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Extraction of Ibuprofen from Bougainvillea Campanulata and Its Method Development and Validation

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ABSTRACT

Background: The extraction of ibuprofen from Bougainvillea campanulata and the creation and approval of a reliable technique for its separation and measurement are the main objectives of this investigation. This study will contribute to the growing body of knowledge regarding the use of plant-based sources for therapeutic compounds and set the stage for future research into Bougainvillea campanulata as a potential natural source of ibuprofen.

Aim: This work aimed to develop and evaluate a UV-visible spectrophotometric approach utilizing FTIR to accurately, precisely, and straightforwardly quantify the concentration of ibuprofen in an extract.

Materials and Methods: In the experiment, methanol served as the solvent. It was determined that the drug's absorption maximum (λmax) was 265 nm. Beer's law was followed between 100 µg/ml and 500 µg/ml.

Results: A correlation coefficient of 1 and the equation y = 0.000023x signify that the approach exhibits linearity with respect to the given concentrations. The extract contained 7 µg/ml of ibuprofen. Ibuprofen extracted from Bougainvillea Campanulata flowers had recovery percentages between 85.9% and 87.6%. The intraday precision was 42.95%, while the interday precision was 51.35%, both quantified as relative standard deviation (RSD%). The quantification limit was 2.70 µg/mL, while the detection limit was 0.90 µg/mL. The method's robustness percentage ranged from 37.43% to 45.86% regarding relative standard deviations.

Conclusion: The proposed methodology was accurate, exact, and economical. This technique may effectively ascertain the methanol concentration in an extract obtained from bougainvillea flowers.

Keywords: *Bougainvillea Campanulata, Ibuprofen, UV, TLC, Method development.*

1. INTRODUCTION

The extraction of bioactive compounds from plants has become an essential area of research, particularly in the field of pharmaceutical sciences [1]. Ibuprofen is a notable molecule, a commonly utilized non-steroidal anti-inflammatory medicine (NSAID) recognized for its analgesic, antipyretic, and anti-inflammatory characteristics [2]. Traditionally, ibuprofen is synthesized chemically; however, the potential of natural sources for ibuprofen extraction is gaining attention. Bougainvillea campanulata, a member of the Bougainvillea genus, has shown promise as a natural source of bioactive molecules, including ibuprofen-like compounds [3].

Bougainvillea campanulata is a flowering plant found in tropical regions and has been traditionally used in herbal medicine for various purposes. Recent studies suggest that its extract contains compounds with properties that resemble those of ibuprofen, making it a candidate for further investigation into its pharmaceutical applications [4, 5].

The extraction process from plant material involves selecting appropriate solvents and methods that can efficiently isolate the desired bioactive compounds while ensuring minimal degradation and loss of the compound [6]. Developing and validating an extraction method is critical to ensure reproducibility, accuracy, and reliability of the results. Method development includes optimizing extraction parameters such as solvent type, temperature, time, and plant material-to-solvent ratio, to maximize the yield of ibuprofen or ibuprofen-like substances [7, 8].

Validation of the extraction method is equally important to confirm its effectiveness and consistency. Validation parameters, such as precision, accuracy, sensitivity, and specificity, are essential to ensure that the developed method can reliably quantify ibuprofen content in Bougainvillea campanulata extracts. The validated method also helps in assessing the potential therapeutic use of this plant as a natural alternative to synthetic ibuprofen [9, 10].

This project is mostly about getting ibuprofen out of Bougainvillea campanulata and making and testing a good way to separate it and measure how much there is. This study adds to what is already known about using plant-based sources for pharmaceutical chemicals and lays the groundwork for more research into Bougainvillea campanulata as a possible natural source of ibuprofen [11, 12].

2. MATERIALS AND METHODS

Method of Extraction:

After measuring approximately 10 g of *Bougainville Campanulata* flowers and 250 ml of water, allow the combination to infuse for five hours. We separated and concentrated the extract for an additional hour on a heating mantle at 50°C before transferring it to a volumetric flask and refrigerating it [13, 14].

