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JOURNAL OF INNOVATIONS IN APPLIED PHARMACEUTICAL SCIENCES

JIAPS
JOURNAL OF INNOVATIONS IN APPLIED PHARMACEUTICAL SCIENCES
ISSN: 2455 - 5177

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JOURNAL OF INNOVATIONS IN APPLIED PHARMACEUTICAL SCIENCES

National Conference on Nano-based Drug Delivery Systems; Recent Developments and Future Prospects conducted By Nirmala College of Pharmacy, Muvattupuzha, in association with Indian Pharmaceutical Association on 7 October 2023

RESEARCH ARTICLE(S)

Online ISSN:2455-5177

CODEN (CAS-USA): JIAPAW

Impact Factor: 5.832

Journal Archived in

PORTICO

KEYWORDS

CURRENT ISSUE

ATOM 1.0
RSS 2.0
RSS 1.0

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J. innov.appl. pharm. Sci, 8[3-S] 2023, 124-130

Journal of Innovations in Applied Pharmaceutical Science [JIAPS]

Content available at: www.saap.org.in ISSN: 2455-5177

EFFICIENT MICROWAVE SYNTHESIS OF COUMARIN DERIVATIVES WITH EVALUATION OF THEIR ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES

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Article History

Received: 06-10-2023
Revised: 27-10-2023
Accepted: 13-10-2023

Keywords: Microwave radiation, Coumarin, Anti-oxidant, Anti-inflammatory

Abstract

Microwave radiation, an electromagnetic radiation, is used as a source of heating in organic synthesis. Coumarin, a benzopyrone derivative, shows various pharmacological activities such as anticoagulant, antimicrobial, analgesic, anti-inflammatory. The prime objective was to compare the synthesis of some coumarin derivatives by microwave assisted methods. Two different coumarin derivatives were synthesized and evaluated for their anti-inflammatory activity and anti-oxidant activity. 3-Acetylcoumarin (3-acetyl-2H-Chromen-2- one) was synthesized by reaction with salicylaldehyde and ethyl acetoacetate in presence of piperidine and the 3-acetyl-6-bromocoumarin (3-acetyl-6-bromo-2H-chromen-2-one) was synthesized by using 5-bromo-salicyladehyde. The synthesized compounds were characterized by MP, TLC, IR and NMR. The antioxidant activity of the compounds was done by reducing power by ferric chloride, nitric oxide and hydrogen peroxide scavenging method. The anti-inflammatory activity of the compounds was done by protein denaturation using BSA method. Out of the two compounds synthesized, bromine-substituted acetylcoumarin showed more anti-oxidant and anti-inflammatory activity when compared to standard.

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https://doi.org/10.37022/jiaps.v8i3-S.523

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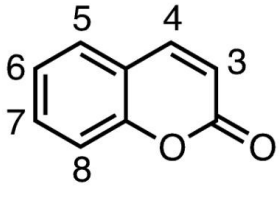
Introduction

Coumarin is classified as a member of benzopyrone family of compounds all of which consist of a benzene ring joined to a pyrone ring. Coumarin derivatives exhibit a wide spectrum of pharmacological activities such as anti-coagulant, antimicrobial, antiviral, analgesic, anti-inflammatory and HIV protease inhibitor.

Chemistry of Coumarin



2H-chromen-2-one

Microwave radiation, an electromagnetic source, is widely used in organic synthesis, leveraging mechanisms like polarization and conduction. It heats by interacting with charged reaction particles. Benefits include faster reaction rates, milder conditions, lower energy use, applicability to solution and solid-phase reactions, and enhanced product outcomes, allowing access to transformations unattainable with conventional heating. However, drawbacks include reduced activation energy and potential high voltage with metal objects in the oven. Various synthesis methods utilize microwaves, e.g., Pechmann condensation, Perkin reaction, Knoevenagel condensation, Wittig reaction, and Baylis-Hillman reaction.

Antioxidants hinder the oxidation of molecules, preventing free radical formation and chain reactions that can damage cells. Antioxidant activity can be assessed through methods like ferric chloride, nitric oxide, and hydrogen peroxide scavenging tests.

Inflammation represents a spontaneous response to injuries, often involving cytokines like IL-1, IL-6, and TNF-α. Anti-inflammatory activity can be evaluated using the bovine serum albumin method.

