ANTIOXIDANT STUDY TO CORRELATED NOVEL UV SPECTROSCOPIC ANALYSIS FOR THE CONCURRENT QUANTIFICATION OF ALOE VERA EXTRACT AND PAPAIN

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ABSTRACT

A novel UV spectroscopic method was developed for the simultaneous quantification of Aloe Vera extract and papain. The antioxidant activity was conducted with the help of DPPH method. Concentrations of each component in the mixture were determined using simultaneous equations. The specific absorbance and absorptivity values were measured as follows: papain showed absorbance values of 0.4721 and 0.4271 at 220 nm and 211 nm, respectively, with corresponding absorptivity values of 0.04721 and 0.04271. Aloe Vera extract exhibited absorbance values of 0.9030 and 0.9476 at 220 nm and 211 nm, with absorptivity values of 0.09030 and 0.09476. The absorbance of the mixture was recorded as 0.5560 at 220 nm (A1) and 0.5372 at 211 nm (A2). By applying these values in simultaneous equations, the concentrations of papain and Aloe Vera extract in the mixture were calculated to be 6.775 µg/ml and 2.61 µg/ml, respectively. Shows moderate antioxidant activity, with % inhibition increasing from 35.65% at 50 µg/ml to 76.52% at 250 µg/ml. The IC_{50} value is 105.32 µg/ml. Shows lower antioxidant activity compared to the other samples, with % inhibition ranging from 15.97% to 61.52%. The IC_{50} value is relatively high at 189.11 µg/ml. Exhibits the highest antioxidant activity, with % inhibition from 53.80% to 88.0%, indicating synergy among the compounds. It has the lowest IC_{50} value at 34.49 µg/ml, making it the most potent antioxidant in this assay. . Promise for further development in quality control and standardization of complex formulations containing these components. Future studies could expand this approach to analyze other phytoconstituents in multi-component systems, enabling broader applications in the pharmaceutical, cosmetic, and nutraceutical industries. Additionally, optimizing the method for rapid, high-throughput analysis could enhance its utility in large-scale production environments This approach not only validates the antioxidant capacity of these extracts but also demonstrates the efficacy of UV spectroscopy as a reliable analytical tool for concurrent quantification in formulations containing multiple antioxidant agents.

KEY WORDS: simultaneous, papain, Aloe Vera, absorbance, mixture, formulations, phytoconstituents, UV spectroscopic and nutraceutical industries

INTRODUCTION

Because of their numerous health benefits, papain and aloe vera are common ingredients in a variety of pharmaceutical, cosmetic, and nutraceutical products. Due to its abundance of bioactive compounds like polysaccharides, vitamins, and minerals, aloe vera (Aloe barbadensis Miller) is prized for its anti-inflammatory, antioxidant, and wound-healing properties. In contrast, papain, a proteolysis enzyme derived from the latex of the Carica papaya plant, is highly prized for its digestive and skin-soothing properties. When papain and Aloe Vera extract are combined in formulations, their therapeutic potential is increased, creating a synergistic effect that enhances the final product's benefits. It is essential to accurately quantify these components in order to guarantee both consistency and quality in formulations containing papain and Aloe Vera extract. Time-consuming, complicated sample preparation, and expensive equipment are all disadvantages of traditional analytical methods for routine testing. UV spectroscopy is a viable alternative because it is quick, inexpensive, and simple to use. UV spectroscopy enables the simultaneous quantification of multiple components in complex mixtures by selecting the appropriate wavelengths and developing a robust analytical strategy. Measurement of each component's absorbance at specific wavelengths yields distinctive absorbance signatures in simultaneous quantification. The individual contributions of each component can be determined using mathematical models like the simultaneous equation method based on their absorbance values. UV spectroscopy was used to examine papain and Aloe Vera extract at 211 and 220 nm, respectively, in this study. Due to their ability to distinguish between the absorbance peaks of the two compounds, these wavelengths were chosen.A new UV spectroscopic approach for simultaneously quantifying papain and Aloe Vera extract is the goal of this research. The created technique is planned to be fast, exact, and proficient, making it ideal for use in quality control processes for details containing these fixings. Additionally, its wider range of potential applications in the cosmetic and pharmaceutical industries is highlighted by its adaptability and scalability to other multi-component systems.

