IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

Synthesis and Biological Screening of a Novel Coumarin Derivative for Antimicrobial Activity

Shweta S. Tarange[1], Rhutuja D. Tarde[2], Kiran R. Ingole[3]
[3]Assistant Professor, Department of Pharmaceutical Chemistry.
[1&2]Student of JBVP's Vidya Niketan Collage of Pharmacy, Lakhewadi, Indapur, Pune, Maharashtra, India

Abstract: *Coumarin derivatives are an important class of heterocyclic compounds known for their wide range of biological and pharmaceutical activities, particularly antimicrobial properties. In the present study, 7-hydroxy-4-methylcoumarin was synthesized by Pechmann condensation reaction using resorcinol and ethyl acetoacetate in the presence of concentrated sulfuric acid as a catalyst. The synthesized compound was purified by recrystallization and characterized using physical observation, melting point determination, Thin Layer Chromatography (TLC), and UV–Visible spectroscopy. The synthesized compound appeared as a pale yellow crystalline solid with a melting point of 120°C. TLC analysis showed a single spot with an Rf value of 0.42, indicating good purity of the compound. UV–Visible spectroscopic analysis revealed an absorption maximum (λmax) at approximately 355 nm, confirming the presence of conjugated chromophoric groups in the coumarin nucleus. The antimicrobial activity of the synthesized compound was evaluated by agar well diffusion method using environmental bacterial culture grown on milk agar medium. A clear zone of inhibition of approximately 1 cm was observed around the well containing the synthesized compound, indicating moderate antimicrobial activity. The results of the present investigation suggest that the synthesized coumarin derivative possesses significant physicochemical characteristics and promising antimicrobial potential. The study highlights the importance of coumarin derivatives in medicinal chemistry and suggests their possible application in the development of new antimicrobial agents.*

Keywords: Medicinal Chemistry, Coumarin Derivatives, 7-Hydroxy-4-methylcoumarin, Pechmann Condensation, Antimicrobial Activity, Thin Layer Chromatography (TLC), UV–Visible Spectroscopy, Agar Well Diffusion Method, Heterocyclic Compounds, Milk Agar Medium.

I. INTRODUCTION

Coumarin is a naturally occurring heterocyclic compound that is widely distributed in various plants, microorganisms, and essential oils.(1,7) The term "coumarin" originated from Coumarou, the traditional name of the Tonka bean (Dipteryx odorata), from which the compound was initially isolated.(1) Structurally, coumarin consists of a benzene ring fused with an α-pyrone ring, forming a benzopyrone nucleus.(4) Because of its conjugated structure and reactive functional groups, coumarin exhibits diverse chemical as well as biological properties. (3,5)Owing to these characteristics, coumarin derivatives have gained considerable attention in medicinal chemistry, pharmaceutical research, and synthetic organic chemistry.(8.30)

A large number of naturally derived and synthetically prepared coumarin derivatives possess important pharmacological activities. These compounds have been reported to exhibit antimicrobial, antioxidant, anti-inflammatory, antiviral, anticoagulant, antitubercular, antidiabetic, and anticancer properties(5,6,8). The biological behavior of coumarin derivatives mainly depends upon the type and position of substituents attached to the coumarin ring system. Modification of the coumarin nucleus can significantly influence biological activity, selectivity, and therapeutic effectiveness.(4)

In recent years, microbial infections caused by pathogenic bacteria and fungi have become a serious global health issue. Continuous and irrational use of antibiotics has led to the emergence of antimicrobial resistance among many

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

microorganisms. As a result, several pathogenic strains have developed resistance against commonly available antimicrobial drugs, thereby reducing treatment effectiveness. This increasing resistance has created an urgent requirement for the development of new antimicrobial agents with improved efficacy and fewer adverse effects.(2,3)

Heterocyclic compounds obtained from natural as well as synthetic sources have attracted significant interest in antimicrobial drug discovery. Among these compounds, coumarin derivatives are considered promising candidates because of their ability to interfere with microbial enzymes, DNA synthesis, and other essential cellular processes. Previous studies have demonstrated that various substituted coumarin derivatives possess antibacterial and antifungal activity against both Gram-positive and Gram-negative microorganisms.

The antimicrobial efficiency of coumarin compounds is strongly affected by the presence of functional groups such as hydroxyl, methoxy, amino, nitro, and halogen substituents on the aromatic ring. Both electron-donating and electron-withdrawing groups can alter the physicochemical and biological properties of the compounds. Researchers have reported that suitable substitution on the coumarin nucleus may enhance antimicrobial potential and improve biological performance. Therefore, synthesis and biological screening of new coumarin derivatives continue to be an important area of pharmaceutical and medicinal research.

Several synthetic methods are available for the preparation of coumarin derivatives, including Pechmann condensation, Knoevenagel condensation, Perkin reaction, and Wittig reaction. Among these approaches, the Pechmann condensation reaction is widely preferred due to its simple procedure, convenient reaction conditions, and high product yield. After synthesis, the compounds are generally purified by recrystallization and characterized by analytical techniques such as melting point determination, Thin Layer Chromatography (TLC), Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectroscopy.

