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A validated comparative study of RP-HPLC, GC-FID and UV spectrophotometric methods for the quantification of eugenol isolated from *Syzygium Aromaticum* L

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Abstract
This study aimed to develop and validate three analytical methods, including reversed-phase high-performance liquid chromatography, gas chromatography with flame ionization detection, and ultraviolet spectrophotometry, for the determination of eugenol isolated from the clove buds. The RP-HPLC technique utilized a C-18 column with a mobile phase composed of 0.1M orthophosphoric acid buffer solution and acetonitrile (70:30, v/v). For GC-FID analysis, DB-1 (USP Phase G2) column was employed for estimation. UV spectroscopy measurements were conducted between 200 and 800 nm using methanol as the solvent, with 282 nm chosen as the analytical wavelength. The developed analytical methods were validated according to the International Conference on Harmonization (ICH) guidelines Q2 (R1) for parameters such as linearity, accuracy, precision, specificity, and robustness. Consequently, each method provided highly dependable results for the quantification of eugenol isolated from *Syzygium aromaticum L.*

Keywords: *Syzygium aromaticum* L, Eugenol, RP-HPLC, GC-FID, UV, ICH guidelines Q2(R1) *etc.*

1. Introduction
Clove *(Syzygium aromaticum (L.) Merr & L. M. Perry)* and cinnamon *(Cinnamomum verum Pres)* are known sources of eugenol, with clove containing 85-90% and cinnamon consisting 25-80% of this phenylpropanoid compound (Figure 1) [1]. Eugenol, also known as 2-methoxy-4-(2-propenyl)-phenol, possesses various pharmacological properties such as anticancer, antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, and analgesic activities [2, 3]. Due to its broad spectrum of biological effects, eugenol is a prime candidate for structural modifications to develop pharmaceutically active compounds [4, 5].

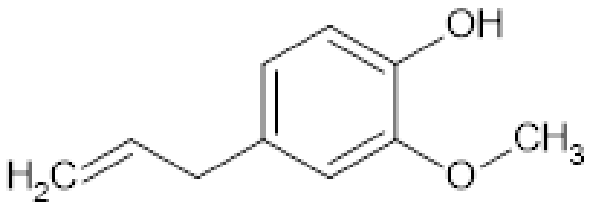


Fig 1: (2-methoxy-4(prop-2en-1yl) phenol)

The widespread acceptance of natural or alternative therapies by the international community hinges on the modernization of herbal medicine. This means that it is crucial to standardize and ensure the quality of herbal materials using modern science and technology. Currently, issues related to quality (such as inconsistency, safety, and efficacy) are casting a shadow over the potential health benefits of various herbal products. The main reason behind these problems appears to be the lack of simple and dependable analytical techniques and methodologies for the chemical analysis of herbal materials [6].
The extensive review of literature revealed that eugenol has been analyzed separately or in combination with other substances by UV spectroscopy [7-10], RP-HPLC [11-14], and Gas chromatography [15-16]. However, there has not been any research that quantified eugenol using UV spectrophotometry, RP-HPLC, and GC-FID simultaneously.

This gap of literature strikes us the current research of innovative, accurate, sensitive and cost-effective methods in quantifying and validating eugenol isolated from *Syzygium aromaticum L.* by RP-HPLC, GC-FID, and UV spectrophotometric methods simultaneously in accordance with ICH guidelines Q2 (R1).

2. Materials and Methods

2.1 Standard: Eugenol working standard (purity 99.5%) free sample was gifted by Poornayu research lab, Bengaluru.

2.2 Reagents and solvents: HPLC grade methanol, acetonitrile and orthophosphoric acid from Merck. HPLC grade water was collected from Sartorius Arium 611 water purification system. 0.45µm nylon syringe filter (Merck), and Class A glassware were used.

2.3 Instruments: HPLC system (Shimadzu Prominence-i LC-2030C plus HPLC) equipped with quaternary pump, DAD (Photo Diode Array Detector) detector, autosampler, thermostat column oven, degasser, and Lab Solutions software. A YMC PACK C-18 column (150 x 4.6 mm, 5µm) was used for estimation. GC system (Shimadzu GC-2010 Plus), AOC-20i Split/Split less Injection Unit, FID detector, column oven, and Lab Solutions software. DB-1, Inner Diameter: 0.32 mm, length: 30 m, film: 1.00 µm, was used for estimation. A UV-Vis spectrophotometer (Shimadzu 1900i) was used for estimation.