Preparation of sample solution

Into a 100 ml volumetric flask Distilled water and filtered water are employed to extract and prepare the flowers of Bougainvillea Campanulata. To prepare a 1000 µg/ml concentration, transfer 10 ml of the previously prepared solution and dilute it with 100 ml of diluent. Subsequently, at elevated quantities, it was diluted with distilled water [15, 16].

Preparation of standard solution

After diluting 0.1g of ibuprofen with 100 mL of distilled water, the mixture is further diluted to produce quercetin concentrations ranging from 100 to 500 µg/ml [17].

Protocol of Method development:

Determination of λ max:

In order to determine the wavelength of maximum absorbance, the test solution was evaluated at a variety of concentrations (100 to 500 µg/ml) by scanning the 200–400 nm wavelength range. At 265 nm, the highest absorption was detected [18, 19].

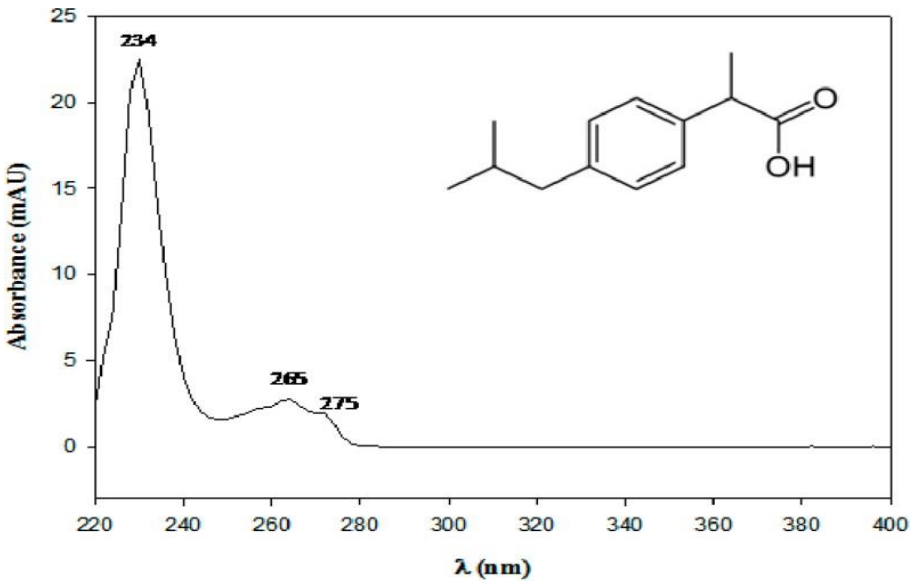

Figure 1: UV Spectrum of Ibuprofen

Protocol for Method Validation:

A variety of metrics, such as precision, accuracy, linearity, robustness, specificity, limit of detection (LOD), limit of quantification (LOQ), and assay, were employed to validate the proposed methodology [20, 21].

Linearity:

Graphing concentration vs matching absorbance facilitated the assessment of linearity. The standard stock solution, which contained 1000 µg/mL, was diluted with a diluent to create solutions ranging from 100 µg/mL to 500 µg/mL. Regression equations were derived and calibration curves were developed with the help of the absorbance versus concentration data [22, 23].