Thin Layer Chromatography is a simple and effective analytical technique commonly employed to monitor reaction progress and evaluate compound purity. In this technique, separation occurs because of differences in the adsorption behavior of compounds between stationary and mobile phases. The retention factor (Rf value) is used for identification and purity assessment of synthesized compounds.(15,16)

Biological screening of coumarin derivatives can be performed by standard antimicrobial methods such as agar well diffusion and broth dilution techniques. In agar well diffusion method, antimicrobial activity is determined by measuring the zone of inhibition formed around the well containing the test compound. The broth dilution method is commonly used for determination of Minimum Inhibitory Concentration (MIC), which indicates the minimum concentration required to inhibit microbial growth.

Different microbial strains including Gram-positive bacteria such as Staphylococcus aureus and Bacillus subtilis, Gram-negative bacteria such as Escherichia coli, and fungal strains like Candida albicans and Aspergillus niger are frequently used for antimicrobial evaluation. Standard antimicrobial drugs are generally used as positive controls, whereas Dimethyl Sulfoxide (DMSO) is commonly employed as solvent control because of its excellent solubility properties for organic compounds.

The present investigation focuses on the synthesis of a coumarin derivative and its evaluation for antimicrobial activity. The synthesized compound was prepared using a suitable synthetic procedure and purified by recrystallization. Characterization of the compound was carried out using melting point determination, Thin Layer Chromatography, and spectroscopic analysis. The antimicrobial potential of the synthesized derivative was studied by standard microbiological techniques.(22,25)

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

The primary objective of the study was to evaluate the antimicrobial effectiveness of the synthesized coumarin derivative and to contribute toward the development of novel antimicrobial agents. The findings of this work may help in understanding the relationship between chemical structure and biological activity of coumarin compounds. Furthermore, the study may provide useful information for future investigations in medicinal chemistry and pharmaceutical drug development.

II. AIM AND OBJECTIVE

Aim

This research work was carried out to prepare a coumarin-based compound and examine its ability to inhibit microbial growth by using microbiological screening methods.(3,8) Coumarin derivatives are widely studied because they possess several important medicinal properties and are considered useful compounds in pharmaceutical research. Since many microorganisms are gradually becoming resistant to existing antimicrobial drugs, researchers are continuously searching for alternative compounds with better therapeutic effects.

In the present investigation, a synthesized coumarin derivative was evaluated for its antimicrobial behavior against selected microorganisms. The study was also intended to examine how structural features of the synthesized compound influence its biological activity. The overall work may provide useful information regarding the medicinal relevance of coumarin derivatives and their future application in development of antimicrobial agents.

Objective

1. To prepare a coumarin derivative under suitable laboratory conditions using appropriate chemical reactions.
2. To obtain the synthesized product in purified form by applying suitable purification techniques.
3. To examine the purity and identity of the prepared compound through analytical methods such as melting point determination and Thin Layer Chromatography.
4. To prepare sample solutions of required concentration for biological testing.
5. To evaluate the antimicrobial property of the synthesized compound against selected microorganisms by agar well diffusion technique.
6. To determine the effectiveness of the prepared compound by comparing its activity with standard antimicrobial agents.
7. To measure the inhibition zone formed around the test sample and interpret the obtained observations.
8. To study the medicinal significance and pharmaceutical importance of coumarin-based compounds.
9. To understand the influence of chemical structure on the biological behavior of the synthesized derivative.
10. To contribute experimental findings related to antimicrobial activity of heterocyclic compounds for future medicinal research.
11. To gain practical experience in synthesis, purification, characterization, and microbiological evaluation procedures.
12. To generate scientific observations that may support further research in antimicrobial drug discovery and heterocyclic chemistry.(1,5)

III. MATERIALS AND METHOD

Material

All chemicals and reagents used during the experimental work were of analytical grade quality and were utilized without additional purification. The chemicals required for synthesis of the coumarin derivative were obtained from standard laboratory chemical suppliers. Proper laboratory precautions and safety measures were followed during handling and storage of all chemicals.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 8, May 2026 Impact Factor: 8.2

Different starting materials including phenolic compounds, β-keto esters, aldehydes, and suitable catalysts were used according to the requirements of the synthetic procedure. Solvents such as ethanol, methanol, acetone, chloroform, ethyl acetate, and Dimethyl Sulfoxide (DMSO) were employed for synthesis, recrystallization, extraction, purification, and preparation of sample solutions. Distilled water was used whenever required for washing and preparation of media.(20,21)

For antimicrobial studies, microbiological media such as nutrient agar and nutrient broth were prepared for cultivation and maintenance of microorganisms. Microbial cultures used during the investigation were obtained from laboratory stock cultures and maintained under sterile environmental conditions to avoid contamination. Periodic subculturing was carried out to maintain proper growth and viability of the microorganisms.