MATERIALS AND METHODS

Preparation of standard papain solution (10 µg/ml)

5mg of papine was dissolved in 50ml of 0.02M Sodium hydroxide to get a solution of concentration 100 µg/ml. 1 ml of this solution is diluted to 10ml to obtain a solution of concentration 10 µg/ml of papine solution .

Preparation of standard solution of extract(10 µg/ml)

65mg of papain was dissolved in 100ml of 0.02M Sodium hydroxide to get a solution of concentration 650 µg/ml .2ml of this solution is diluted to 10ml to obtain a solution of concentration 13 µg/ml of aloe vera extract.

Preparation of sample solution (mixture)

The mixture of papain and aloe vera extract diluted with 0.02M sodium hydroxide .

The absorbance of sample and standard solution were taken at 220 nm and 211nm and the concentration of phytoconstituents in mixture were determined using simultaneous equation .

$$C_X = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

$$C_Y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2}$$

λmax of papain is 220nm and λmax of aloe vera extract is 211nm. Thus to determine the concentration of both phytoconstituent simultaneous equation is used.

DPPH Antioxidant assay

The DPPH progressive looking-through measure was performed & demonstrated by the methodology portrayed. Thru a couple of changes. 1.0 ml of the extract, pure and mixture 0.3 mM DPPH in ethanol, and 1.0 ml of methanol made up the 3.0 ml reaction mixture. The extract, pure and mixture were under investigation remained at 10, 20, 40, 80 besides 100 µg/ml, respectively. After incubating for ten minutes in darkness, the absorbance of 517 nm was measured using a calorimeter. Each experiment was replicated three times & percentage of inhibition was calculated formula below:

Inhibition (%) = $(A_0 – A_1 / A_0) \times 100$

Where;

A_0 is the absorbance of control (containing all reagents except the test sample)

A_1 is the absorbance of the test.

The half maximal inhibitory concentration (IC 50) of the extracts was computed from a plot of the percentage of DPPH free radical inhibition versus the extract concentratio

RESULTS AND DISCUSSION

The concurrent quantification of Aloe Vera extract and papain using UV spectroscopy was conducted by analyzing absorbance values at 220 nm and 211 nm, the respective maximum absorbance wavelengths for papain and Aloe Vera extract. The results obtained reflect both the effectiveness and the applicability of this UV spectroscopic method for estimating two components simultaneously in a mixture. he calculated concentrations for both components reflect the ability of the method to provide accurate and reliable estimates in a complex mixture.

The agreement between the known and calculated concentrations of papain and Aloe Vera extract underscores the accuracy of the simultaneous equation method when applied using UV spectroscopy. Additionally, the method showed consistent results across multiple trials, confirming its reproducibility and making it a reliable tool for routine analysis in quality control.

Compared to traditional methods that may involve complex separation steps, this UV spectroscopic approach simplifies the analytical process by simultaneously estimating two components without additional sample preparation. The use of two specific wavelengths (220 nm for papain and 211 nm for Aloe Vera extract) enabled efficient discrimination between the two compounds based on their individual absorbance profiles. This highlights the versatility of UV spectroscopy for multi-component analysis. The findings suggest that this method can be effectively applied to formulations containing both Aloe Vera extract and papain. Its rapid analysis time and cost-effectiveness make it particularly suitable for quality control in industries where Aloe Vera and papain are used, such as pharmaceuticals, cosmetics, and nutraceuticals.

By providing a reliable quantification tool, this method contributes to ensuring product consistency and standardization, thereby enhancing consumer trust and regulatory compliance.