2.4 Extraction and Isolation of Eugenol from clove buds: Extraction and isolation of eugenol from *Syzygium aromaticum L* was carried out at the laborites of department of pharmaceutical chemistry, Government College of pharmacy, Bengaluru in our previous research carried out. The isolated eugenol is characterized by FTIR, GCMS and ^{1}HNMR [17].

2.5 RP-HPLC method

2.5.1 Preparation of mobile phase: The mobile phase used in the method was composed of 0.1% orthophosphoric acid buffer and acetonitrile (30:70, v/v) in isocratic elution. A 0.1% buffer solution was prepared in a 1 L volumetric flask by diluting 1 mL of orthophosphoric acid in millipore water. The mobile phase was sonicated for 15 minutes. The mobile phase was filtered through a membrane 0.45 µm, sonicated for 10 min.

2.5.2 Preparation of diluent: A solvent system of acetonitrile and water in a 70:30 ratio was used as the diluent.

2.5.3 Preparation of standard stock solution: 30 mg of eugenol standard was precisely weighed and transferred to a clean, dry 50 ml volumetric flask. The eugenol was dissolved and diluted with methanol to achieve a concentration of 600 µg/mL.

2.5.4 Preparation of solution for standard calibration curve: Standard solutions for calibration were made from standard stock solutions at concentrations ranging from 6 to 125 µg/mL by transferring aliquots of 0.5 mL, 1.0 mL, 2.5 mL, 5.0 mL, 6.25 mL, 7.5 mL, and 10.0 mL from the standard stock solutions to a 50 mL volumetric flask. The final volume was made up to the mark using diluent and then filtered through a 0.45 µm nylon filter. The filtered solution was then injected to RP-HPLC system. The calibration curve was plotted as a standard curve with peak area versus drug concentration.

2.5.5 Preparation of sample solution: 30 mg of isolated eugenol was accurately weighed and taken in a 50 mL volumetric flask. It was dissolved with methanol, and the volume was then made up to 50 mL with methanol. Subsequently, 5 mL was transferred to a 50 mL volumetric flask and made up to the mark with diluent to achieve a concentration of 60 µg/mL.

2.5.6 Chromatographic conditions: The chromatographic parameters are presented in Table 1.

Table 1: Chromatographic conditions

Conditions		Value
Column	:	YMC PACK C-18 column (150 x 4.6 mm, 5µm)
Flow rate	:	1.0 mL/min
Injection volume	:	20 µL
Wavelength	:	281 nm
Temperature	:	27°C
Elution type	:	Isocratic
Run time	:	8 min

2.5.7 System Suitability: The method's suitability was assessed based on system suitability factors such as the tailing factor (T), number of theoretical plates (N) and the percentage relative standard deviation (% RSD) values derived from five replicate injections of peak area and retention time (min).

2.6 UV spectrophotometric method

2.6.1 Wavelength of maximum absorbance (λmax) of eugenol: The wavelength at which eugenol exhibited maximum absorbance was determined by scanning a 10µg/ml eugenol solution using a UV instrument that ranged from 200 to 800 nm, and the maximum absorbance was observed at 282 nm.

2.6.2 Preparation of standard stock solution

25 mg of eugenol standard was precisely weighed and then transferred to a clean, dry 100 ml volumetric flask. The eugenol was dissolved and diluted with methanol to achieve a concentration of 250 µg/ml.

2.6.3 Preparation of solution for standard calibration curve: Calibration standard solutions were made from standard stock solutions at concentrations ranging from 5 to 75 µg/mL by transferring aliquots of 0.5 mL, 1.0 mL, 1.5 mL, 3.0 mL, 4.5 mL, 5.0 mL, and 7.5 mL to a 25 mL volumetric flask, and the final volume was made up to the mark using diluent, then filtered using a 0.45 µm nylon filter. The filtered solution was then measured absorbance maxima at 282 nm. The calibration curve was plotted as a standard curve with absorbance versus drug concentration.