Standard antibiotic discs or solutions were used as reference standards during antimicrobial evaluation, whereas DMSO served as the solvent control. Laboratory apparatus including Petri dishes, cotton swabs, test tubes, conical flasks, measuring cylinders, micropipettes, capillary tubes, filter paper, glass rods, and cork borers were used during different stages of the experiment.(31,35)

Before use, all glassware was properly washed, rinsed with distilled water, and dried completely. Sterilization of microbiological media and laboratory equipment was performed using an autoclave under suitable temperature and pressure conditions to maintain aseptic conditions throughout the experiment.

The instruments employed in the present work included an analytical balance, magnetic stirrer, hot plate, reflux condenser, melting point apparatus, UV chamber for TLC analysis, incubator, autoclave, laminar airflow chamber, pH meter, micropipettes, and refrigerator for storage of chemicals and microbial cultures.

Appropriate safety precautions were maintained during the entire experimental work. Laboratory coat, gloves, face mask, and protective goggles were used to minimize exposure to chemicals and microbial contamination.

Method

The synthesis of the coumarin derivative was carried out by an appropriate organic synthetic procedure under controlled laboratory conditions. Accurately weighed quantities of reactants and reagents were taken using an analytical balance. The reactants were mixed in a clean round-bottom flask containing a suitable solvent system. The reaction mixture was subjected to continuous stirring and heating under reflux conditions for a specified duration to ensure completion of the reaction.

Progress of the reaction was monitored at regular intervals using Thin Layer Chromatography with a suitable mobile phase. After completion of the reaction, the mixture was allowed to cool to room temperature. Formation of precipitate indicated completion of the synthesis process.

The obtained solid product was separated by filtration using Whatman filter paper and washed repeatedly with distilled water to remove remaining impurities and excess reagents. The crude product was then dried and purified by recrystallization using an appropriate solvent such as ethanol or methanol.

Finally, purified crystals of the synthesized coumarin derivative were collected and dried properly. Percentage yield of the prepared compound was calculated and recorded. Physical characteristics including colour, appearance, texture, and solubility of the synthesized compound were carefully examined and noted.(22,23)

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

VI. SYNTHESIS OF COUMARIN DERIVATIVE

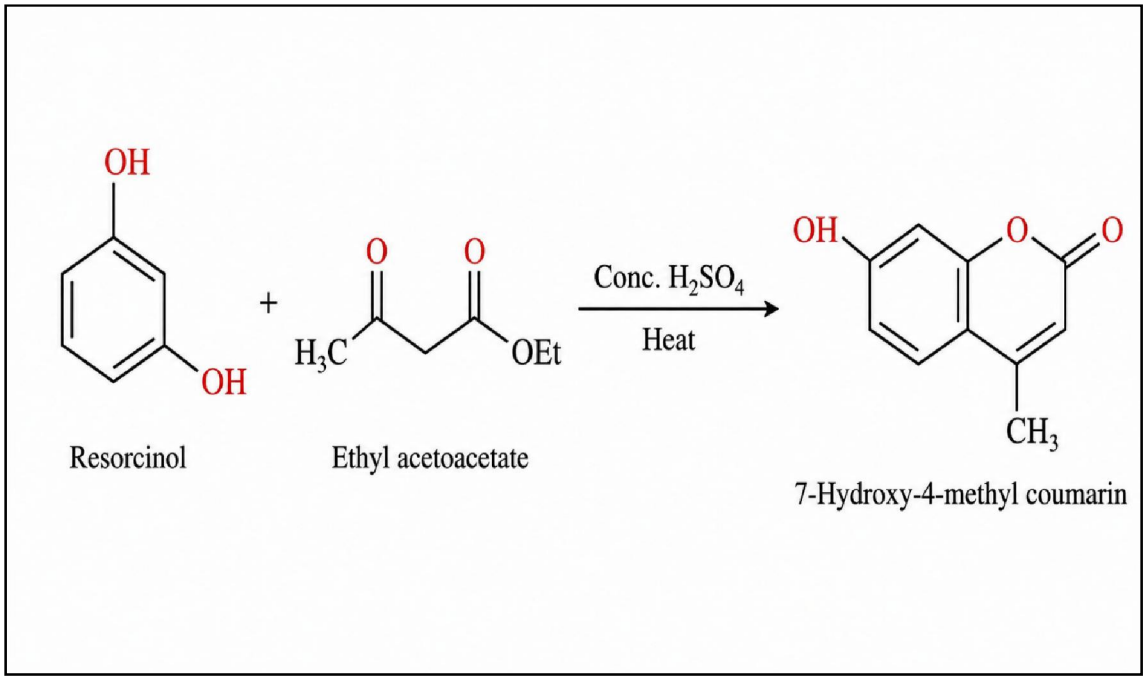
Principle

The synthesis of 7-hydroxy-4-methylcoumarin was carried out by Pechmann condensation reaction, which involves the reaction between resorcinol and ethyl acetoacetate in the presence of concentrated sulfuric acid acting as a catalyst. During the reaction, condensation of the reactants occurs followed by cyclization and dehydration, resulting in formation of the coumarin ring structure.