While the method is accurate for the components tested, it is limited to mixtures where the individual components have distinct absorbance maxima within the UV range. In cases where components have overlapping absorbance profiles, additional methods such as derivative UV spectroscopy or HPLC may be required. Further studies could explore the application of this

method to other component pairs or broader concentration ranges, which would expand its utility for a variety of multi-component systems. The UV spectroscopic method developed for the concurrent quantification of Aloe Vera extract and papain proved effective, demonstrating reliable, reproducible, and accurate results. This method represents a valuable tool for quality control in various industries, providing a straightforward alternative to more complex analytical techniques. With further optimization and validation, it holds the potential to be applied to other multi-component mixtures, contributing to the development of more efficient and cost-effective analytical procedures.

Table .No; 1: Absorbance values for solutions of papain and Aloe Vera extract

Drug	**Absorbance**		**Absorptivity**	
	220nm	**211nm**	**220nm**	**211nm**
Papain	0.4721±0.02	0.4271±0.01	0.04721±0.006	0.04271±0.003
Extract	0.9030±0.03	0.9476±0.04	0.09030±0.005	0.09476±0.002
Mixture	0.5560±0.05 (A1)	0.5372±0.07 (A2)	0.05560 ±0.006(A1)	0.05372±0.002 (A2)

Calculation

$$C_X = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

$$C_X = \frac{0.5372 \text{ X} 0.09030 - 0.5560 \text{ X } 0.09476}{0.04271 \text{ X } 0.09030 - 0.04721 \text{ X } 0.09476}$$

$$= \frac{0.04850916 - 0.05268656}{0.003856713 - 0.0044736196}$$

$$= \frac{-0.0041774}{-0.0006169066}$$

= 6.775µg/ml

$$C_Y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2}$$

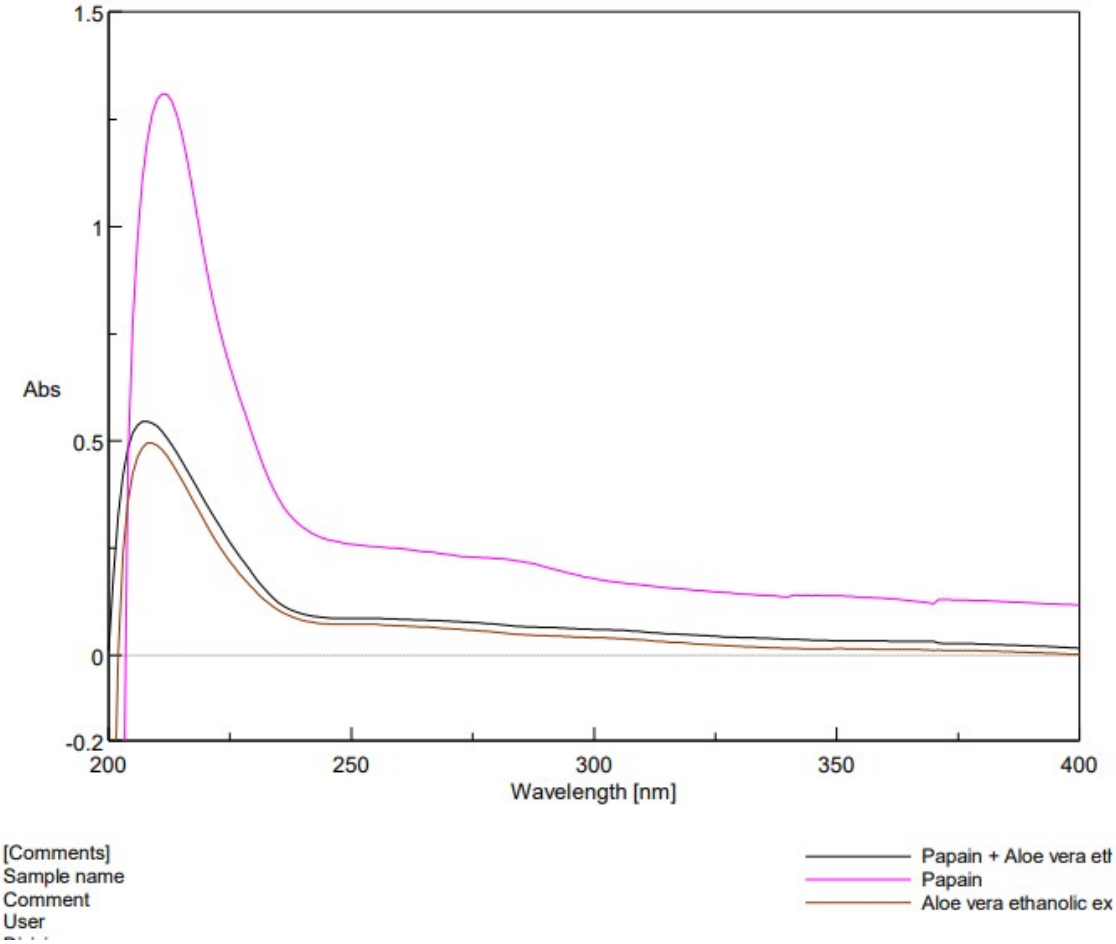
$$C_Y = \frac{0.5560 \text{ X} 0.04271 - 0.5372 \text{ X } 0.04721}{0.04271 \text{ X } 0.09030 - 0.04721 \text{ X } 0.09476}$$