Materials and methods

Microwave assisted synthesis

A) Synthesis of compound 1

Mixed salicylaldehyde [.01M, 1.22g] and ethyl acetoacetate [.01M, 1.266ml] and 4-5 drops of piperidine placed in a beaker and were irradiated [350W] in a microwave synthesizer for 3

min, at the end of exposure to microwave, reaction mixture cooled at room temperature. Cooled product was recrystallized from ethanol.

B) Synthesis of compound 2

Mixed 5-bromo salicylaldehyde [.01M, 2.0102mg] and ethyl acetoacetate [0.01M, 1.266ml] and 4-5 drops of piperidine placed in a beaker and was irradiated [350W] in a Microwave synthesizer for 3min, at the end of exposure to microwave, reaction mixture cooled at room temperature Cooled product was recrystallized from ethanol.

1. TLC

Chromatographic characterization for synthesized compounds was done by TLC.

Procedure: 10µl of solution of Compound-1 and Compound-2 was spotted on TLC plate coated on silica gel, the plate was developed in the solvent system and dried at room temperature. TLC of compound was characterized under iodine chamber. Rf value of coumarin was compared.

Solvent system used- methanol: water [1:1]

Amount applied-10 microliter

Detected by- iodine chamber

Total number of spots-1

2. Melting point

Melting point of synthesized compound were determined using open capillary tubes on Thomas Hoover melting point apparatus.MP of Compound-1 was found to be 80°- 88°C & for Compound-2 it was within 118-125°C

Anti-oxidant studies

In different concentration of test sample of 10, 20, 30, 40, 50 mg/ml were mixed with 2.5 ml of 1% potassium ferric cyanide & incubated at 50°C for 20 minutes. 1.5 ml of 10% trichloroacetic acid was then added to the reaction mixture the contents were centrifuged at 3000 rpm for 10 minutes. 1.5 ml of supernatant was collected & mixed with 1 ml of distilled water & 0.5 ml of 1% ferric chloride. Control was prepared similarly with water.

Evaluation

The difference in absorbance between test & control was calculated & expressed as percentage of reducing power was calculated by using the equation.

Reducing power effect = 1- Absorbance of sample÷ Absorbance of control × 100

Hydrogen peroxide scavenging assay procedure

1mL of standard and test solution was added to 0.6 ml hydrogen peroxide solution. After 10 min the absorbance of the solution was measured at 230mm using UV-Vis spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of both standard and test compound was determined.

Evaluation

The percentage inhibition was calculated for the standard and samples using the following equation.

Scavenging effect in percent =1- Absorbance of sample÷ Absorbance of control × 100

Nitric oxide free radical scavenging method

Sodium nitroprusside 5mmol phosphate buffer of pH 7.4 saline was mixed with different concentration of extract and incubated at 25°C for 150 minutes.1.5 ml of incubated solution was mixed with 1.5ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 1% naphthalene diamine dihydrochloride) Ascorbic acid was used as standard.

Evaluation

The difference in absorbance between test and control was calculated using the following equation.

Percentage nitric oxide radical scavenging activity=1- Absorbance of sample÷ Absorbance ofcontrol × 100

Anti-inflammatory assay (In vitro)

Bovine serum albumin method

- Weighed 10 mg of test compounds & standard drug & transferred to 100 mlstandard flask which was previously washed with distilled water followed by DMF.
- Dissolved them in minimum amount of DMF [maximum 2 ml] and made up thevolume with freshly prepared phosphate buffer [.02M, pH 7.4]
- From this stock solution of each compound pipette 1, 2,3 ml to three 10 ml standard flask and made up the volume with phosphate buffer to get 10, 20,30 µ/ml respectively.
- From each 10 ml standard flask pipette 1.5ml of the solution into a clean & dry test tube, mixed 1.5 ml of 2 mM of BSA [1.329 gm in 10 ml phosphate buffer] and incubated [BOD (cooling) incubator, Rotex] at 27+/-1°C for 15min.
- Mixed 1.5 ml of the buffer solution and 1.5 ml of 2 mM BSA in another test tube to make control. Along with the other test tubes incubated the control also [as above step].
- Induced denaturation by keeping the reaction mixture at 60±1°C in Ika make rotary evaporator electronic water bath for 10 min.
- Cooled them to room temperature and measured the turbidity at 660 nm from Shimadzu make UV-visible spectrophotometer taken buffer solution as blank.
- Calculated the percentage inhibition of denaturation from control where no drug is added. Each experiment was done in triplicate and average is taken.
- The percentage inhibition was calculated from the following formula:

Percentage inhibition = [1- Absorbance of test] ÷ Absorbance of control × 100

3. IC_{50} Value

IC_{50} value is the concentration of compound required to scavenge 50% of radicals and was determined for their anti-oxidant and anti-inflammatory activity. Various concentrations of (100-500 µg/ml) of samples were taken for study.

IC_{50} values were calculated using regression analysis in MS-Excel. All experimental measurements were carried out in triplicate and were expressed as average of 3analysis ± standard deviation.

4. Spectral analysis

IR Spectroscopy

The range of electromagnetic radiation between 0.8 and 500µm is referred as infrared radiation, which is represented with percent transmittance as the ordinate and the wave number (cm^{-1}) as the abscissa. It is an important record which gives sufficient information about the structure of a compound.

NMR Spectroscopy

NMR spectroscopy is an important tool for determining the structure of a molecule NMR spectrum can give almost detailed information about molecular structure .^{1}H and ^{13}C NMR helps to determine the number of protons and carbon atoms present

in the molecule. NMR spectral study was done with Bruker Fourier NMR spectrometer. DMSO is used as solvent.

Results and Discussion

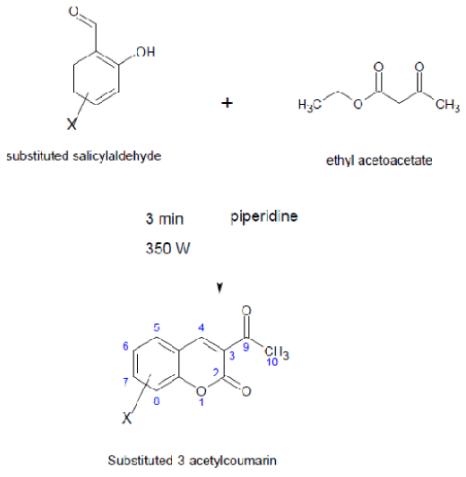
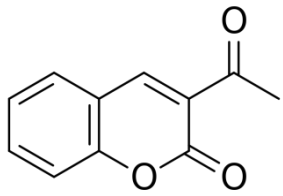


Fig 1 X=H, Br etc

COMPOUND 1	3-acetylcoumarin
COMPOUND 2	3-acetyl-6-bromocoumarin

Compound 1



3-acetyl-2H-chromen-2-one

Molecular formula of salicylaldehyde -$C_4H_4(OH)$

Molecular weight of salicylaldehyde-122.12gm

Molecular formula of compound 1 - $C_{11}H_8O_3$

Molecular weight of compound 1-188gm

Hence, 1.22gm of salicylaldehyde produces=188/122.1x1.22 = 1.87gm

Theoretical yield-1.87gm

Practical yield-1.65gm (0.0089 M)

Percentage yield-0.0089/0.01x 100 = 89.03%

Melting point-80°C -85°C

Rf value-0.51

Spectral Analysis

^{1}H NMR (400MHz, DMSO) δ (ppm): 8.710(s, Ar 1H), 8.009 (d, J= 9.6 Hz, Ar 1H), 7.80625 (dd, J=17.6 Hz, Ar 1H), 7.53-7.45 (m, Ar 2 H), 2.646 (s,3H)

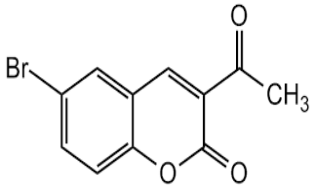
[**Reference values**: ^{1}H NMR (400 MHz, $CDCl_3$) δ:8.51 (s, 1H),7.67(m, 2H), 7.39 m, 2H), 2.73 (s.3H)]

^{13}C NMR (100MHz, DMSO)δ (ppm): 195.081, 158.394, 154.583, 146.985, 134.44, 130.733, 124.908,124.456,118.141, 116.082, 60.434, 49.563, 29.966, 13.956

