two techniques provides a comprehensive analytical platform where GC first separates the components of a mixture, and MS subsequently detects and identifies them based on their mass spectra.

Figure 3: Gas Chromatography Mass Spectrometry

The principle of GC-MS relies on two sequential processes. In the gas chromatography stage, the sample is vaporized and injected into a chromatographic column, typically coated with a liquid stationary phase. An inert carrier gas (such as helium or nitrogen) moves the vaporized sample through the column. Different compounds travel at different speeds depending on their chemical properties, such as volatility and interaction with the stationary phase, resulting in their separation. As each compound elutes from the column at a specific retention time, it enters the mass spectrometer, where it undergoes ionization. In most GC-MS systems, Electron

Ionization (EI) is employed, where molecules are bombarded with high-energy electrons, causing them to ionize and fragment in predictable ways. These ions are then sorted by their mass-to-charge ratio (m/z) using a mass analyzer, such as a quadrupole or time-of-flight system. The resulting signal is detected and plotted as a mass spectrum, which displays the intensity of ions versus their m/z values.

The output of a GC-MS run consists of a total ion chromatogram (TIC), showing retention times of separated compounds, and individual mass spectra for each peak. By comparing the acquired spectra with reference libraries such as NIST or Wiley, compounds can be identified with high confidence. Quantification is achieved by analyzing the peak areas relative to calibration standards. The strength of GC-MS lies in its ability to separate complex mixtures and confirm compound identities based on fragmentation patterns, making it an indispensable tool in the detection of drugs, pollutants, flavor compounds, pesticides, and biomarkers. Recent advancements have improved the sensitivity, speed, and robustness of GC-MS, including the development of tandem MS (GC-MS/MS), high-resolution mass analyzers, and advanced sample preparation techniques like solid-phase microextraction (SPME).

GC-MS continues to be a gold standard in analytical laboratories. For example, studies such as Wan *et al.* (2023) used GC-MS to analyze volatile organic compounds in clinical diagnostics, while Gawalko *et al.* (2022) applied GC-MS/MS to detect trace-level pesticide residues in food samples. These applications demonstrate the method’s reliability in both routine testing and advanced research.

Here are the applications of Gas Chromatography Mass Spectrometry (GC-MS) listed in concise points, covering various scientific and industrial fields:

1. **Environmental Analysis**
    - Monitoring pesticide and herbicide residues in soil and water.
    - Identification of pollutants in industrial emissions and wastewater.
2. **Forensic Science**
    - Analysis of drugs of abuse in biological samples (blood, urine, hair).
    - Identification of accelerants in arson investigations.
    - Detection of poisons and toxins in toxicological screenings.
3. **Pharmaceuticals**
    - Quality control and purity testing of drug formulations.
    - Detection of residual solvents in active pharmaceutical ingredients (APIs).
    - Identification of drug metabolites in pharmacokinetic studies.
4. **Food and Beverage Industry**
    - Detection of food additives, preservatives, and contaminants.
    - Analysis of flavor and aroma compounds in beverages.
    - Monitoring pesticide residues in fruits, vegetables, and grains.
5. **Clinical and Biomedical Research**
    - Biomarker identification for disease diagnosis through breath, blood, or urine analysis.

- Metabolomics studies involving profiling of small molecules in biological samples.

6. Petrochemical and Chemical Industry

- Characterization of hydrocarbons in petroleum products.
- Quality control of fuels, lubricants, and chemical intermediates

7. Toxicology

- Identification of exposure to hazardous substances.
- Screening of environmental and occupational toxins.

8. Flavor and Fragrance Industry

- Profiling of essential oils and perfumes.
- Detection of synthetic additives or adulterants in natural products.

4.2 Methodology:

4.2.1 General method for synthesis of Coumarin Derivatives

In a round bottom flask, a mixture of phenol derivative (10 mmol), ethyl acetoacetate (10 mmol), and catalyst (0.1 g) was refluxed at 70^{o}C for around 4 hours with the solvent ethanol (10 ml) . Then, the resulting reaction mixture was poured into crushed ice. The separated solid was filtered and washed with ice-cold water. product was recrystallized from ethanol.

a) Synthesis of 4-methyl 2H-benzo Chromen-2-one (CO1)

The compound (a) is prepared by re-fluxing beta-naphthol (10mmol) and ethyl acetoacetate (10mmol) in the presence of Oxalic acid catalyst(0.1 g) at 70^{o}C for 4

hrs. and then pouring into crushed ice and then filtered and compound was recrystallized using ethanol.