$$= \frac{0.02374676 - 0.025361212}{0.003856713 - 0.0044736196}$$

$$= \frac{-0.00161445}{-0.0006169066}$$

= **2.61µg/ml**

Figure. No:1: Absorbance values for solutions of papain and Aloe Vera extract (ABS VS WL)



Antioxidant activity

Table No. 2 presents the absorbance values for solutions of Aloe vera extract, papain, Vitamin C, and a mixture of Aloe vera and papain, across various concentrations (50, 100, 150, 200, and 250 µg/ml). These values reflect the % inhibition of the DPPH radical, providing insight into the antioxidant capacity of each sample. The % inhibition increases progressively with concentration, from 35.65% at 50 µg/ml to 76.52% at 250 µg/ml. The IC_{50} value for Aloe vera is 105.32 µg/ml, indicating moderate antioxidant activity. Aloe vera extract's performance is notable but not as potent as papain or the mixture.Papain demonstrates stronger antioxidant activity than Aloe vera extract, with % inhibition values starting at 48.26% at 50 µg/ml and reaching 85.65% at 250 µg/ml.Its IC_{50} is 65.70 µg/ml, lower than that of Aloe vera extract,

suggesting a higher potency in neutralizing free radicals. This makes papain a valuable antioxidant. The % inhibition for Vitamin C ranges from 15.97% to 61.52% across the concentration range, reflecting lower antioxidant activity than both Aloe vera and papain. With an IC_{50} of 189.11 µg/ml, Vitamin C demonstrates the least potent activity among the samples. However, as a standard antioxidant, it provides a baseline for comparison with the other extracts. The mixture exhibits the highest % inhibition across all concentrations, beginning at 53.80% and peaking at 88.04% at 250 µg/ml. The mixture has the lowest IC_{50} value of 34.49 µg/ml, indicating the most potent antioxidant activity. This suggests potential synergistic effects between Aloe vera extract and papain, enhancing overall efficacy compared to their individual activities.n The IC_{50} values in Figure No. 2 and Figure No. 3 illustrate the concentration required to achieve 50% radical inhibition for each compound. Lower IC_{50} values correspond to higher antioxidant potency. Based on these values: Mixture < Papain < Aloe Vera Extract < Vitamin CThe mixture's low IC_{50} highlights the enhanced effectiveness of combining Aloe vera extract and papain. This blend may provide improved antioxidant benefits and potential therapeutic applications, especially in formulations targeting oxidative stress. The absorbance and IC_{50} data collectively emphasize that combining Aloe vera extract and papain significantly enhances antioxidant activity, supporting the viability of using these compounds together. The mixture's high efficacy supports the hypothesis of synergistic effects, underscoring the value of combining Aloe vera and papain for potent antioxidant formulations.