Table 1: Ibuprofen Calibration curve

Sr. No	**1**	**2**	**3**	**4**	**5**	**6**
Concentration (µg/ml)	0	100	200	300	400	500
Absorbance	00	0.001	0.002	0.003	0.004	0.005

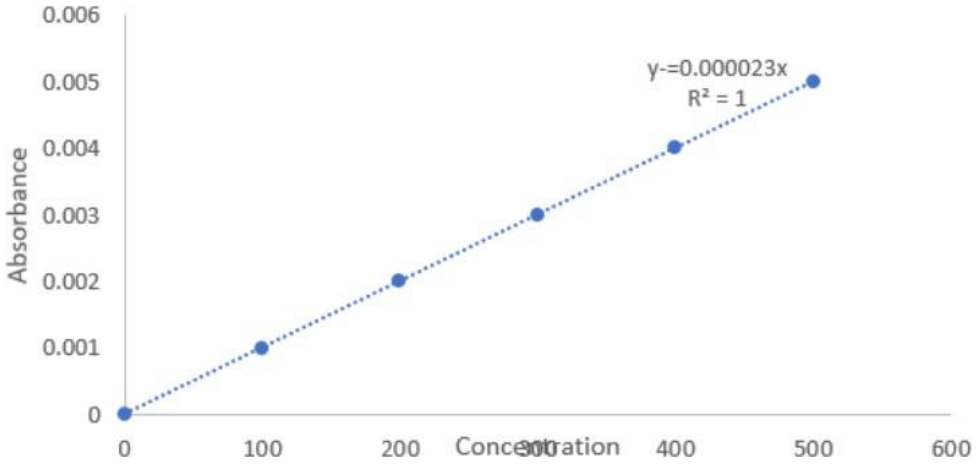

Figure 2: Graphical Representation for Ibuprofen Calibration curve

Precision:

1. **Intra-day Precision:**

Following that, the test sample was diluted until it reached a concentration of 100–500 µg/ml. Six replicates were measured, and the percentage relative standard deviation was calculated.

2. **Inter-day Precision:**

The % RSD was calculated when the selected concentrations for the intra-day precision study were re-evaluated over three days [24].

Table 2: Results of Intra-day and inter-day Precision

Sr. No	1	2	3	4	5	6	Mean	SD	%RSD
Intra-day Precision	0.003	0.006	0.007	0.009	0.012	0.013	0.007	0.003	42.95
Inter-day Precision	0.005	0.009	0.013	0.016	0.018	0.019	0.009	0.004	51.35

Study of Accuracy and Recovery:

Recovery tests were conducted at three specific levels (75%, 100%, and 125%) employing the standard addition method to evaluate the precision of the approach and examine the impact of excipients. The initial recovery experiment focused on the excipient combination (placebo), accomplished by incorporating accurately measured quantities of almond extract into the excipient mixture and calculating the percentage recovery for each instance [24, 25].

Table 3: Study/Results of Recovery

Sr. No	Level % Recovery	% Recovery	Mean % Recovery	SD	%RSD
1	75%	88.67	87.6	0.81	0.92
2		87.54			
3		86.69			
4	100%	85.64	85.90	0.20	0.23
5		85.97			
6		86.12			
7	125%	87.32	87.36	0.442	0.05
8		86.85			
9		**87.93**			

Specificity Study with excipients:

The specificity test was conducted using only excipients. At various time intervals, measurements of the blank and sample spectra were made and compared [26].

Table 4: Specificity study with excipients Results

Time	0	2	4	6	Mean	SD	%RSD
Standard	0.003	0.005	0.008	0.011	0.005	0.002	41.09
Sample	0.007	0.009	0.014	0.017	0.010	0.002	29.43

Robustness Study:

The robustness of the analytical product impeded the measurement of the medication. The approach assesses its capacity to endure deliberate slight alterations in method parameters, indicating its reliability under standard operating settings. The analysis was conducted at three specific wavelengths: 265 nm, 266 nm, and 267 nm. The test was conducted six times, and the absorbance was recorded [27, 28].