In acidic medium, the carbonyl group of ethyl acetoacetate becomes activated, which facilitates electrophilic substitution with resorcinol. Subsequent intramolecular cyclization leads to formation of the heterocyclic coumarin nucleus. Final dehydration and rearrangement steps produce the desired coumarin derivative, namely 7-hydroxy-4-methylcoumarin.(20,21,34)

Reaction

The reaction can be represented as follows:



Reaction Mechanism

Protonation of ethylacetoacetate

The carbonyl oxygen of ethyl acetoacetate gets protonated by sulfuric acid, increasing the electrophilicity of the carbonyl carbon

Electrophilic attack by resolcinol

The activated β-keto ester undergoes electrophilic substitution with resorcinol at the ortho position relative to the hydroxyl group.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

Cyclization

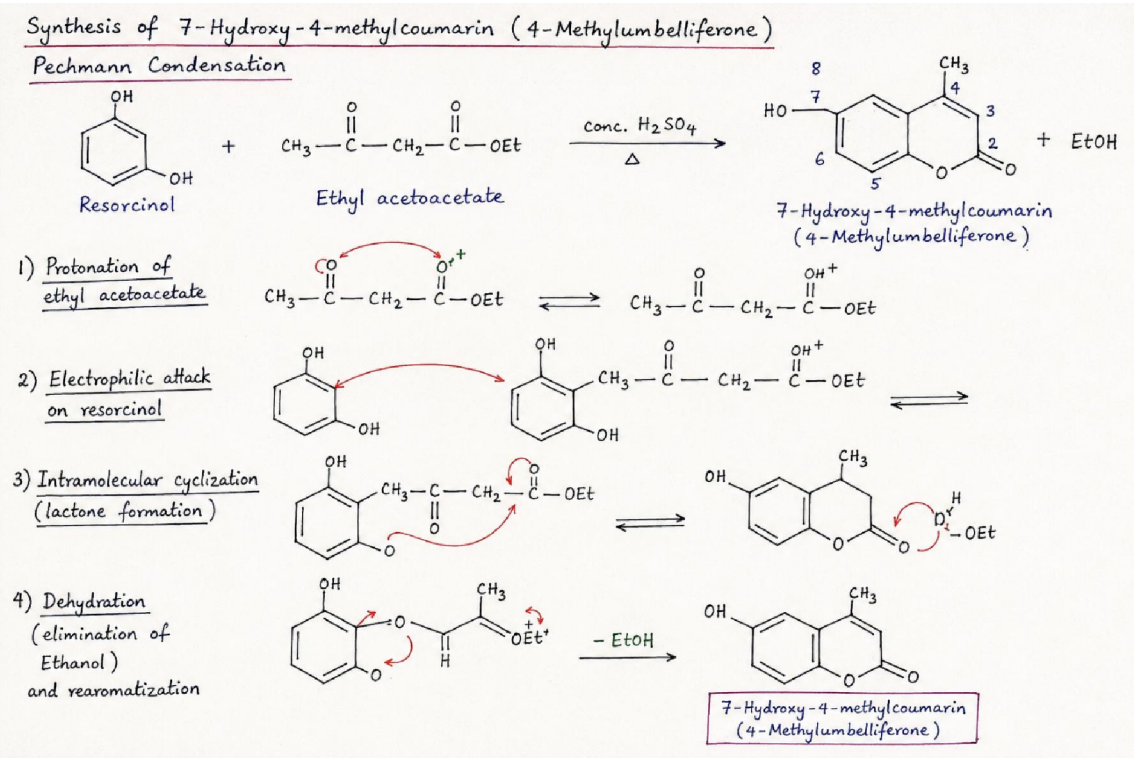
The hydroxyl group attacks the ester carbonyl intramolecularly, leading to ring closure and formation of the coumarin nucleus

Dehydration

Loss of ethanol and proton rearrangement finally produces 7-hydroxy-4-methylcoumarin.

Final Product

7-Hydroxy-4-methylcoumarin



Reaction Mechanism

Procedure

A suitable quantity of resorcinol was transferred into a clean and dry beaker. Ethyl acetoacetate was then added gradually with continuous stirring to obtain a uniform reaction mixture. Concentrated sulfuric acid was carefully introduced dropwise while maintaining constant stirring conditions.

The reaction mixture was heated gently on a water bath for a specific time period to promote completion of the condensation reaction. During heating, gradual thickening of the reaction mixture was observed, indicating formation of the product.

After completion of the reaction, the mixture was allowed to cool and then slowly poured over crushed ice with continuous stirring. Formation of a solid precipitate of 7-hydroxy-4-methylcoumarin was observed.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

The precipitated product was collected by filtration and washed several times with cold distilled water to remove acidic impurities and unreacted materials. The crude product was dried and further purified by recrystallization using ethanol as solvent.

The purified crystals obtained after recrystallization were dried properly, and parameters such as percentage yield, colour, and melting point were determined and recorded.