[**Reference values**: ^{13}C NMR (75.5 MHz $CDCl_3$) δ (ppm): 195.5, 159.2, 155.3.147.4, 134.4, 130.2, 125.0, 124.6, 118.3, 116.7]

IR (KBr) cm^{-1}: 1755.22(C=O), 2931.80(C-H aliphatic stretching), 1575.84(C=C aromatic stretching)

Coumpound 2



3-acetyl-6-bromo-2H-chromen-2-one

Molecular formula of 5-bromosalicylaldehyde- $C_4H_3(OH)$ Br

Molecular weight of 5-bromosalicylaldehyde - 201.02gm

Molecular formula of compound 2- $C_{11}H_7O_3Br$

Molecular weight of compound 2-266.904gm

Hence, 2.01 gm of 5-bromosalicylaldehyde produces = 266.9/201.02x2.01= 2.66gm

Theoretical yield-2.66gm

Practical yield-2.18gm (0.0076 M)

Percentage yield-0.0076/0.01x100=76%

Melting point- 118°C -125°C

Rf value-0.42

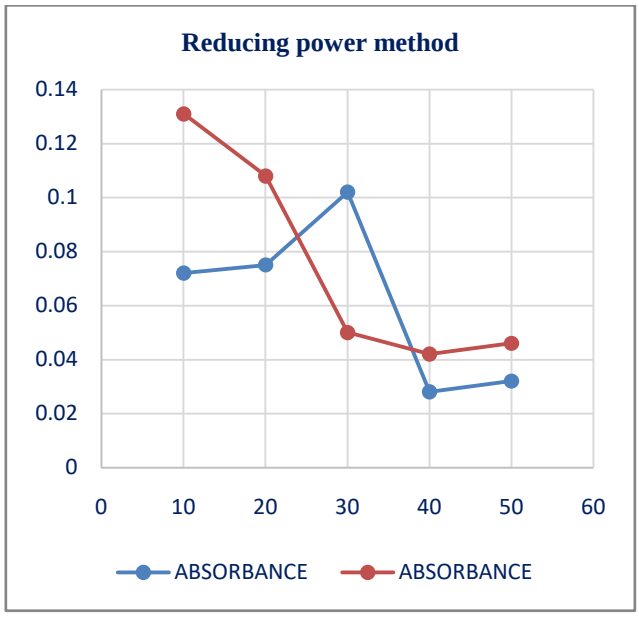
Spectral Analysis

IR (KBr) cm^{-1}: 1755(C=O), 2800(C-H aliphatic stretching),1685 (C=C aromatic stretching),765 (mono-substitution in aromatic ring)

Reducing power method

Table 1: showing inhibitory activity of compounds of reducing power

Concentration (µg/ml)	Absorbance	
	Compound 1	**Compound 2**
10	0.356±0.072	0.439±0.131
20	0.423±0.075	0.484±0.108
30	0.522±0.102	0.592±0.050
40	0.514±0.028	0.631±0.042
50	0.559±0.032	0.664±0.046



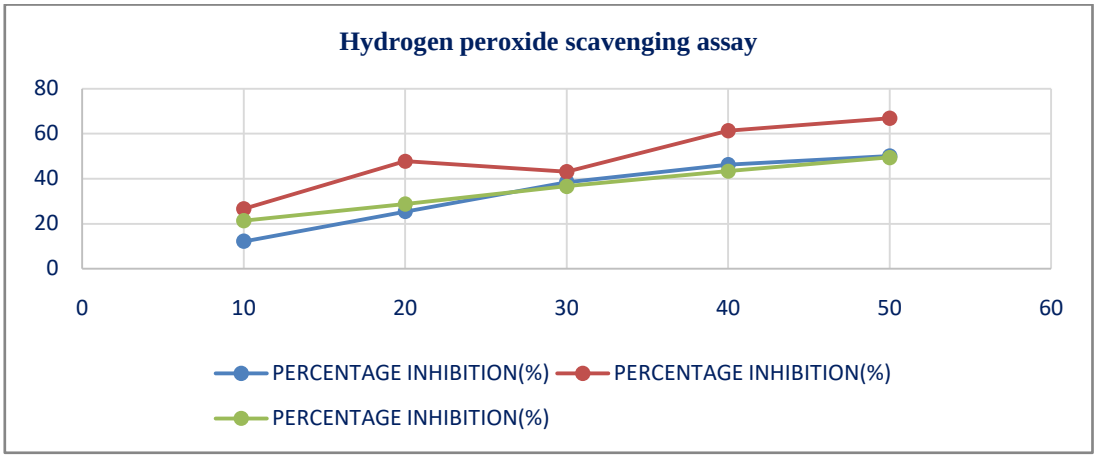
Concentration (µg/ml)

