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Quantitative determination of Eugenol in aqueous extract of *Ocimum sanctum* by High Performance Thin Layer Chromatography

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ABSTRACT

Background: The present study describes development and validation of new, rapid, accurate and precise, high performance thin layer chromatographic (HPTLC) method for quantitative determination of eugenol in herbal extract of *Ocimum sanctum* with densito-metric detection. **Methods**: High-performance thin layer chromatography (HPTLC) studies were carried out using CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3 and winCATS 3 software were used. HPTLC on aluminium-backed silica gel 60 F_{254} plates with toluene-ethyl acetate-formic acid, 90: 10: 01 (v/v) as mobile phase was followed by densito-metric measurement at 280 nm. **Results and Discussion**: This system was found to give compact bands for eugenol (R_F 0.59). The accuracy of the method was checked by conducting recovery studies for two different levels of eugenol; the average recovery was found to be 98.39%. The average eugenol content, as estimated by use of the proposed method, was 46.6 ng μL^{-1}. **Conclusion:** The method was found to be simple, sensitive, selective and accurate. This study reports a routine quantitative method for the analysis of eugenol, in the leaf extract of *Ocimum sanctum*.

KEYWORDS: Eugenol, *Ocimum sanctum*, HPTLC, Densitometric, Mobile phase

1. INTRODUCTION

Ocimum sanctum is a member of the Lamiaceae family, commonly known as 'Tulsi' in Hindi and 'Holy Basil' in English and is used as food seasoning. Tulsi is a shrub reaching a height of 0.5 to 1.5 m. The leaves are 2-4 cm in length[1], covering the entire Indian sub continent, ascending up to 1800 m in the Himalaya and as far as the Andaman and Nicobar islands[2]. Tulsi has been well documented for its therapeutic potentials in Ayurveda described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna)[3]. Species of the tulsi are also valuable due to their pharmaceutical properties; for example, antipyretic, anti-inflammatory[4], cardioprotective, central nervous system (CNS) depressant[5], chemopreventive, antiulcer and anticancer[6]. Many scientific studies showed that sweet basil extract is a strong radical scavenger and can be considered as a good source of natural antioxidants[7].

Leaves of Tulsi contain a bright yellow volatile oil, which is useful against insects and bacteria. The principal constituents of this essential oil are eugenol, methyl eugenol, carvacrol, caryophyllene, and they also yield other substances such as ursolic acid and apigenin[8]. Eugenol (4-allyl 2-methoxyphenol) a naturally occurring phenolic compound is a major component of essential oil[9] extracted from different parts of Tulsi plant. In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of eugenol for therapeutic potentials of Tulsi[10].

Recently many works has been directed to investigate the estimation of eugenol including non-aqueous titration[11], thin layer chromatography[12], high-performance liquid chromatography[13] and gas chromatography[14]. Most of those techniques were expensive and have not been extensively used on a large scale. HPTLC has emerged as an efficient tool for phytochemical evaluation of herbal drugs, because of its simplicity, specificity, low cost and the minimum sample cleanup requirement[15]. HPTLC technique provides both a separation efficiency that is considerably better than that in conventional TLC and reproducible gradient on the thin layer in a cost effective way. The present method was an attempt to develop a fully validated HPTLC analysis for quantification of eugenol in herbal formulations and can be used for routine quality control and standardization of *Ocimum sanctum*.

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MATERIAL AND METHODS

1.1. Reagents and Standard

Analytical grade methanol and chlorophorm were obtained from

Qualigens Fine Chemicals, Mumbai, India. Eugenol standard was procured from Sigma–Aldrich Chemie (Steinheim, Germany).

1.2. Plant material

Fresh leaves of *Ocimum sanctum* were collected around Bhopal and Khandwa (India) in the month of January-February. The plant materials were identified and authenticated by Dr. S.S. Khan of Botany Department, Saifia College, Bhopal. A voucher specimen (286/Bot/Saifia/11) is deposited at herbarium of Department of Botany, Saifia College Bhopal. The collected leaves were shade dried under normal environmental condition, powdered, stored in a closed container for further use.

1.3. Extraction of eugenol

The extraction of eugenol from leaf extracts of Tulsi was carried out as per the method of Thakur and Pitre[16] with some modification. Briefly apparatus was assembled for steam distillation using a 250 ml round bottom flask. 120 g of ground dried basil leaves were taken and water was added to approximately three-fourths full. The flask was heated using a heating mantle in order to boil the water. The volatile oil along with the water vapour condensed in the condenser and accumulated in a graduated side arm of the Clavenger apparatus. Distillation was continued until there was no difference in successive readings of the oil volume. The oil was then transferred to a separating funnel with some drops of water, extracted with chlorophorm. The solvent was removed on a steam bath. Pure eugenol was obtained as pale yellow oil.