Fig. Synthesis of 7-Hydroxy-4-methylcoumarin

Percentage Practicle Yield

The percentage yield of the prepared sample was calculated to evaluate the efficiency of the preparation method. The obtained percentage yield was found to be **97.97%**, indicating good recovery and minimum loss of sample during the preparation process. The high percentage yield suggests that the adopted method was efficient and suitable for preparation of the sample with satisfactory productivity.

The obtained result demonstrates that the experimental procedure was successfully carried out with high accuracy and minimal material wastage. The high yield also indicates that the selected preparation conditions were appropriate and effective for obtaining

V. CHARACTERIZATION

The synthesized compound was characterized by:

- Observation of physical appearance
- Melting point determination
- Thin Layer Chromatography (TLC)
- UV Visible Spectroscopy

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

The purity of the compound was confirmed by obtaining a sharp melting point and a single spot on TLC plate.

Physical Observations

Parameter	Observation
Color	Pale yellow to cream colored
Nature	Crystalline solid
Solubility	Soluble in ethanol and DMSO
Odour	Pleasant aromatic odour.

Melting Point

Melting point is the temperature at which a solid substance changes into a liquid state under atmospheric pressure. Pure crystalline compounds generally possess a sharp and characteristic melting point range.

The principle of melting point determination is based on the fact that when heat is supplied to a solid compound, the intermolecular forces holding the molecules together weaken, causing the substance to convert into liquid form. The temperature at which the first drop of liquid appears and the temperature at which the entire substance completely melts are noted as the melting point range.

Pure compounds usually show a narrow melting point range, whereas impure substances exhibit a lower and broader melting point range due to the presence of contaminants.

Melting point determination is commonly used for:

- Identification of compounds
- Determination of purity
- Characterization of synthesized compounds

Fig. Melting Point Apparatus

The melting point of the synthesized 7-Hydroxy-4-methylcoumarin was determined using the capillary tube method. The observed melting point of the compound was found to be **187°C**. The sharp melting point indicates the purity and proper formation of the synthesized compound.(15,17,18)

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

Thin Layer Chromatography

Thin Layer Chromatography (TLC) is an analytical technique used for the separation and identification of compounds present in a mixture. It is based on the difference in the adsorption and solubility of compounds between the stationary phase and the mobile phase.

In TLC, a thin layer of silica gel coated on a plate acts as the stationary phase, while a suitable solvent system acts as the mobile phase. When the solvent moves upward through the plate by capillary action, the sample components travel at different rates depending on their polarity and affinity towards the stationary and mobile phases.

Compounds having greater affinity for the mobile phase move faster and travel a longer distance, whereas compounds strongly adsorbed on the stationary phase move slowly. This results in the separation of components as distinct spots on the TLC plate.

The separated compounds are identified by calculating the Rf value using the formula:

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Preparation Of TLC Chamber

A clean and dry TLC chamber was taken. The mobile phase was prepared by mixing **7 mL of hexane** and **3 mL of ethyl acetate** in the chamber. The solvent mixture was added to a depth of approximately 0.5 cm. A strip of filter paper was placed along the inner wall of the chamber to ensure proper saturation of solvent vapours. The chamber was then closed with a lid and allowed to equilibrate for about 10–15 minutes before development of the TLC plate.(15,16)

Preparation of TLC Plate

A silica gel coated TLC plate was taken and a straight pencil line was drawn approximately 1 cm above the lower edge of the plate. This line served as the origin line.

Sample Application

The synthesized sample of 7-Hydroxy-4-methyl coumarin was dissolved in a suitable solvent. Using a capillary tube, a small spot of the sample solution was carefully applied on the origin line. The spot was allowed to dry.

Development of TLC Plate

The spotted TLC plate was carefully placed inside the chamber ensuring that the sample spot remained above the solvent level. The chamber was closed properly and the solvent was allowed to rise through the plate by capillary action.

Removal of Plate

When the solvent front reached approximately three-fourths of the plate length, the TLC plate was removed from the chamber. The solvent front was immediately marked using a pencil and the plate was dried.

Visualization

The developed TLC plate was observed under UV light for detection of spots. The separated spots were marked carefully.