2.6.4 Preparation of sample solution: 25 mg of isolated eugenol was accurately weighed and taken in a 100 mL volumetric flask. It was dissolved with methanol, and the volume was then made up to 100 mL with methanol. Subsequently, 3.0 mL was transferred to a 25 mL volumetric flask and made up to the mark with diluent to achieve a concentration of 30 µg/mL.

2.6.5 System Suitability: The% RSD values derived from five replicate injections of absorbance.

2.7 GC-FID method

2.7.1 Preparation of standard stock solution: Precisely 100 mg of eugenol standard was weighed and then transferred to a clean, dry 100 ml volumetric flask. It was dissolved and diluted with methanol to achieve a concentration of 1000 µg/ml.

2.7.2 Preparation of solution for standard calibration curve: Solutions for calibration were made from standard stock solutions at range of 10 to 200 µg/mL by transferring aliquots of 0.5 mL, 2.5 mL, 3.75 mL, 5.0 mL, 6.25 mL, 7.5 mL, 8.75 mL, and 10.0 mL to a 50 mL volumetric flask. The final volume was made up to the mark using diluent, then filtered using a 0.45 µm nylon filter. The filtered solution was then injected to GC-FID system. The calibration curve was plotted as a standard curve with peak area versus drug concentration.

2.7.3 Preparation of sample solution: About 100 mg of the isolated eugenol was accurately weighed and taken in a 100-mL volumetric flask. It was then dissolved with methanol, and the volume was made up to 100 mL with methanol. Further, 5 ml was transferred to a 50 ml volumetric flask and made up to the mark with diluent to achieve a concentration of 100 µg/ml.

2.7.4 Chromatographic conditions: The chromatographic parameters are presented in Table 2.

Table 2: Chromatographic conditions

Conditions		Value
Column	:	DB-1, Inner Diameter: 0.32 mm, Length: 30 m, Film: 1.00 µm
Carrier gas	:	Nitrogen
Flow rate	:	1.0 mL/min
Injection volume	:	1µl
Injector temperature	:	250°C
Detector temperature	:	280°C
Run time	:	5.65 min
Makeup gas	:	FID makeup flow-30ml/min FID H2 flow-40ml/min FID Air flow-300ml/min

2.7.5 System Suitability: The system suitability of the established method was evaluated based on factors such as the USP tailing factor (T), asymmetry, and the number of theoretical plates (N). These factors also included injection precision for retention time (min), injection precision for peak area (n = 5).

2.8 Method validation [18]

The developed methods for the quantification of eugenol have been validated in accordance with ICH Q2 (R1) guidelines, encompassing specificity, precision, and linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, ruggedness, and robustness.

2.8.1 Specificity: The specificity of the analytical method for the analyte was assessed by analyzing and comparing the chromatograms obtained through RP-HPLC, GC-FID, and UV-Vis scans of eugenol standard, sample and blank solution. The findings indicate that the peak corresponding to the analyte is free from interference by other components present in the blank solution.

2.8.2 Precision: To assess precision, both repeatability and inter-day as well as intraday measurements were employed. The standard deviation (SD) and percentage relative standard deviation (%RSD) values were found to be below 2, demonstrating the method's precision.

2.8.3 Linearity and range: The linearity of the methods was evaluated using standard calibration solutions, with concentration ranges of 6.0-125.0 µg/mL for RP-HPLC, 5.0-75.0 µg/mL for UV, and 10.0-220.0 µg/mL for GC-FID. Calibration curves were generated by plotting concentration against absorbance or peak area for each analytical method, and the resulting data on regression analysis via the least squares method. The linearity of eugenol was determined through the response ratios of the drug, calculated by dividing the absorbance or peak area by the corresponding concentration.

2.8.4 Accuracy as recovery: The accuracy of the method was assessed through recovery studies employing the standard addition technique. Pre-analyzed samples were spiked with additional amounts of standard eugenol at levels of 80%, 100%, and 120%. These mixtures were subsequently analyzed using the proposed methods. The experiment was performed in triplicate. Standard deviation (SD) and percentage relative standard deviation (%RSD) values below 2 demonstrate the method's accuracy. The total quantity of the drug detected and the percentage recovery were computed.