1.4. HPTLC profile of eugenol

1.4.1. Preparation of eugenol Standard Solution

A stock solution of eugenol (1 mg mL^{-1}) was prepared by dissolving 50 mg accurately weighed eugenol in methanol and diluting to 50 mL with methanol. Aliquots (2.0 mL to 12.0 mL) of this stock solution were transferred to 10 mL volumetric flasks and the volume of each was adjusted to 10 mL with methanol, to obtain working standard solutions containing 200 to 1200 µg mL^{-1}.

1.4.2. Calibration Plot for eugenol

Chromatography was performed on 20 cm × 10 cm TLC plates precoated with 0.2 mm layers of silica gel 60 F254 (Merck); before use the plates were prewashed with methanol and activated at 100ºC for 10 min. Working standard eugenol solutions (10 µL) of different concentration were applied to the plates, as 7 mm bands, by means of a Camag Linomat V automatic sample applicator filled with a 100 µL syringe (Hamilton, Bonaduz, Switzerland).

A constant spot application rate of 150 nL^{-1} was used. Plates were developed, at 25 ± 2°C, with toluene-ethyl acetate-formic acid, 90 : 10 : 1 (v/v) as mobile phase in a Camag (Muttenz, Switzerland) glass twin-trough chamber; the development distance was 8.0 cm. The plates were then dried in air and scanned at wavelength (λ_{max}) 280 nm by means of a Camag TLC Scanner 3 in reflectance-absorbance mode, under control of Camag winCATS 3 planer chromatography manager software versions 1.4.4. The slit dimention were 6 ×0.30 mm and the scanning speed was 100nmS^{-1}. Peak areas were recorded for eugenol and a calibration plot was obtained by plotting peak area against eugenol concentration.

1.4.3. Estimation of eugenol in Samples of Ocimum sanctum

The sample solution (10 µL) was applied, in triplicate, to the precoated silica gel 60 F254 plates, again with the Camag Linomat IV. Each plate was developed and scanned as described above. The peak areas and absorption spectra were recorded. The amount of eugenol in the *Ocimum sanctum* sample was calculated for each solution by use of the calibration plot.

2. RESULT AND DISCUSSION

Nowadays, the interest in the study of natural products and their formulation is growing rapidly, especially as a part of drug discovery programs but they lack in standardization, and hence there is a need to develop suitable analytical methods for such formulations. In case of natural product analysis, HPTLC is more widely used than other chromatographic methods[16, 17].

Among various mobile phases like Meoh: Chloroform[18], Meoh: Water[19], N-hexane: ethyl acetate[20] etc. used for characterization of eugenol from leaf extract of *Ocimum sanctum*, the toluene–ethyl acetate–formic acid (90: 10: 01) is found to be more efficient. The retention factor (Rf) of eugenol in the methanolic extract was recorded as 0.58 (Table 1). The identity of eugenol band in the sample extract was confirmed by overlaying the UV absorption spectrum of the sample with that from the reference standard, obtained by use of the Camag TLC Scanner (Fig. 1A). The results showing the chromatograph of HPTLC analysis and Rf value of the active ingredient eugenol in plant extract having the same values as that of the standard eugenol (Fig. 1B).

The reproducibility of the method was determined by different analysts using the samples from the same homogenous batch and repeatability was determined. The response to eugenol was found to be linearly dependent on concentration in the range 200 to 1200 µg mL^{-1}, with a correlation coefficient of 0.98553±2.03 (Fig. 2). The linearity of calibration graph and adherence of the system to Beer's law was validated by high value of correlation co-efficient.

Table 1: Showing the results of the HPTLC of *Ocimum sanctum*

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
Sd	1							Not used
Sd	1							Not used
Sd	1							Not used
Sd	1	0.58	5.000 µg	385.27		16074.27		
Sd	1	0.58	6.000 µg	417.73		17927.45		
Sd	1	0.58	7.000 µg	446.29		19617.88		
Sd	1	0.58	8.000 µg	468.03		21027.87		
Sd	1	0.58	9.000 µg	486.20		22171.5		
Sd	1	0.58	10.000 µg	505.25		23591.45		
Sd	1	0.59	12.000 µg	531.63		25571.05		
Smp	2	0.58		375.81	4.500 µg	16567.36	4.969 µg	BT-HPTLC-763
Smp	2	0.58		439.01	6.990 µg	20337.98	7.761 µg	BT-HPTLC-763
Smp	2	0.59		479.48	8.942 µg	23083.16	9.793 µg	BT-HPTLC-763
Smp	2	0.59		506.07	10.22 µg	25122.39	11.30 µg	BT-HPTLC-763
Smp	2	0.59		523.71	11.07 µg	26757.32	12.51 µg	BT-HPTLC-763
Smp	2	0.59		533.64	>11.55 µg	27744.48	>13.20 µg	BT-HPTLC-763
Smp	2	0.59		531.82	11.47 µg	27635.83	13.16 µg	BT-HPTLC-763