DOI: 10.48175/IJARSCT-35588

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

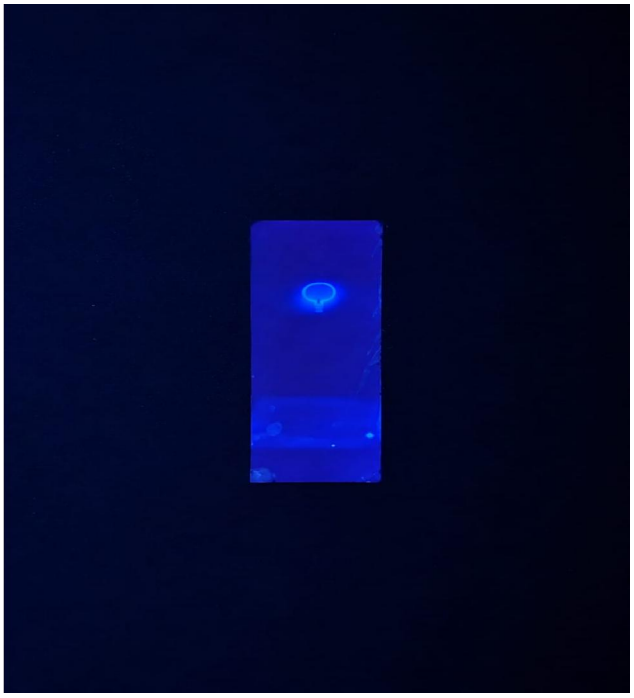


Fig. TLC Plate

TLC Analysis

Parameter	Observation
Mobile Phase	Hexane : Ethyl Acetate
No. Of Spots	1
Rf Value	0.42
Detection	UV Chamber

VI. UV VISIBLE SPECTROSCOPY

Principle

UV–Visible spectroscopy is an analytical technique used to determine the absorption of ultraviolet or visible light by a compound. When electromagnetic radiation passes through a sample solution, molecules absorb light at specific wavelengths corresponding to electronic transitions. The amount of absorbed light is measured as absorbance, which helps in characterization of the synthesized compound.

Procedure

For UV–Visible spectroscopic analysis, approximately **1 g of synthesized sample** was dissolved in methanol to prepare the sample solution. Methanol was used as the solvent because of its good solubility and transparency in the UV region.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

A clean quartz cuvette was taken for analysis. The blank solution was prepared using only methanol solvent and placed in the reference cuvette. The prepared sample solution was then placed in another cuvette.(17,18)

The absorbance of the sample was recorded using a UV–Visible spectrophotometer over the required wavelength range, and the absorption maxima of the synthesized compound were observed and noted.

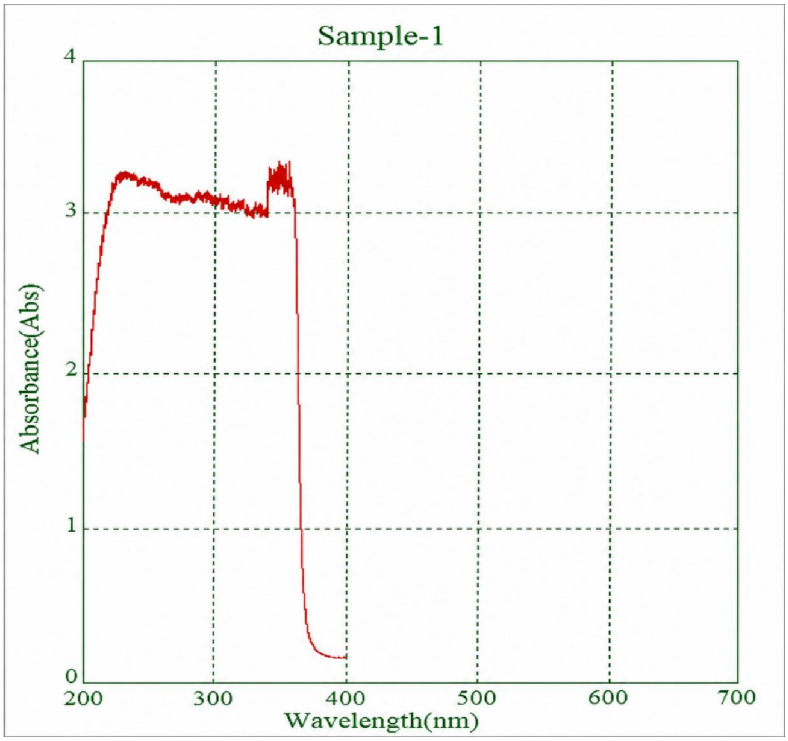


Fig.UV Spectrum

UV Visible Spectroscopy Analysis

Parameter	Observation
Solvent	Methanol
Wavelength Range	200 to 400 nm
λmax	Approximately 355 nm
Observation	In UV region

Antimicrobial Activity

Milk agar media was prepared by using milk and distilled water in the ratio of 1:1. The required quantity of milk and distilled water were mixed properly to obtain a uniform solution. About 2 gm of agar was added to the mixture as a solidifying agent. The media was heated gently with continuous stirring until complete dissolution of agar.

The prepared media was sterilized and then poured into sterile petri plates under aseptic conditions.
The plates were allowed to solidify at room temperature

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

After solidification, the plates were exposed to the surrounding environment for a certain period of time to allow collection and growth of environmental microorganisms. The contaminated plates containing microbial growth were then used for antimicrobial activity studies

The plates were then kept in an incubator for microbial growth under suitable conditions.

After sufficient growth of microorganisms, wells were made in the agar media and the synthesized compound was added into the wells for evaluation of antimicrobial activity by agar well diffusion method.(22,25)

Fig. Agar Well Diffusion Method

The plate was again incubated for a specific period under suitable conditions. After incubation, the antimicrobial activity of the synthesized compound was evaluated by observing the zone of inhibition formed around the wells.