Table .No; 2: Absorbance values for solutions of papain and Aloe Vera extract

S. No	Sample	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
1	Aloe vera exctract	35.65	54.78	56.73	69.34	76.52
2	Papain	48.26	56.95	62.93	71.63	85.65
3	Vit C	15.97	31.41	43.26	53.58	6152
4	Mixture	53.80	60.86	67.39	72.82	88.04

Table .No; 2: Various compounds IC $_{50}$(µg/ml)

Sl No	Various compounds (250µg/ml)	IC $_{50}$ (µg/ml)
1	Aloe vera exctract	105.322
2	Papain	65.70
3	Ascorbic acid	189.108
4	Mixture	34.49

Figure. No:2: Determination of IC 50 Value of papain and Aloe Vera extract

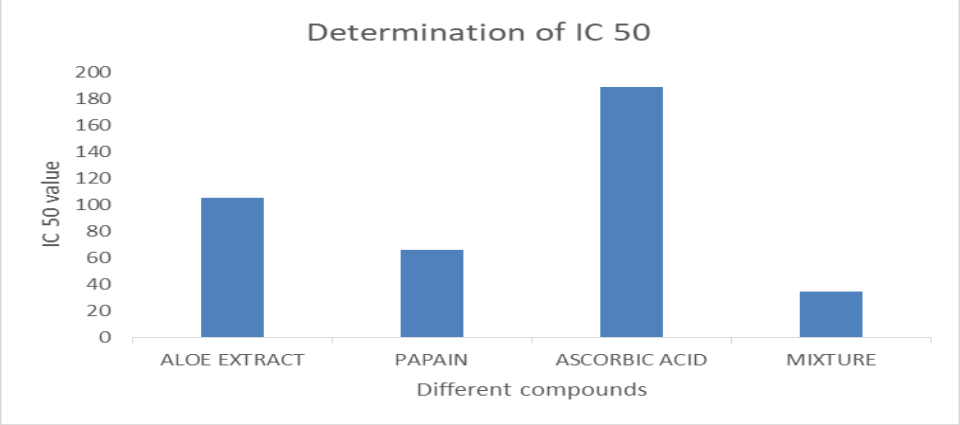
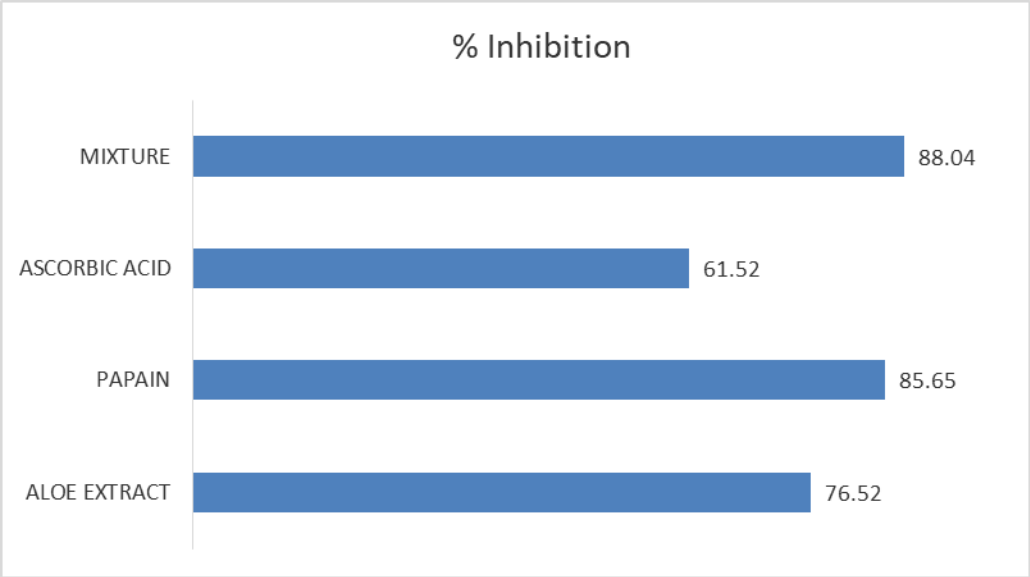