2.8.5 Sensitivity: The limit of detection (LOD) refers to the lowest quantity of analyte present in a sample that can be identified, although it may not be quantifiable. Conversely, the limit of quantification (LOQ) indicates the minimum amount of analyte that can be measured with adequate precision and accuracy. Both the LOD and LOQ were established using the standard calibration curve method, as described by the following equations:

$$LOD = 3.3\sigma/S \quad (1)$$
$$LOQ = 10\sigma/S \quad (2)$$

In these equations, σ represents the standard deviation of the calibration plot intercept, while S denotes the slope of the calibration curve.

2.8.6 Ruggedness and robustness: The ruggedness and robustness of the method were evaluated by implementing minor modifications in the analytical procedure at a single concentration level (100%). The robustness of the proposed method was assessed through various approaches, including intentional alterations in the mobile phase composition, flow rate, and detection wavelength during the analysis. To evaluate the robustness of the method, the percentage of relative standard deviation (%RSD) was calculated based on the experimental results.

2.8.7 Assay of isolated eugenol: A stock solution of eugenol was prepared at a concentration of 1000 µg/mL. This stock solution was subsequently diluted to the necessary concentrations for analysis using RP-HPLC, GC-FID, and UV spectrophotometry methods, with the final volume adjusted using appropriate diluents. The solutions were then filtered through a 0.45 µm nylon filter.

The percentage assay was determined using the specified equation.

$$\text{Assay} = \frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Standard Weight}}{\text{Sample Weight}} \times \frac{\text{Sample dilution}}{\text{Standard dilution}} \times \frac{\text{Purity}}{100} \times 100$$

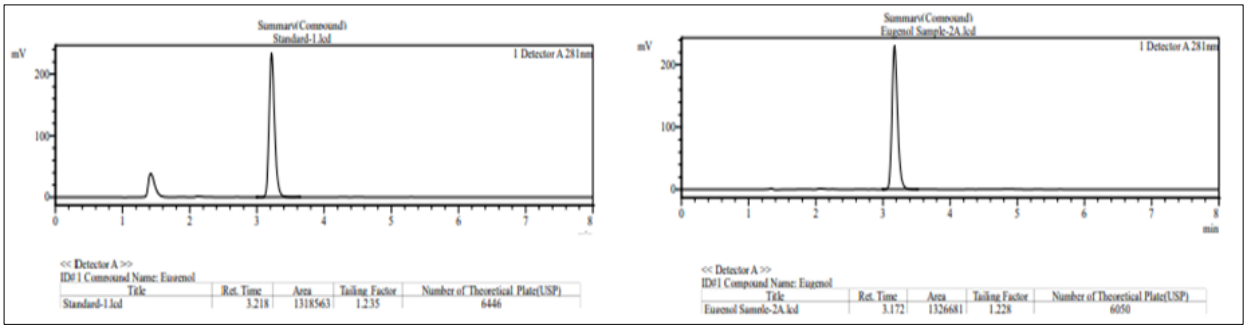
3. Results and Discussion

In-house RP-HPLC separation studies were carried out with a mobile phase composed of methanol and water in an isocratic elution pattern. Several trials were conducted utilizing different isocratic elution patterns, but system suitability requirements were not reached. As a result, testing was conducted at a higher pH (weakly acidic pH). Finally, acetonitrile in a 0.1% orthophosphoric acid buffer solution with an isocratic elution pattern and a flow rate of 1.0 mL/min was found to be optimal. The method has a total runtime of 8 minutes. (Figure 2). Methanol was chosen as the solvent

because of the high solubility of eugenol. Further dilutions were made with the diluent acetonitrile and water in the ratio of 70:30.