Graph 1: showing inhibitory activity of compounds of reducing power

Hydrogen peroxide scavenging assay

Table 2: showing inhibitory activity of compounds on H_2O_2 scavenging assay

Concentration (µg/ml)	Percentage inhibition (%)		
	Compound 1	Compound 2	Ascorbic acid
10	12.1±0.019	26.6±0.0331	21.3±0.0423
20	25.4±0.0083	47.7±0.170	28.7±0.0425
30	38.5±0.013	43.1±0.0385	36.6±0.022
40	46.2±0.008	61.3±0.0929	43.3±0.078
50	50.1±0.0341	56.8±0.190	49.4±0.086



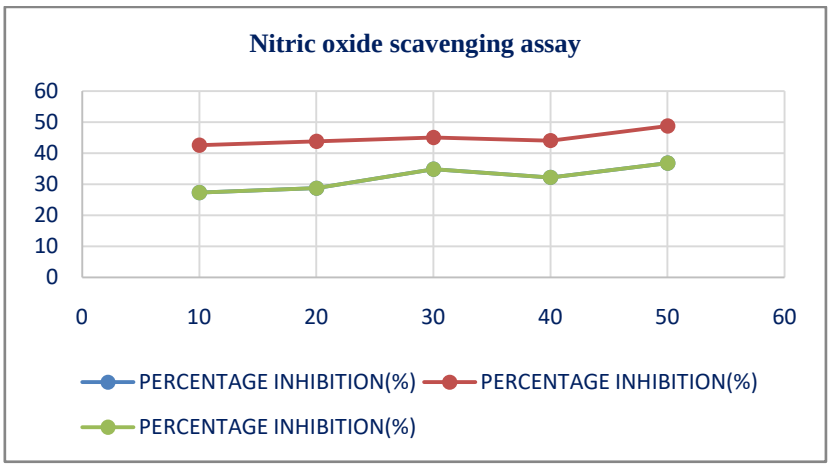
Concentration (µg/ml)

Graph 2: Showing inhibitory activity of compounds on H_2O_2 scavenging assay

Nitric oxide radical scavenging method

Table3: showing inhibitory activity of compounds on nitric oxide scavenging assay

Concentration (µg/ml)	Percentage inhibition (%)		
	Compound 1	Compound 2	Ascorbic acid
10	27.3±0.0023	42.5±0.0025	27.3±0.00231
20	28.7±0.0023	43.75±0.0007	28.7±0.00234
30	34.8±0.0023	45±0.0013	34.8±0.0023
40	32.2±0.0045	44±0.003	32.2±0.045
50	36.8±0.0037	48.75±0.001	36.8±0.0037