Table 5: Results of robustness study:

Sr. No	Wavelength		
	265 nm	266 nm	267 nm
1	0.004	0.006	0.005
2	0.006	0.009	0.007
3	0.009	0.011	0.009
4	0.013	0.014	0.012
5	0.016	0.018	0.017
6	0.018	0.020	0.019
Mean	0.010	0.012	0.010
Standard Deviation	0.005	0.006	0.004
% Relative Standard Deviation	45.90	37.50	42.98

Calculation of Limit of Quantitation and Limit of Detection:

As a result of the findings of the linearity investigations, the Limit of Detection and the Limit of Quantification were established. A determination was made on the slope of the linear plot. An analysis was performed to assess the standard deviation of the responses for each of the ten replicate measurements that were performed at the same doses [29-34]. For the purpose of determining the detection limit, the following formula can be utilized:

$$LOD = 3.3\ \sigma / S = 0.9\ \mu g/ml$$

The slope and standard deviation of the response can be employed to determine the limit of quantitation.

$$LOQ = 10\ \sigma / S = 2.7\ \mu g/ml$$

Where,

σ = Standard deviation of the response;

S = Slope of the calibration curve.

Analytical Assay Performance of *Bougainville Campanulata* flowers extract:

To ascertain the quantity of flower within the vial, a 100-milliliter volumetric flask was diluted with water. The floral extract comprised 100 milligrams of powdered Bougainville Campanulata flower. The solution was further diluted with water to ascertain the final concentration of Bougainville Campanulata flower extract, which was established at 100µg/mL. The analyzed proportion of the medication was ascertained. In every instance, the decision was rendered thrice [35-42].

Table 6: Assay performance Results

Drug Sample	Concentration (µg/ml)	Amount found Concentration (µg/ml)	Amount found (%)
1	100	8.10 ± 0.01	8.10 ± 0.01
2	100	8.20 ± 0.01	8.20 ± 0.01
3	100	8.00 ± 0.01	8.00 ± 0.01

IR Spectrum Interpretation:

FTIR analysis is employed to identify functional groups, with peak values in the infrared spectrum utilized to ascertain active components. The ibuprofen interpretation and the *Bougainville Campanulata* extract are found to be within the same proximity [31, 43-48].

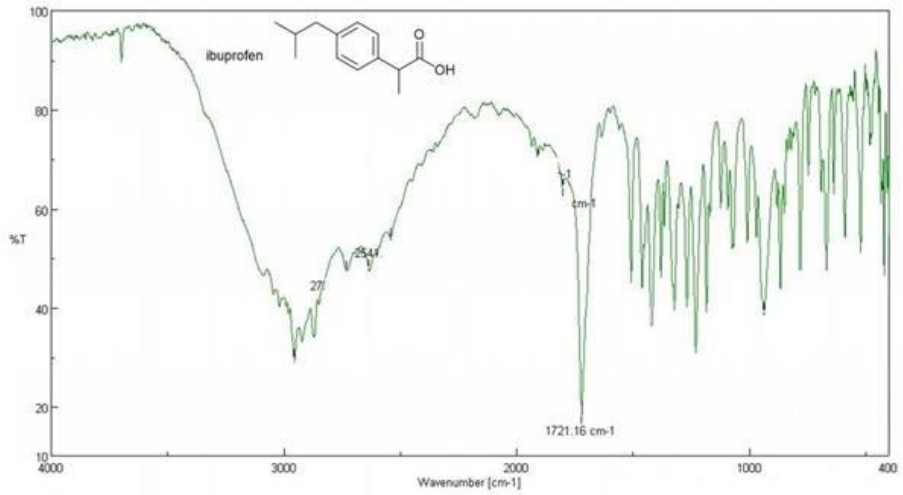

Figure 3: Ibuprofen IR Spectrum

Table 7: Interpretation IR Spectrum of Ibuprofen

Sr. No	Functional Group	Type of Peak	Observed Absorption Value (cm^{-1})
1	C-H	Def	1483
2	CONH-R	Stretching	1543
3	C=C	Stretching	1679
4	C-H	Bending	2778
5	C-H	Stretching	3069
6	N-H	Stretching	3233