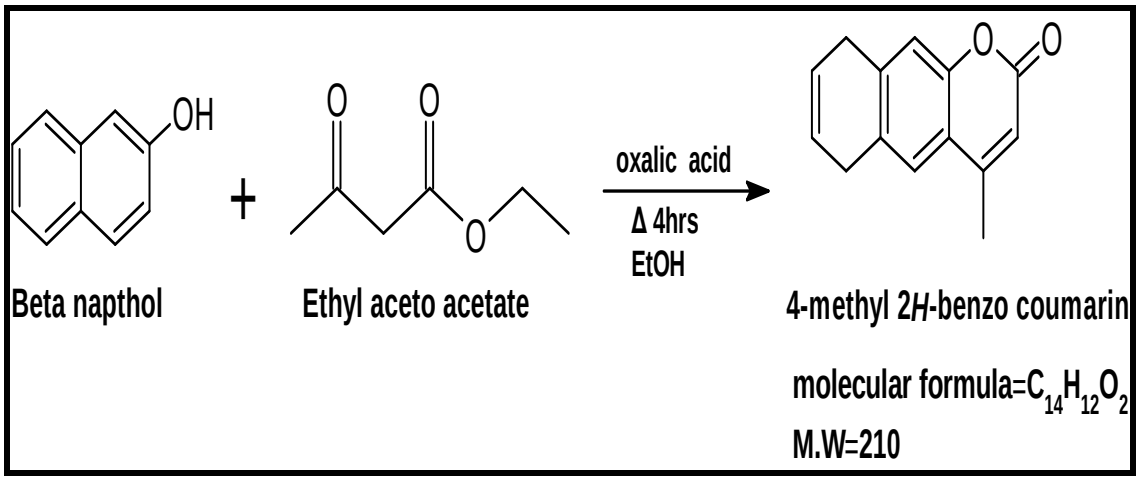

Figure 4: Scheme of synthesis of Compound (a) CO1

b) Synthesis of 4-methyl 2H-benzo Chromen-2-one (CL1)

The compound (b) is prepared by re-fluxing beta-naphthol (10mmol) and ethyl acetoacetate (10mmol) in the presence of lemon juice extract catalyst at 70^{o}C for 4 hrs. and then pouring into crushed ice and then filtered and compound was recrystallized using ethanol.

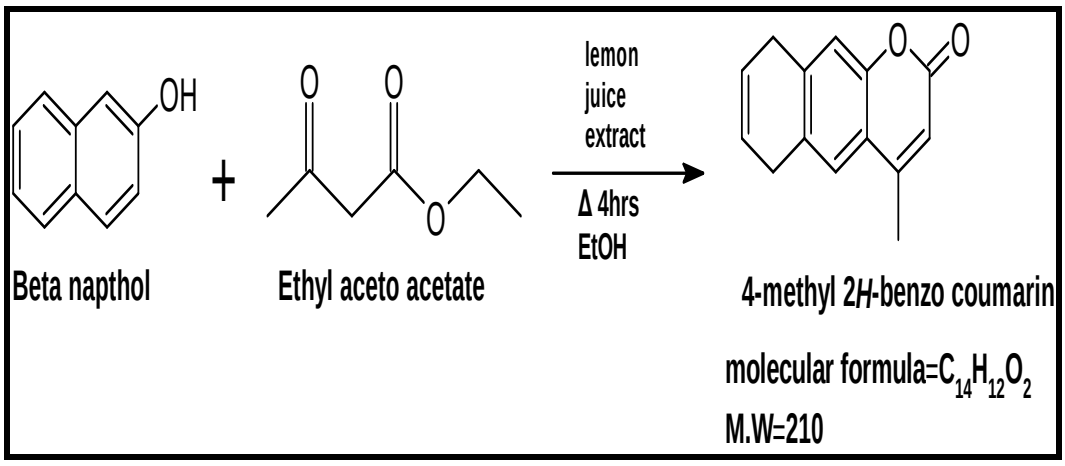

Figure 5: Scheme of synthesis of Compound (b) CL1

4.2.2 ANTIBACTERIAL STUDIES

- Antibacterial behaviour may be measured using agar diffusion techniques.
- By scattering a volume of bacterial culture over the entire agar plate surface, the entire agar plate surface is injected.
- A 3 to 4 mm radius cavity is aseptically drilled with a sterile cork borer, and extract solution or antimicrobial agent is pumped into the well at the desired concentration.
- Agar plates are incubated under suitable conditions based on the test Micro organism.
- The zone of inhibition is the area around the discs that would be apparent if bacteria grew thickly around it.
- To determine antibacterial efficacy, the diameter of the region of inhibition is determined.