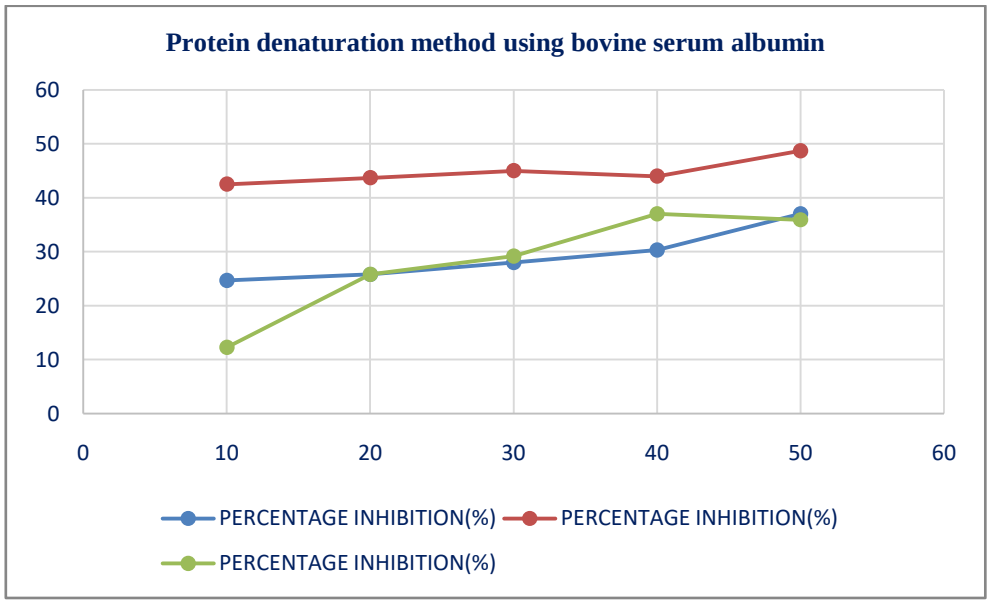
Concentration (µg/ml)

Graph 3: showing inhibitory activity of compounds on nitric oxide scavenging assay

Anti-inflammatory activity-protein denaturation method using bovine serum albumin

Table 4: showing percentage inhibition of protein denaturation

Concentration (µg/ml)	Percentage inhibition (%)		
	COMPOUND 1	COMPOUND 2	ASCORBIC ACID
10	24.7±0.0073	42.5±0.0269	12.3±0.057
20	25.8±0.00923	43.7±0.0009	25.8±0.0066
30	28±0.00655	45±0.0016	29.2±0.0046
40	30.3±0.0655	44±0.0042	37±0.0013
50	37±0.087	48.7±0.0014	35.9±0.0153



Graph 4: showing percentage inhibition of protein denaturatio

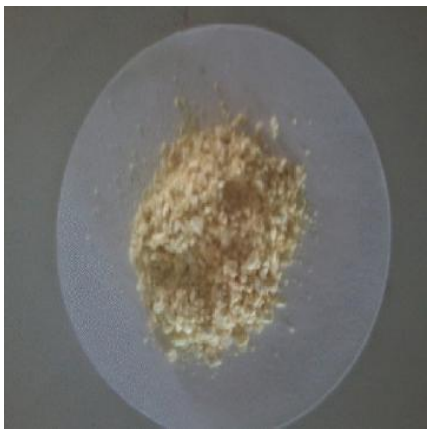


Fig 1. Image of Compound 1

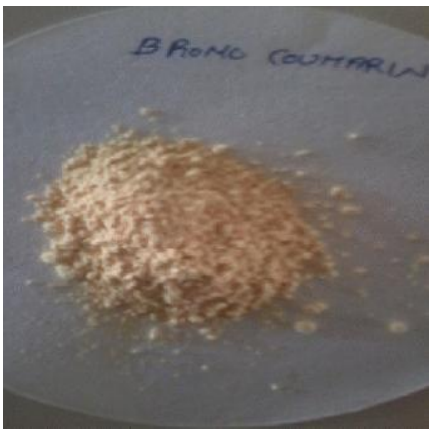


Fig 2. Image of Compound 2

Determination of IC_{50} Value

Antioxidant Activity

Reducing power method

This antioxidant screen method shows a positive result with increasing absorbance with respect to concentration

Hydrogen peroxide scavenging assay

Sl.no	Compound	IC_{50}
1	Compound 1	45.37
2	Compound 2	34.88
3	L-Ascorbic acid	46.98

Nitric oxide radical scavenging method

Sl.no	Compound	IC_{50}
1	Compound 1	67.66
2	Compound 2	42.94
3	L-Ascorbic acid	64.95

Assay of Anti-Inflammatory Activity

Protein denaturation method using bovine serum albumin

Sl.no	Compound	IC_{50}
1	Compound 1	45.48
2	Compound 2	42.85
3	Ibuprofen	42.85

In this study, coumarin was chosen as the basic nucleus for the synthesis of two derivatives, 3-acetyl coumarin and 3-acetyl 6-bromo coumarin, using a microwave-assisted method. These newly synthesized compounds underwent various physical and spectral evaluations to confirm their identity and purity.