**** Sd = standard***

Smp= our samples

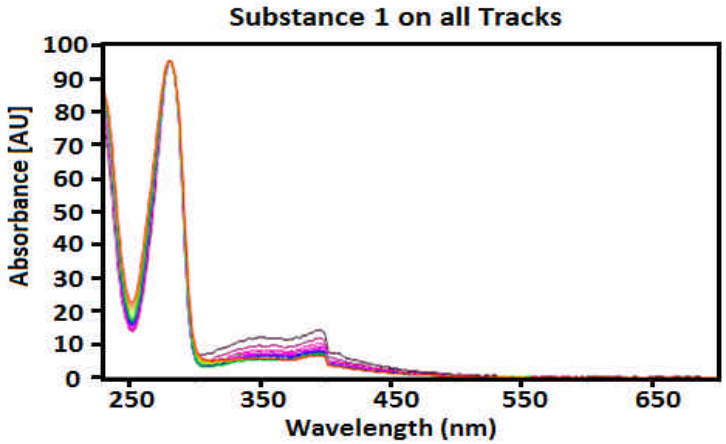

Figure 1A: Absorption spectra of standard eugenol and leaf extract samples at Peak start, peak maximum and Peak end in Absorption mode in the UV range, taken on the CAMAG TLC Scanner 3.

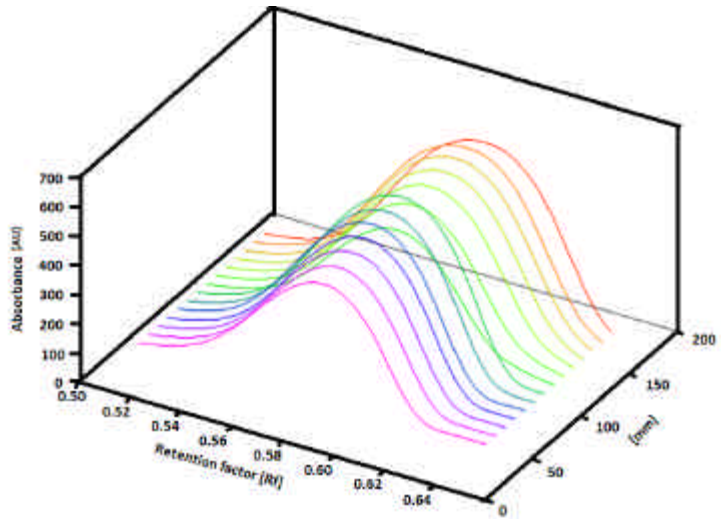

Figure 1B: Tracks (Standard and plant extract) having maximum absorption at 280 nm wavelength indicating the same Rf value.

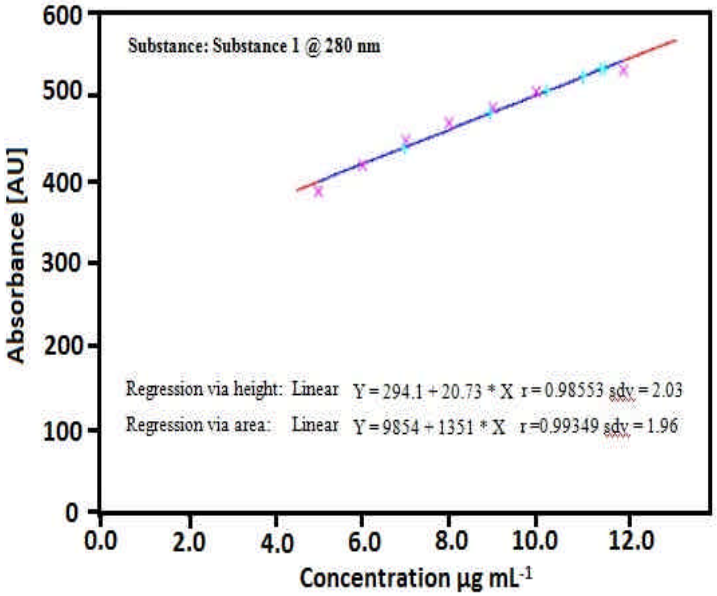

Figure 2: Calibration curve for Eugenol

The limit of detection and quantification was found to be 33.0 ng μL^{-1} and 46.6 ng μL^{-1}. The intraday and interday precision studies, expressed as coefficient of variation (% CV), were found to be 3.0418 and 3.4674, respectively indicating good precision. The accuracy of the method was determined from recovery studies. The average recovery of eugenol at two different levels was found to be 98.39%.This method was found superior in linearity, recovery, and sensitivity compared to a validated HPTLC method reported earlier[20] for quantitative estimation of eugenol in the herbal extraction of *Ocimum sanctum.*

CONCLUSION

The developed HPTLC method is simple, specific and accurate, economic and can be utilized for the routine analysis and quantification

determination of eugenol from *Ocimum sanctum* plant samples and other herbal preparation. Statistical analysis proves that the method is reproducible and selective.