Fig. Zone Of Inhibition

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

Evaluation Of Antimicrobial Activity

Test Organism	Zone Of Inhibition	Activity
Environmental Bacterial Culture	Approximately 1 cm	Antimicrobial Activity Observed

"The sample exhibited antimicrobial activity against environmental bacterial culture with a zone of inhibition of approximately 1 cm."

VII. RESULT AND DISCUSSION

The present study was carried out to evaluate the physicochemical characteristics and antimicrobial potential of the prepared sample using Thin Layer Chromatography (TLC), UV-Visible spectroscopy and agar well diffusion method.

Thin Layer Chromatography analysis was performed to identify the presence of different chemical constituents in the sample. A distinct spot was observed on the TLC plate after development, indicating successful separation of the compound present in the sample. The calculated Rf value confirmed the movement of the compound in the selected solvent system and suggested the presence of moderately polar constituents. TLC analysis indicated the purity and chromatographic behavior of the sample.

UV-Visible spectroscopic analysis was carried out in the wavelength range of 200–400 nm using methanol as blank solution. The sample solution was prepared by dissolving the required quantity of sample in methanol. The obtained spectrum showed significant absorption in the ultraviolet region with λmax observed approximately at 355 nm. The absorption in the UV region indicates the presence of chromophoric and conjugated functional groups in the sample.

The UV spectrum further confirmed that the sample contains UV active compounds capable of absorbing radiation in the ultraviolet range. Negligible absorption was observed in the visible region, indicating that the sample mainly exhibits UV absorption characteristics.

Antimicrobial activity of the sample was evaluated by agar well diffusion method using environmental bacterial culture grown on milk agar medium. Clear zone of inhibition was observed around the well containing the sample. The sample exhibited a zone of inhibition of approximately 1 cm, indicating moderate antimicrobial activity against the tested microorganisms. The antimicrobial effect may be attributed to the presence of biologically active phytoconstituents or chemical compounds present in the sample.

The observed antimicrobial activity suggests that the sample possesses inhibitory action against bacterial growth. The results obtained from antimicrobial studies support the potential application of the sample as an antimicrobial agent. Overall, the combined results of TLC, UV-Visible spectroscopy and antimicrobial evaluation confirmed the presence of active chemical constituents and moderate antimicrobial potential of the prepared sample.(15,17,22)

VIII. CONCLUSION

The present research work was carried out to investigate the physicochemical characteristics and antimicrobial potential of the prepared sample using Thin Layer Chromatography (TLC), UV-Visible spectroscopy and agar well diffusion method. The study provided important information regarding the chemical nature and biological activity of the sample.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 8, May 2026 Impact Factor: 8.2

TLC analysis confirmed the presence of chemical constituents in the sample through the appearance of distinct spots on the TLC plate. The obtained Rf value indicated proper migration and separation of compounds in the selected solvent system, suggesting the presence of moderately polar components. The chromatographic analysis also helped in preliminary characterization of the sample.

UV-Visible spectroscopic analysis was performed in the wavelength range of 200–400 nm using methanol as blank. The spectrum showed characteristic absorption in the ultraviolet region with λmax observed approximately at 355 nm, confirming the presence of chromophoric and conjugated functional groups within the sample.

The UV spectral study suggested that the sample contains UV active compounds which may contribute to its biological activity. The observed absorption behavior further supported the presence of important chemical constituents in the prepared sample.

Antimicrobial activity of the sample was evaluated by agar well diffusion method against environmental bacterial culture grown on milk agar medium. The sample exhibited a measurable zone of inhibition of approximately 1 cm, indicating moderate antimicrobial activity against the tested microorganisms. The inhibitory effect observed around the well suggested that the sample contains bioactive compounds capable of restricting microbial growth.

Overall, the results obtained from TLC analysis, UV-Visible spectroscopy and antimicrobial studies confirmed the presence of chemically active constituents and demonstrated the antimicrobial potential of the sample .(15,17,22) The study indicates that the prepared sample may have possible pharmaceutical and biological applications. Further studies such as isolation, purification and detailed characterization of active compounds can be carried out to explore its therapeutic potential in greater detail.