5.1 Mass spectral Study :

High-resolution mass spectrometry (HRMS) analysis of the synthesized compound, 4-methylbenzo[c]coumarin ($C_{14}H_{10}O_2$), was performed using electrospray ionization in positive mode (ESI^+). The spectrum exhibited a prominent **molecular ion peak at *m/z* 211.1186,** corresponding to the protonated molecular ion **[M + H]**$^+$. This closely matches the calculated monoisotopic mass of **211.0756** for $C_{14}H_{10}O_2 + H^+$, confirming the expected molecular composition. A minor peak at *m/z* 212.1231 is attributed to the naturally occurring **^{13}C isotope** peak, and the signal observed at *m/z* 251.0791 is likely due to an adduct or trace impurity. The HRMS data is in excellent agreement with the proposed structure, supporting the successful synthesis and molecular identity of 4-methylbenzo[c]coumarin.

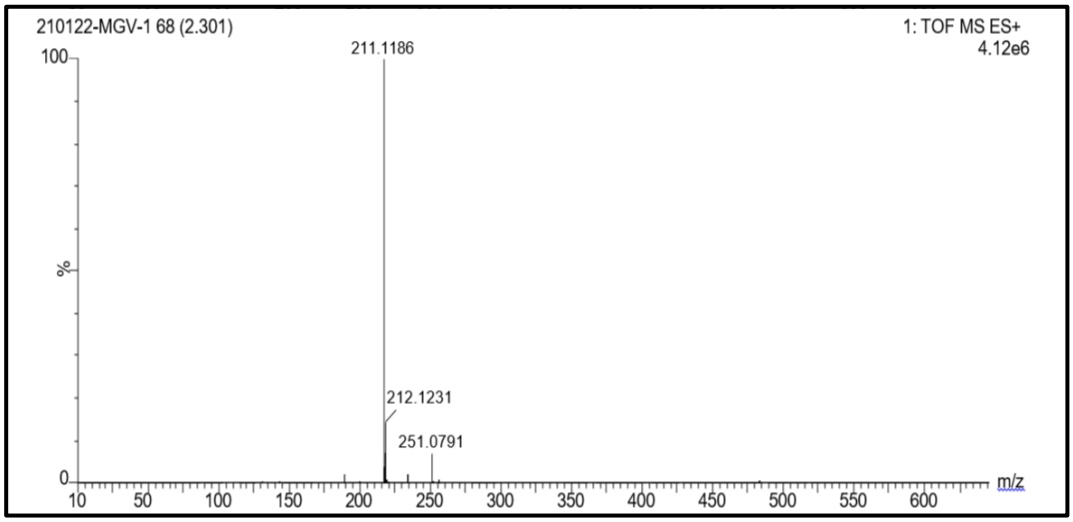

Figure 6:Mass Spectrum of compound (a) and (b)

5.2 FTIR Studies:

The FTIR spectrum of 4-methylbenzo[c]coumarin exhibits a strong and sharp absorption at **1723.68 cm^{-1}**, confirming the presence of a **lactone carbonyl group**, characteristic of the coumarin moiety. The **aromatic C=C stretching vibrations** observed at **1604.09 and 1545.5 cm^{-1}** indicate an extended **conjugated fused ring system**, consistent with the benzo[c]fused coumarin core. Peaks at **2924.94 and 2854.27 cm^{-1}** confirm the presence of a **methyl group**, which is further supported by CH_3 bending vibrations at **1468.89 and 1381.98 cm^{-1}**. Strong **C–O stretching** between **1250–1100 cm^{-1}** confirms the presence of **ether-type linkages** in the lactone. Additionally, multiple bands in the **900–700 cm^{-1}** region correspond to **aromatic out-of-plane C–H bending**, indicating characteristic substitution patterns on the benzo coumarin skeleton.