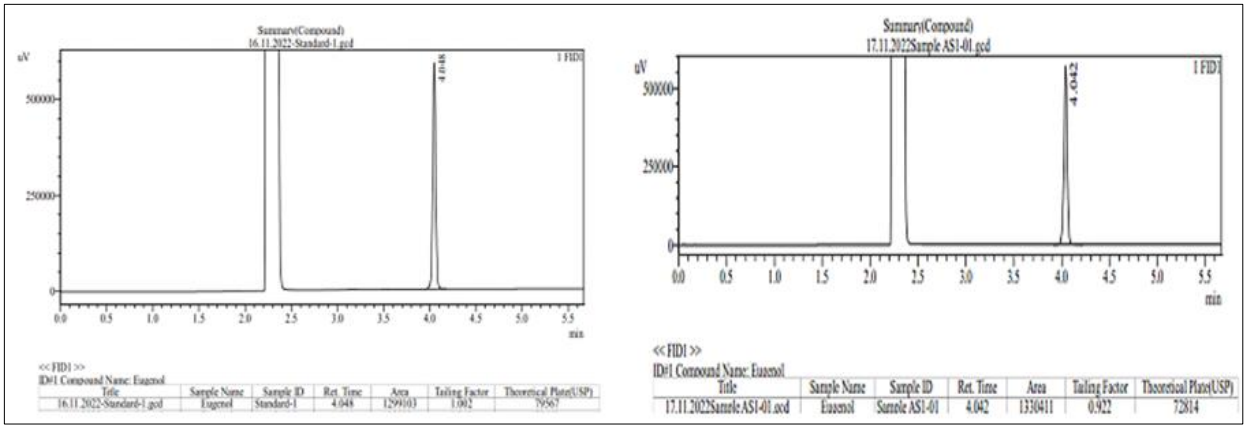
In GC-FID, the column DB-1, 100% dimethylpolysiloxane, Inner Diameter: 0.32 mm, Length: 30 m, Film: 1.00 m, is used for eugenol analysis, which is nonpolar, low-bleed, and operates at high temperatures. It is cross-linked and solvent-rinsible. Which results in a good peak shape and retention time. Nitrogen is used as the carrier gas or mobile phase for the analysis of eugenol because it is inert, safe, and cost-effective. The column oven temperature, which has a significant impact on the performance of chromatographic separation, is a critical component for controlling GC. The analysis of eugenol carried out with an initial temperature, a temperature ramp in degree increases per minute, and a final temperature to achieve good peak shape and retention time of the peak. An auto injector with split mode was chosen for analysis of eugenol due to the higher concentration level (Figure 3).

The UV-visible range of the standard solution of 30µg/ml eugenol was scanned (200-800 nm). The resulting spectra were documented, and the wavelength corresponding to the maximum absorbance of the solution was designated as lambda max. The absorption spectrum at 282 nm was chosen for eugenol analysis (Figure 4).



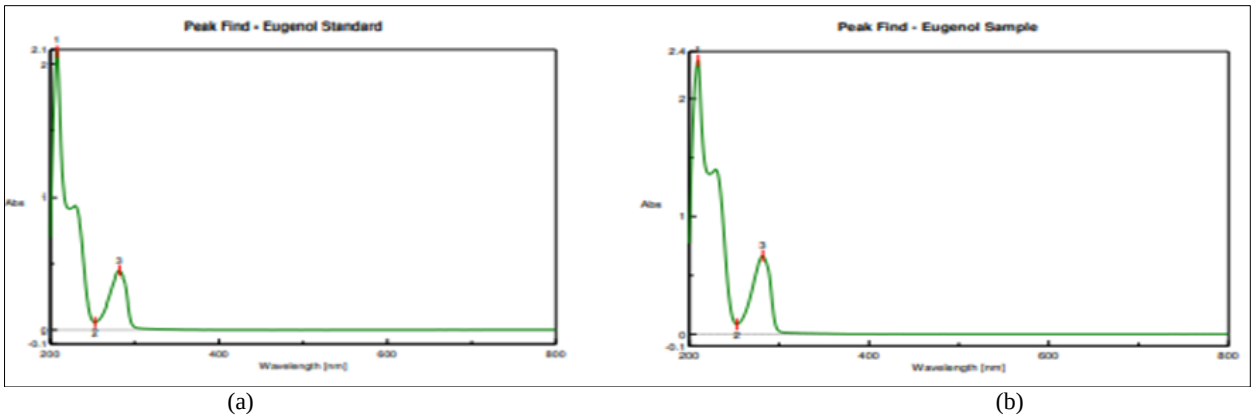
(a) (b)

Fig 2: (a) RP-HPLC chromatogram of eugenol standard. (b) RP-HPLC chromatogram of isolated eugenol



(a) (b)

Fig 3: (a) GC-FID chromatogram of eugenol standard (b) GC-FID chromatogram of isolated eugenol