The physical properties of the compounds were determined, including the Rf (Retention Factor) values and melting points. The Rf values were 0.51 for Compound1 and 0.42 for Compound2. The melting point of Compound-1 was in the range of 80-88°C, while Compound-2 exhibited a melting point range of 118-125°C. These differing values from their reactants confirmed the formation of new chemical compounds, indicating the success of the synthesis.

Further spectral analysis through IR and NMR provided insights into the chemical structures of the compounds. IR spectral data for Compound-2 revealed distinct peaks, including a strong band at 1751 cm^{-1}, indicating C=O stretching. A peak at 3032 cm^{-1} suggested aromatic alkene CH stretching, while a peak at 1680 cm^{-1} indicated C=C stretching. Notably, a peak at 765cm^{-1} implied a mono-substituted benzene ring. In contrast, Compound-1 lacked the 765 cm^{-1} peak, confirming that its aromatic ring was unsubstituted.

NMR data for Compound-1 showed the presence of 8 protons, with singlets at the 3rd and 4th positions representing the aliphatic and lactone ring protons, respectively. In the aromatic region, signals corresponding to different carbons were observed, and their chemical shifts were analyzed to confirm the structural composition of the synthesized products.

Both compounds were evaluated for their antioxidant activity using different methods. They exhibited concentration-dependent reducing power, which is indicative of their ability to react with free radical ions and terminate radical chain reactions. In the presence of these compounds, Fe^{3+} was converted to Fe^{2+}, highlighting their strong reducing action and suggesting a correlation between reducing power and antioxidant activity.

The compounds were also tested for their nitric oxide scavenging activity. Nitric oxide, a free radical, can lead to inflammation and tissue injury when produced excessively. The synthesized compounds showed significant nitric oxide scavenging activity, making them potential candidates for anti-inflammatory applications.

Furthermore, a hydrogen peroxide scavenging assay was performed, comparing the synthesized coumarin derivatives with the standard ascorbic acid. Compound-2 exhibited the highest antioxidant property with an IC_{50} value of 34.88 µg/ml.

The anti-inflammatory activity of the compounds was assessed using a bovine serum albumin procedure. Both compounds displayed concentration-dependent percentage inhibition, with IC_{50} values of 45.48 µg/ml for Compound-1 and 42.85 µg/ml for Compound-2. Compound-2 demonstrated anti-inflammatory activity comparable to the standard drug Ibuprofen.

In summary, this research successfully synthesized two coumarin derivatives and confirmed their purity and identity through physical and spectral analysis. These compounds displayed promising antioxidant and anti-inflammatory properties, making them potential candidates for further study as therapeutic agents in the fields of chemistry and medicine.

Conclusion

The current research work, which has been undertaken, is BONAFIED and novel for the synthesis of coumarin derivatives. The extensive reviews of literature were carried out from chemical abstracts, periodicals and with different websites. Microwave assisted synthesis gave a better percentage yield in less time. In this study the 2 coumarins, namely 3-acetylcoumarin and 3-acetyl 6-bromocoumarin have been successively synthesized and the synthesized compounds were confirmed by MP, TLC, IR and NMR spectroscopy. The anti-oxidant activity of the compounds was initially tested and showed that synthesized coumarins have improved properties compared to standard ascorbic acid. Among these compounds, Compound-2 with ICs 34.88, 42.94 using hydrogen peroxide scavenging assay and nitric oxide scavenging assay procedures respectively, exhibited maximum anti-oxidant activity when compared to standard ascorbic acid. From the anti-inflammatory assay procedure, it was confirmed that Compound-2 exhibit anti- inflammatory activity comparative to that of standard ibuprofen.
Since these compounds exhibit anti-oxidant activity, this can be further studied for anticancer activity.

Acknowledgement

I extend heartfelt thanks to my dedicated mentor, Mrs. Saranya T.S, a lecturer at Nirmala College of Pharmacy, Muvattupuzha, for unwavering guidance and support. Gratitude to the entire Nirmala College of Pharmacy community for their contributions. I'm also thankful to Amrita School of Pharmacy, Kochi, for enabling the project's success**.**

Conflict of interest

Authors are declared that no conflict of interest.

Funding

Nirmala College of Pharmacy, Muvattupuzha.

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