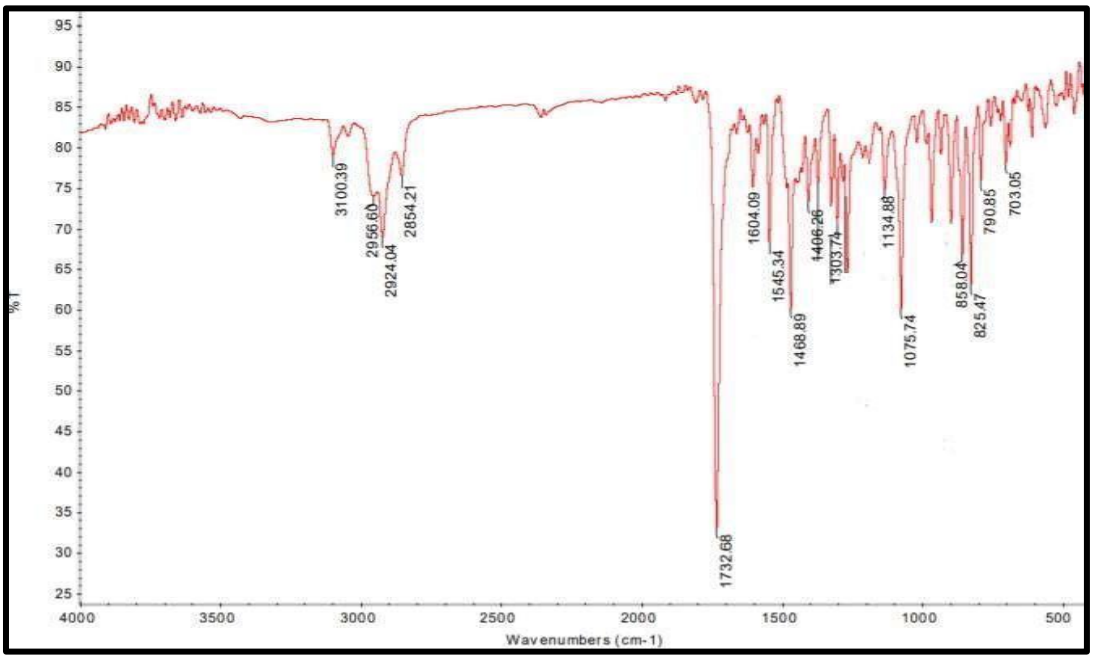

Figure 7 : FTIR Spectrum of compound of (a) and (b)

5.3 Antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method:

A comparative analysis between the two synthesized compounds, CL-1 and CO-1, reveals distinct differences in their antioxidant potential as assessed by the DPPH free radical scavenging assay. CL-1 exhibited a lower IC_{50} value of 33.55 µg/mL compared to CO-1, which showed an IC_{50} of 35.12 µg/mL. This indicates that CL-1 has a relatively higher free radical scavenging ability than CO-1. Additionally, the percentage inhibition values at each concentration were consistently higher for CL-1, reflecting a stronger and more efficient antioxidant response. The linear regression data further support this observation, with CL-1 demonstrating a better correlation ($R^2 = 0.9931$) than CO-1 ($R^2 = 0.9845$), suggesting greater consistency in its dose-dependent activity. Overall, CL-1 outperforms CO-1 in antioxidant efficacy and appears to be a more promising candidate for further development in biomedical applications.

Table 1: Antioxidant properties of samples by DPPH Method results expressed in IC_{50} (µg/mL)

Sl. No.	Samples	DPPH IC_{50} (µg/mL)
1	**CL-1**	33.547
2	**CO-1**	35.120
3	**Vit-C**	26.384