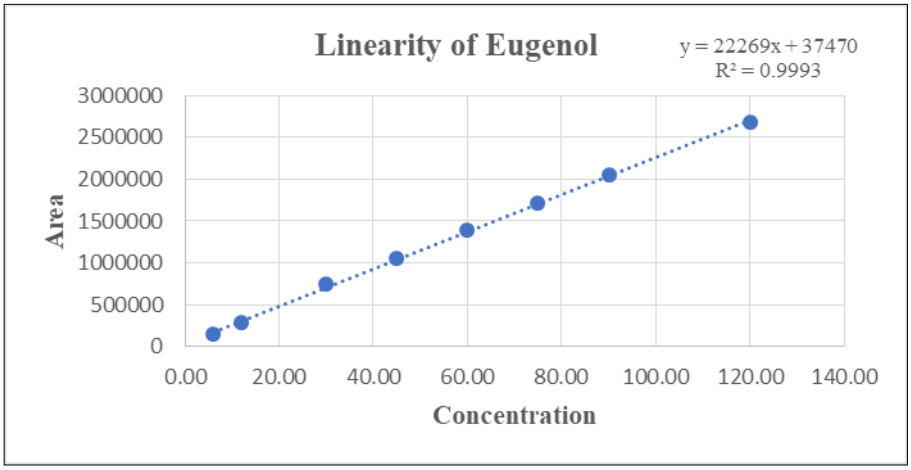


(a) (b)

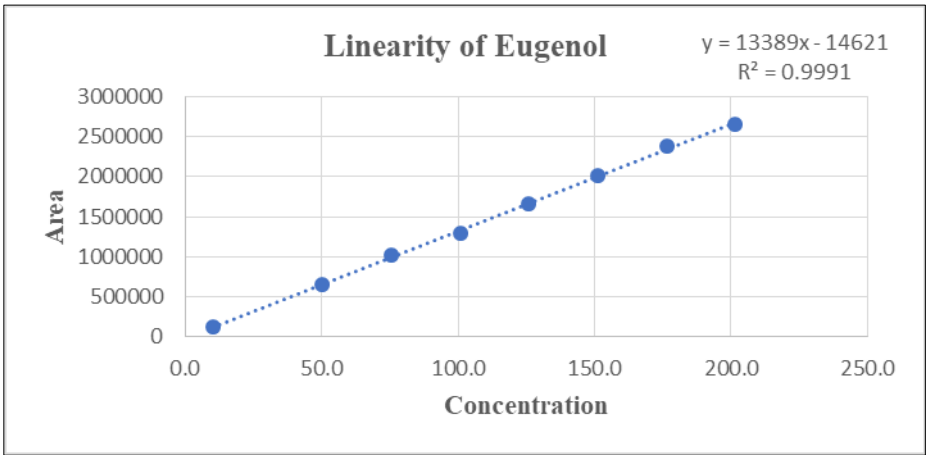
Fig 4: (a) UV spectrum of eugenol standard. (b) UV spectrum of isolated eugenol

Specificity of the established method for analysis of isolated eugenol was confirmed by comparing the chromatograms or spectra obtained from both standard and sample evaluations. Linearity was investigated in replicate by injecting standard solution concentrations ranging from 6.0-125.0 µg/mL into the RP-HPLC. From 10-200 µg/mL to the GC-FID method and 5-75 µg/mL to the UV method. The regression square equation was generated by plotting peak area versus

concentration y = 22269x + 37470 (R^2 = 0.9993) for RP-HPLC, for GC-FID y = 13389x-14621 (R^2 = 0.9991), and for UV method by plotting absorbance versus concentration y = 0.0175x + 0.0058 (R^2 = 0.9999). The standard calibration curve was used to calculate the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were found to be 0.3 µg/mL and 0.91 µg/mL for RP-HPLC, 0.7 µg/mL and 2.1 µg/mL for GC-FID, and 0.9 µg/ml for LOD and 2.72 µg/ml for UV method, respectively, based on the standard calibration curve (Fig 5).