REFERENCE

1. Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. Medical Microbiology. Elsevier, 2016.
2. Pelczar, M. J., Chan, E. C. S., & Krieg, N. R. Microbiology. McGraw Hill, 2005.
3. Aneja, K. R. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International, 2003.
4. Harborne, J. B. Phytochemical Methods. Chapman and Hall, 1998.
5. Silverstein, R. M., Webster, F. X., & Kiemle, D. Spectrometric Identification of Organic Compounds. Wiley, 2005.
6. Vogel, A. I. Textbook of Practical Organic Chemistry. Longman, 1978.
7. Skoog, D. A., Holler, F. J., & Crouch, S. R. Principles of Instrumental Analysis. Thomson Brooks, 2007.
8. Sharma, B. K. Instrumental Methods of Chemical Analysis. Goel Publishing House, 2011.
9. Lambert, J. B., Shurvell, H. F., & Lightner, D. A. Organic Structural Spectroscopy. Pearson Education, 2010.
10. Furniss, B. S., Hannaford, A. J., Smith, P. W. G., & Tatchell, A. R. Vogel's Textbook of Practical Organic Chemistry. Pearson, 1989.
11. Pavia, D. L., Lampman, G. M., & Kriz, G. S. Introduction to Spectroscopy. Cengage Learning, 2008.
12. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare, 2018.
13. United States Pharmacopoeia (USP 41–NF 36), United States Pharmacopoeial Convention, 2018.
14. Khandelwal, K. R. Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan, 2008.
15. Chatwal, G. R., & Anand, S. Instrumental Methods of Chemical Analysis. Himalaya Publishing House, 2007.
16. Wilson, K., & Walker, J. Principles and Techniques of Biochemistry and Molecular Biology. Cambridge University Press, 2010.
17. Prescott, L. M., Harley, J. P., & Klein, D. A. Microbiology. McGraw Hill, 2002.
18. Cappuccino, J. G., & Sherman, N. Microbiology: A Laboratory Manual. Pearson Education, 2014.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, May 2026
Impact Factor: 8.2

19. Singleton, P. Bacteria in Biology, Biotechnology and Medicine. Wiley, 1999.
20. Cruickshank, R. Medical Microbiology. Churchill Livingstone, 1975.
21. Kokate, C. K. Practical Pharmacognosy. Vallabh Prakashan, 2010.
22. Indian Council of Medical Research (ICMR). Guidelines for Antimicrobial Testing, 2019.
23. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. "Antibiotic Susceptibility Testing by Standardized Single Disk Method." American Journal of Clinical Pathology, 1966, 45(4), 493–496.
24. Cheesbrough, M. District Laboratory Practice in Tropical Countries. Cambridge University Press, 2006.
25. Collins, C. H., & Lyne, P. M. Microbiological Methods. Butterworth-Heinemann, 1989.
26. Cowan, M. M. "Plant Products as Antimicrobial Agents." Clinical Microbiology Reviews, 1999, 12(4), 564–582.
27. Nostro, A., Germano, M. P., D'Angelo, V., Marino, A., & Cannatelli, M. A. "Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity." Letters in Applied Microbiology, 2000, 30(5), 379–384.
28. Eloff, J. N. "Which Extractant Should be Used for the Screening and Isolation of Antimicrobial Components from Plants?" Journal of Ethnopharmacology, 1998, 60(1), 1–8.
29. Hammer, K. A., Carson, C. F., & Riley, T. V. "Antimicrobial Activity of Essential Oils and Other Plant Extracts." Journal of Applied Microbiology, 1999, 86(6), 985–990.
30. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute, 2020.
31. Kuster, R. M., & Rocha, L. M. "Coumarins: Pharmacological and Toxicological Aspects." Revista Brasileira de Farmacognosia, 2001, 11(2), 103–110.
32. Venugopala, K. N., Rashmi, V., & Odhav, B. "Review on Natural Coumarin Lead Compounds for Their Pharmacological Activity." BioMed Research International, 2013, 1–14.
33. Borges, F., Roleira, F., Milhazes, N., Santana, L., & Uriarte, E. "Simple Coumarins and Analogues in Medicinal Chemistry." Current Medicinal Chemistry, 2005, 12(8), 887–916.
34. Kostova, I. "Synthetic and Natural Coumarins as Cytotoxic Agents." Current Medicinal Chemistry – Anti-Cancer Agents, 2005, 5(1), 29–46.
35. O'Kennedy, R., & Thornes, R. D. Coumarins: Biology, Applications and Mode of Action. Wiley, 1997.
36. Kennedy, J. F., & Thorley, M. Pharmaceutical Substances. Wiley-VCH, 2001.
37. Pandeya, S. N., Sriram, D., Nath, G., & Clercq, E. D. "Synthesis and Antimicrobial Activity of Schiff and Mannich Bases of Isatin." European Journal of Pharmaceutical Sciences, 1999, 9(1), 25–31.
38. Singh, H., Kapoor, V. K. Medicinal and Pharmaceutical Chemistry. Vallabh Prakashan, 2006.
39. Patrick, G. L. An Introduction to Medicinal Chemistry. Oxford University Press, 2013.
40. Foye, W. O. Principles of Medicinal Chemistry. Lippincott Williams & Wilkins, 2008.
41. Ashutosh Kar. Medicinal Chemistry. New Age International Publishers, 2005.
42. Rang, H. P., Dale, M. M., Ritter, J. M., & Flower, R. J. Rang and Dale's Pharmacology. Churchill Livingstone, 2012.
43. Tripathi, K. D. Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers, 2013.
44. Remington. The Science and Practice of Pharmacy. Lippincott Williams & Wilkins, 2006.
45. Goodman, L. S., & Gilman, A. The Pharmacological Basis of Therapeutics. McGraw Hill, 2011.