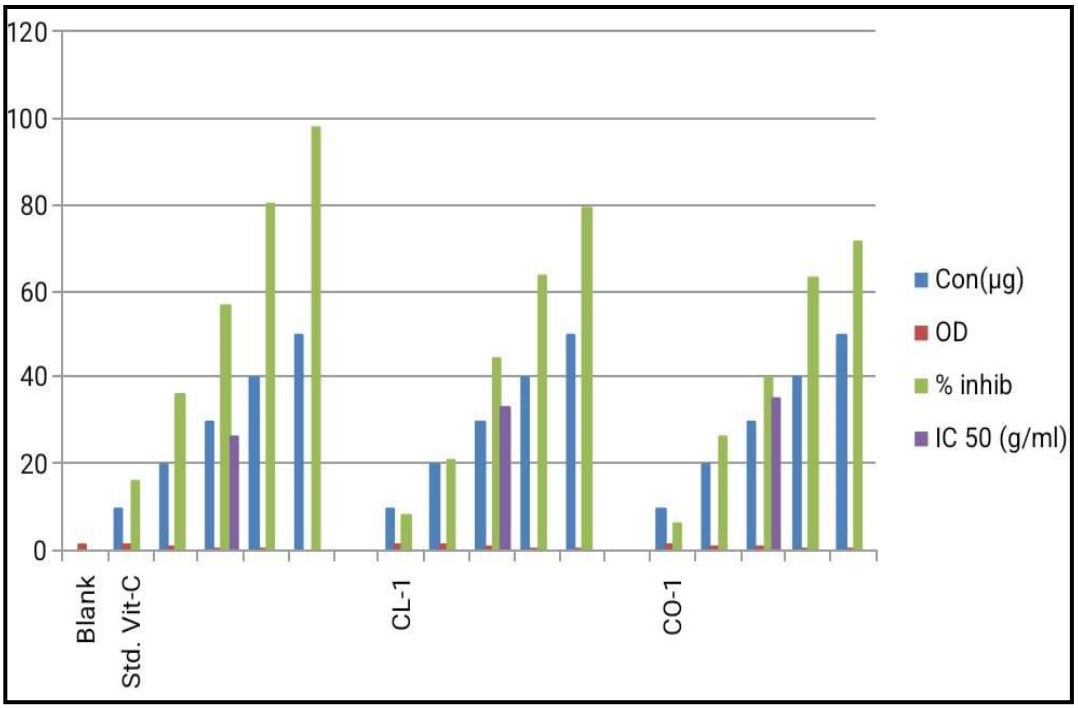

Figure 8:Antioxidant study with respect to compound(a) and (b)

5.4 DOCKING STUDY

The molecular docking image shows a coumarin derivative interacting with specific amino acid residues in the protein binding site. The notable residues include:

- VAL A:54 (Valine)
- LEU A:138 (Leucine)
- GLU A:139 (Glutamic acid)
- TYR A:50 (Tyrosine)

These interactions involve hydrophobic and polar contacts, with distances (in A^0) ranging from 3.41 A^0 to 5.86 A^0, suggesting moderate to strong non-covalent interactions such as hydrogen bonding or π–π stacking.

Binding Affinity Data:

The most favorable model is Model 1 with a binding affinity of –6.409 kcal/mol, indicating stronger and more stable binding. Models with binding energy < –6 kcal/mol (Models 1–3) are considered significantly active and promising.

Table 2: Calculated binding affinities for 10 docking models (in kcal/mol)

Model	Binding Affinity(kcal/mol)
1	**-6.409 (best binding)**
2	**-6.230**
3	**-6.064**
4	**-5.709**
5	**-5.649**
6	**-5.619**
7	**-5.570**
8	**-5.408**
9	**-5.354**
10	**-5.173**

The docking study suggests that coumarin derivatives show good binding affinity with the target protein, particularly in the top 3 models. The interaction with residues VAL-54, LEU-138, GLU-139, and TYR-50 indicates that these amino acids play a crucial role in ligand binding. Model 1 is the most stable complex and can be selected for further pharmacological evaluation or molecular dynamics simulation.

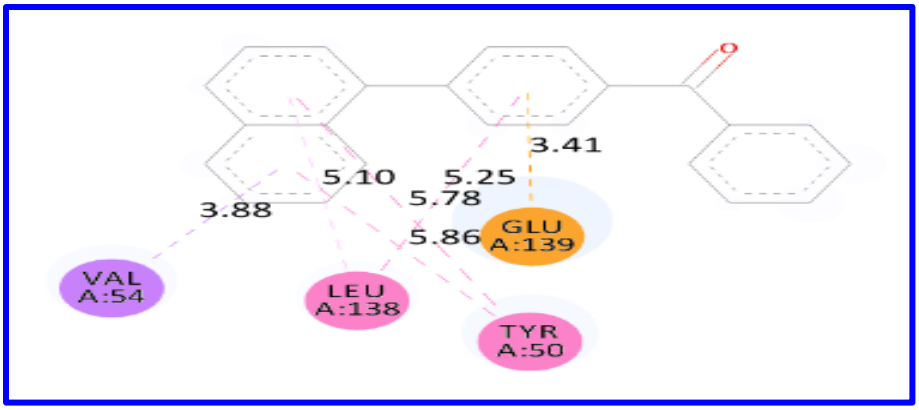

Figure 9:Docking study diagram of compound (a) and (b)

The present study successfully demonstrates the green synthesis of coumarin derivatives using eco-friendly catalysts such as lemon juice and oxalic acid, highlighting a sustainable approach to chemical synthesis. The synthesized compounds were structurally confirmed through FTIR and GC-MS analyses, validating the efficiency of the adopted methodology. Antibacterial screening revealed that the coumarin derivatives exhibit promising inhibitory activity, indicating their potential as therapeutic agents. Furthermore, molecular docking studies confirmed their effective interaction with biological targets, providing insight into their possible mechanisms of action.

By integrating green chemistry principles with conventional synthetic methods, this work contributes to the advancement of sustainable practices in medicinal chemistry. The use of safe, low-cost, and biodegradable catalysts not only minimizes environmental impact but also offers an accessible route for the synthesis of pharmacologically active compounds. Overall, the project opens new avenues for the design and development of coumarin-based molecules with enhanced biological activities, supporting future research in drug discovery and green chemistry.

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