(a)



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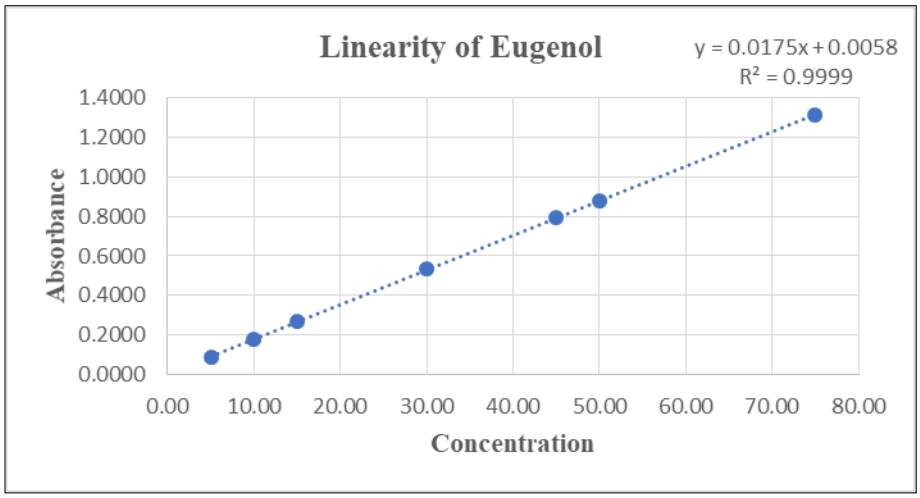

(c)

Fig 5: (a) Eugenol standard calibration curve using RP-HPLC method. (b) Eugenol standard calibration curve using GC-FID method. (c) Eugenol standard calibration curve using UV method

The method precision was determined for the standard and sample solution intraday, the %RSD were found to be 0.569-0.897%, 0.966-1.07%, and 0.078-1.41%, respectively, for the RP-HPLC, GC-FID, and UV methods. The studies were also repeated inter-day using freshly prepared standards and samples, the %RSD were found to be 0.416-0.679%, 1.041-1.403%, and 0.093-0.731%, respectively, for the RP-HPLC, GC-FID, and UV methods.

The method accuracy was determined by injecting standard spiked samples at three levels 80%, 100%, and 120% in

triplicate, then determining percentage recoveries. The recovery was found to be 99.15-101.48%, 100.11-101.49%, and 98.72-99.89%, respectively, for RP-HPLC, GC-FID, and UV methods. To ensure the insensitivity of the three methods to minor changes in experimental conditions, it is important to demonstrate robustness of method by modification in flow rate, detection wavelength, column temperature for RP-HPLC, change in carrier gas flow rate (±10%), detector temperature (±5°C), column ramp temperature (±5%), etc. for GC-FID, and detection wavelength for UV method. None of the methods caused a significant change in resolution.

The established analytical method was applied to quantitatively analyze eugenol in extracted and isolated clove. The content of eugenol is listed in Table 3.

Table 3: Summary of the developed methods along with their validation parameters

SL. No.	Parameters	RP-HPLC	GC-FID	UV	Acceptance criteria
1	Specificity	There is no interference of blank and matrix of the sample at the retention time of eugenol			No interference of blank and other matrix of the sample
2	System suitability % RSD Tailing factor Theoretical plates	0.659% 1.229 6262	0.966% 1.002 79567	0.056% - -	NMT 2.0% NMT 2.0 NLT 1500
3	Linearity and range Regression equation Regression coefficient (R^2)	Y = 22269x + 37470 0.9993	y = 1347.1x-14428 0.9991	y = 0.0175x + 0.0058 0.9999	≥0.999
4	**Sensitivity**				
	LOD LOQ	0.3 µg/ml 0.9 µg/ml	0.7 µg/ml 2.1 µg/ml	0.9 µg/ml 2.7 µg/ml	- -
5	**Intraday precision**				
	System precision Method precision	0.569% 0.897%	0.966% 1.07%	0.078% 1.41%	NMT 2.0%
	Intraday precision				
	System precision Method precision	0.416% 0.679%	1.041% 1.403%	0.093% 0.731%	
6	Accuracy	99.15-101.48%	100.11-101.49%	98.72-99.89%	90-110%
7	Robustness (Avg %)	100.2%	99.3%	99.5%	-
8	Assay (%)	99.9%	99.3%	99.6%	-

4. Conclusion

In this study, we have successfully developed and validated innovative analytical techniques, including RP-HPLC, GC-FID, and UV, for the analysis of isolated eugenol. These methods are simple, accurate, and cost-effective in manner, as they do not require the complex treatments and procedures associated with other methodologies. This provides a distinct advantage over other techniques and confirms their suitability for routine eugenol analysis for control purposes of pharmaceutical and cosmetic products containing eugenol. Good agreement was seen in the assay results of isolated eugenol by the developed methods. It can be concluded that all the proposed methods are good approaches for obtaining reliable results and were found to be suitable for the routine quality control analysis of eugenol-containing herbal plants and formulations.