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REFERENCES

1. Hall MG (2001). Pharmacokinetics and pharmacodynamics of NTBC (2-(2-nitro-4-fluromethylbenzoyl)-1,3-cyclohexanedione) and mesotrione, inhibitors of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) following a single dose to healthy male volunteers. *British Journal of Clinical Pharmacology* 52 (2), 169.
2. Farooqi AA and Sreeramu BS (2003). Cultivation of medicinal and aromatic crops. Universities press (India) private limited ISBN 81 7371 5041, 531.
3. Sirkar NN (1989). Pharmacological basis of Ayurvedic therapeutics. In: Cultivation and utilization of medicinal plants. Editors: Atal CK and Kapoor BM (Published by PID CSIR).
4. Singh S and Majumdar DK (1997). Evaluation of anti-inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J. Exp. Biol.* 35: 380-383.
5. Devi UP, Gonasoundari A, Vrinda B, Srinivasan KK, Unnikrishanan MK (2000). Radiation protection by the *Ocimum sanctum* flavonoids orientin and vicenin: Mechanism of action. *Radiat. Res.* 154: 455- 460.
6. Madhuri S and Pandey G (2010). Effect of ProImmu, a herbal drug on estrogen caused uterine and ovarian cytotoxicity. *Biomed.* 5: 57-62.
7. Abas F, Lajis NH, Israf DA, Khozirah S, Kalsom YU (2006). Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chemistry*, 95(4): 566–573.
8. Hsieh WC (2007). Map of used Chinese medicine commonly. Committee on Chinese Medicine and Pharmacy, Taipei Publishers, Taiwan, pp.108-109.
9. Skalicka WK, Ludwiczuk A, Widelski J, Filipe JJ, Asakawa Y, Glowniak K (2009). Volatile constituents of *O. minimum* herb cultivated in Portugal. *Nat. Prod. Commun.* 4: 1383-1386.
10. Yuwono M, Siswandono, Hajid AF, Poernomo AT, Agil M, Indrayanto G, Ebel S (2002). Analytical Profiles and Drug Substances and Excipients, Academic Press, Elsevier, USA, 149–177.
11. Covello M, Ciampa G, Rotonda MILa. (1966). Determination of Eugenol in the essence of "*Eugenia caryophyllata*." Titration in non-aqueous solvent and comparison with other methods of analysis. *Boll. Chim. Farm.*, 105 (11):799–806.
12. TLC Densitometric method for the Quantification of Eugenol and Gallic acid in clove (2004). *Chromatographia* 60: 241-244.
13. Fischer IU and Dengler HJ (1990). Sensitive high-performance liquid chromatographic assay for the determination of eugenol in body fluids. *J. Chromatogr.* 525 369–377.
14. Myint S, Daud WR, Mohamad AB, Kadhum AA (1996). Gas chromatographic determination of eugenol in ethanol extract of cloves. *J. Chromato B: Biomed Sci Applic*. 679:193–195.
15. Thennarasan S, Murugesan S, Subha TS. HPTLC finger printing profile of brown alga *Lobophora variegate* (J.V. Lamouroux) (2014). *Journal of Chemical and Pharmaceutical Research*, 6(1):674-677.
16. Thakur K and Pitre KS (2009). Anti-Inflammatory activity of extracted eugenol from *Ocimum sanctum* L. Leaves. *RJCABP* Vol 2 (2): 472-474.
17. Suganthi A, Sai Lakshmi CH, Sruthi V, Ravi TK (2014). Development and Validation of UV Spectroscopic and HPTLC Methods for the determination of Bosentan from Tablet Dosage Form *Sch. Acad. J. Pharm.*, 3(2): 123-127.
18. Bhatt MK, Shankar MB, Saluja AK, Dholwani KK, Captain AD (2012). Evaluation of anti-microbial activity of *Ocimum sanctum* methanolic extract. *Journal of Pharmaceutical and Scientific Innovation* 1: 39-41.
19. Sharma PA, Singh GP (2013). Determination of Eugenol in Human Plasma by High Performance Liquid Chromatography. *International Journal of Applied Research & Studies* 2: 3
20. Alam P, Gupta J, Firdouse S, Firdouse A, Afshan J (2012). HPTLC method for qualitative and quantitative estimation of eugenol from *ocimum sanctum* linn in polyherbal formulation. *International Journal of Comprehensive Pharmacy* 3: 10 (07).

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