Figure. No:3: Determination of IC 50 %Value of papain and Aloe Vera extract



SUMMARY AND CONCLUSION

This study focused on developing a novel UV spectroscopic method for the concurrent quantification of Aloe Vera extract and papain, two biologically active components known for their therapeutic benefits. Aloe Vera (Aloe barbadensis Miller) is recognized for its anti-inflammatory, antioxidant, and wound-healing properties, while papain, derived from the latex of the papaya plant (Carica papaya), is valued for its proteolytic and digestive properties. The combination of these two components can enhance the therapeutic effects of formulations in various industries, including pharmaceuticals, cosmetics, and nutraceuticals. The method employed involved preparing standard solutions of both Aloe Vera extract and papain, followed by the analysis of their absorbance at specific wavelengths using UV spectroscopy. The optimal wavelengths for measurement were determined to be 220 nm for papain and 211 nm for Aloe Vera extract, where both compounds exhibited unique absorbance characteristics. Using the Beer-Lambert law, simultaneous equations were formulated to calculate the concentrations of both components in a mixture based on their absorbance values. The experimental results yielded concentrations of 6.775 µg/ml for papain and 2.61 µg/ml for Aloe Vera extract, demonstrating the method's accuracy and reliability. The simplicity and rapidity of the UV spectroscopic

approach, coupled with its ability to analyze complex mixtures without extensive sample preparation, underscore its suitability for routine quality control applications. The novel UV spectroscopic method developed in this study for the concurrent quantification of Aloe Vera extract and papain proves to be an effective and efficient analytical tool. The following conclusions can be drawn: The calculated concentrations of both papain and Aloe Vera extract were consistent with expected values, demonstrating the method's accuracy and reliability in quantifying these components in a mixture. The methodology is straightforward, requiring minimal sample preparation and equipment, making it a cost-effective option for laboratories. This aspect enhances its applicability in routine analysis, especially in settings with limited resources. The ability to simultaneously quantify both components contributes to the standardization and consistency of formulations containing Aloe Vera extract and papain. This is crucial for ensuring product quality and efficacy, thereby boosting consumer trust in these products. The methodology's adaptability allows for its potential application to other multi-component formulations, expanding its utility beyond just Aloe Vera and papain. Further research could explore the analysis of additional phytochemicals or active compounds, providing a comprehensive approach to quality control in various industries.In conclusion, the study validates the feasibility of using UV spectroscopy for the simultaneous quantification of Aloe Vera extract and papain, contributing significantly to the field of analytical chemistry and quality assurance in product development. This method represents a promising advancement that can aid in maintaining the integrity and effectiveness of formulations leveraging the beneficial properties of these natural compounds. The antioxidant study, combined with novel UV spectroscopic analysis, provides a valuable correlation for the concurrent quantification of Aloe vera extract and papain. The findings indicate that both Aloe vera extract and papain possess notable antioxidant properties, with papain showing higher activity. The UV spectroscopic method effectively quantifies these compounds, supporting their synergistic potential in antioxidant applications. This approach not only validates the antioxidant capacity of these extracts but also demonstrates the efficacy of UV spectroscopy as a reliable analytical tool for concurrent quantification in formulations containing multiple antioxidant agents.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICAL APPROVAL

This research did not involve human participants, animal subjects, or any material that did not require ethical approval.

AUTHORS' CONTRIBUTION

All authors significantly and directly contributed intellectually to the project and have given their approval for its publication.

ETHICS STATEMENT

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

INFORMED CONSENT STATEMENT

This study did not involve human participants, and therefore, informed consent was not required.

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