5. Conflict of interest

The authors declare they have no conflicts of interest.

6. Acknowledgement

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7. References

1. Evans DWC. *Trease and Evans' Pharmacognosy*. 16th Ed. London: Elsevier; c2009, p. 263-303.
2. Al-Sharif I, Remmal A, Aboussekhra A. Eugenol triggers apoptosis in breast cancer cells through E2F1/survivin down-regulation. BMC Cancer. 2013;13:600.
3. Ulanowska M, Olas B. Biological properties and prospects for the application of eugenol-A review. Int J Mol Sci. 2021;22(7):3671.
4. Da Silva FFM, Monte FJQ, de Lemos TLG, do Nascimento PGG, de Medeiros Costa AK, de Paiva LMM. Eugenol derivatives: synthesis, characterization, and evaluation of antibacterial and antioxidant activities. Chem Cent J. 2018;12(34).
5. Alam MM. Synthesis and anticancer activity of novel eugenol derivatives against breast cancer cells. Nat Prod Res. 2023;37(10):1632-1640.
6. Yun SM, Lee MH, Lee KJ, Ku HO, Son SW, Joo YS. Quantitative analysis of eugenol in clove extract by a validated HPLC method. J AOAC Int. 2010;93(6):1806-1810.
7. Mota LB, Da Silva Campelo M, Silva GA, Oliveira CLCG, Gramosa NV, et al. Spectrophotometric method for quantification of eugenol in volatile oil of clove buds and nanoemulsion. *Órgão Oficial Da Sociedade Brasileira de Farmacognosia. 2022;32(6):912*-920.
8. Indalkar YR, Aloorkar NH. Validation of eugenol in ethanol by UV spectrophotometric method. Asian J Pharm Ana. 2015;5(4):178-180.
9. Nilawar N, Khan T. Analytical method development and validation studies for simultaneous estimation of luliconazole and eugenol using UV spectroscopy. IJPSR. 2024;15:2088-2093.
10. Vineeta V, Khanvilkar PP, Bhavin R, Shruti S, Mandle PR. Estimation of eugenol content in marketed tulsi drop preparations by simple UV spectrophotometric method. IJCRT. 2023;11(6):61-62.
11. Yun SM, Lee MH, Lee KJ, Ku HO, Son SW, Joo YS. Quantitative analysis of eugenol in clove extract by a validated HPLC method. J AOAC Int. 2010;93(6):1806-1810.

12. Pramod K, Ilyas UK, Kamal YT, Ahmad S, Ansari SH, Ali J. Development and validation of RP-HPLC-PDA method for the quantification of eugenol in developed nanoemulsion gel and nanoparticles. J Anal Sci Technol. 2013;4(1):16.
13. Saran S, Menon S, Shailajan S, Pokharna P. Validated RP-HPLC method to estimate eugenol from commercial formulations like Caturjata Churna, Lavangadi Vati, Jatiphaladi Churna, Sitopaladi Churna and clove oil. J Pharm Res. 2013;6(1):53-60.
14. Domlur Thyagaraj V, Koshy R, Kachroo M, Mayachari AS, Sawant LP, Balasubramanium M. A validated RP-HPLC-UV/DAD method for simultaneous quantitative determination of rosmarinic acid and eugenol in Ocimum sanctum L. Pharm Methods. 2013;4(1):1-5.
15. Sruthi BYK, Gurupadayya BM, Sairam KV, Kumar T. Development and validation of GC method for the estimation of eugenol in clove extract. Int J Pharm Pharm Sci. 2014;6(2):473-476.
16. Gotmare S, Tambe S. GC-FID method development and method validation of eugenol in clove oil extracted by hydrodistillation. Int J Adv Res. 2021;9(5):501-511.
17. Mamatha M, Chaluvaraju KC, Shantharam U, Ranjeetha M, Darshitha R, Varsha CA. Study on isolation and purification of eugenol from clove by column chromatography. EJPMR. 2018;5(5):383-386.
18. ICH. Validation of analytical procedures: text and methodology Q2(R1). [Internet]. Available from: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf. Published 6 November 1996.