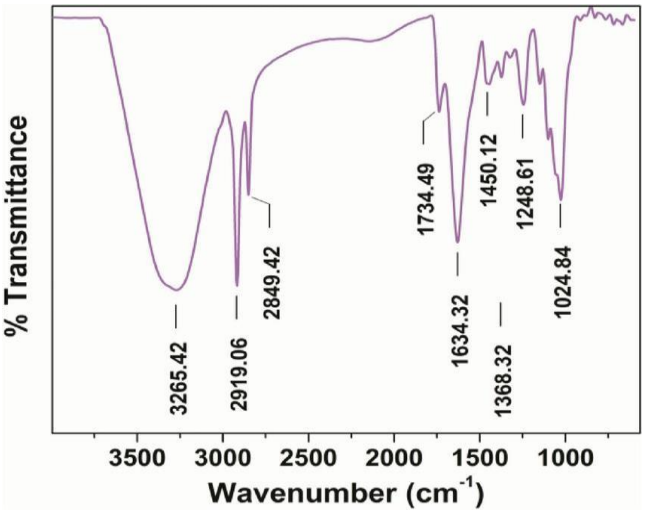

Figure 4: *Bougainville Campanulata* extract IR Spectrum

Table 8: Interpretation of *Bougainville Campanulata* extract IR Spectrum

Sr. No	Functional Group	Type of Peak	Observed Absorption Value (cm^{-1})
1	C-H	Def	1450.12
2	C=O	Stretching	1634.32
3	C=O	Stretching	1734.49
4	C-H	Bending	2849.42
5	N-H	Stretching	2919.06
6	N-H	Stretching	3265.42

Thin layer chromatography:

The Rf value of the extract in TLC was 0.870, utilizing a methanol: water ratio of 60:40 [49-52].

3. RESULTS AND DISCUSSION

In this article, a method that is both speedy and accurate for evaluating the extract of Bougainville Campanulata flowers is presented. Following the examination of a number of different concentrations, it was discovered that the highest absorption wavelength was 265 nm. Within the concentration range of 100–500 µg/ml, the drug was found to be in compliance with Beer's law, as evidenced by the linear equation y=0.000023x, which has a correlation coefficient of 1. Based on the extract of Bougainvillea campanulata flowers, the amount of ibuprofen present was 0.007 micrograms per milliliter. In order to determine the limit of detection and limit of quantification of the method that was developed, it was tested six times using just small volumes of the standard solution. The findings of the study indicated that the Limit of Quantification was determined to be 2.7 µg/mL, while the Limit of Detection was detected to be 0.9 µg/mL. Recovery rates ranging from 85.64 percent to 88.67 percent were observed for ibuprofen that was extracted from Bougainville Campanulata flowers. The relative standard deviation for the intraday precision was 42.95%, while the RSD for the interday precision was 51.35%. Furthermore, the limits of detection and quantification were determined to be 0.9 µg/mL and 2.7 µg/mL, respectively. The relative standard deviations were used to establish the robustness percentage of the approach, which ranged from 37.43% to 45.86%. Additionally, it was discovered that the infrared spectra of the functional groups of ibuprofen and the extract from Bougainville Campanulata flowers were identical. As a result, the approach that was proposed was precise, accurate, and cost-effective. For the purpose of determining the amount of ibuprofen present in a flower extract from Bougainville Campanulata, this method might prove to be an effective instrument.

4. CONCLUSION

Two hundred sixty-five nanometers was found to be the peak absorption wavelength after the investigation of several concentrations. To adhere to Beer's law, the drug exhibited a strong linear relationship within the concentration range of 100–500 µg/ml. This relationship was defined by the equation y=0.000023x and a correlation coefficient of 1. A total of 7 micrograms per milliliter of ibuprofen was found in the extract of Bougainville Campanulata flowers. It has been confirmed by us that the approach that was provided was accurate, exact, and cost-effective. The use of this method has the potential to properly determine the amount of ibuprofen that is present in a flower extract derived from Bougainville Campanulata.

5. DECLARATIONS

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

Not applicable.

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