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Quantitative Estimation of Eugenol in Different Formulations of *Ocimum Sanctum* by HPTLC Method

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***Abstract - Aim*: The purpose of the study was to quantify eugenol in different formulations of *Ocimum sanctum* via HPTLC.**
***Materials and Methods*: Formulations were Tulsi Ghanvati tablets, Dabur Honitus Hotsip, Himalaya Tulsi tablets and Divya Coronil tablets; Chemicals were eugenol, methanol, toluene, ethyl acetate and formic acid. Extracts were prepared by Maceration and Reflux Condensation. Quantitative analysis was done by HPTLC.**
***Results*: Quantitative determination of eugenol has been done successfully. The minimum and maximum yield calculated was 0.04 and 1.42 respectively. For the most beneficial and efficient usage of tulsi, it can be employed for the standardisation and quantification of eugenol in various herbal compositions.**

Keywords:- *Eugenol, HPTLC, Tulsi, Marketed formulations, Extraction, Marker, Standar.*

I. INTRODUCTION

Ocimum sanctum is one of the few "wonder" herbs because of its powerful healing power. This has led to the plant being referred to as sacred and worthy of worship, known as Tulsi or holy basil in India. Different parts of the plant have reportedly demonstrated a variety of therapeutic effects, including analgesic, antioxidant, antibacterial, and anthelmentic action. Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and - caryophyllene are the principal chemical constituents of Tulsi.[12] *Ocimum sanctum* Linn (Tulsi) contains eugenol, a phenolic component (l-hydroxy-2-methoxy4- allylbenzene), which is one of the most significant bioactive compounds range from 40 and 70%, which varies from plant to plant. The well acknowledged functional qualities of eugenol include its capacity to scavenge free radicals and its ability to inhibit the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This substance can be utilised as a marker compound to assess and standardise Tulsi-containing extracts and formulations. A literature review finds that there aren't many HPTLC methods that have been validated for estimating eugenol in extracts and formulations.

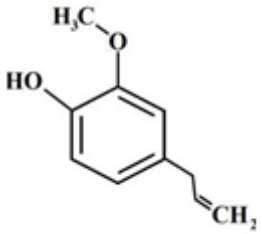

Fig. 1: Molecular Structure of Eugenol [2]

II. MATERIALS AND METHODS

A. Materials

Himalaya Tulsi Tablets, Divya Coronil Tablets, Tulsi Ghanvati, Dabur Honitus Hotsip were collected from local markets of Rohtak.

B. Chemicals and Reagents

Standard eugenol was procured from Yucca Chemicals. Methanol, toluene, ethyl acetate, glacial acetic acid was procured from Loba Chemicals. HPTLC Silica gel $60F_{254}$ prepared plates were procured from Sigma.

C. *Instrumentation*

The Linomat V Sample Spotter is a device for spotting samples in the HPTLC system (CAMAG), along with a 100 ul Hamilton Syringe. TLC Scanner 3 connected to WinCATS software for densitometric analysis and TLC chamber for the creation of TLC plates.

D. *Preparation of Standard Solution of Eugenol*

Eugenol stock solution (2 mg/ml) was created by carefully weighing 20 mg of the marker component and dissolving it in 10 ml of methanol in a volumetric flask of 10 ml.

E. Preparation of Sample Solution

By precisely weighing 50 mg of extract and dissolving it in 10 ml of methanol using a volumetric flask, a 5 mg/ml solution of each extract was created using methanol as the solvent.

F. Conditions for HPTLC

Without prewashing, the experiment was conducted using silica gel 60F HPTLC plates (20 x 10 cm) with a 0.2 mm thickness. Samples were applied to the plates in bands of 6mm width spaced 8mm apart using a LINOMAT-5 applicator. The plates were developed using the ascending

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method in a glass chamber with a stainless-steel top using a mobile phase made up of toluene, ethyl acetate, and formic acid (7:3:0.2). The 20-minute chamber saturation period was maintained. Plates were developed, dried on a TLC hot plate, viewed in a UV cabinet, and then scanned using a CAMAG TLC scanner with Win CATS software (version 3.5) at 254 and 366 nm wavelengths. Deuterium and Tungsten lamp for 254 nm and a Mercury lamp for 366 nm were used for the spectrum investigation. Eugenol's R_f value was determined to be 0.719.

G. Methodology

Extraction of the formulations was done by two methods: Maceration; dissolve 20 g of powdered medication formulation in 100 cc of methanol. Place it in beakers with foil paper covering and leave for three days. After three days, the mixture was filtered, maintained in beakers covered in foil with holes drilled into it to drain the solvent, and kept for a further three to four days. The solvent evaporates after 4 days, leaving a dried extract in the beaker. Scrape the extract into a china dish, collect it, and keep it in a desiccator with foil covering it so that it does not absorb moisture. Calculate the percentage yield. Reflux Condensation; in a round bottom flask, weigh 20g of the drug's powdered form and dissolve it in 200 ml of methanol. Install a reflux condenser assembly. The condensation process lasted for 8 hours at a temperature of 40 to 45 degrees. Filter the mixture after condensation, collect the filtrate in a beaker, cover it with foil paper, poke holes in it to let the solvent escape, and keep it there for 3–4 days. Place the leftover mixture in a hot air oven for 2 hours, keeping the temperature at 30–40 degrees. Weigh the dried extract and calculate the percentage yield. After extraction, in 10 ml of methanol, 50 mg of dry extract was weighed, dissolved, and stored in vials. All of the solutions were refiltered and an HPTLC was performed. Calculate the peak regions, Rf values and determine their concentration and % yield with help of equation y = mx+c.

III. RESULTS AND DISCUSSION

TABLE I: Percentage Yield of Extract

Sr. No.	Sample	Percentage Yield of Extract
1.	Ghanvati M	4.05%
2.	Honitus M	3.95%
3.	Himalaya M	11.35%
4.	Coronil M	2%
5.	Ghanvati R	8.05%
6.	Honitus R	4.95%
7.	Himalaya R	6.5%
8.	Coronil R	1.67%

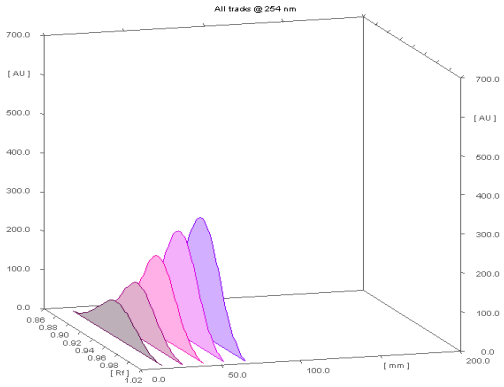

Fig. 2: 3D Graph of Standard Eugenol

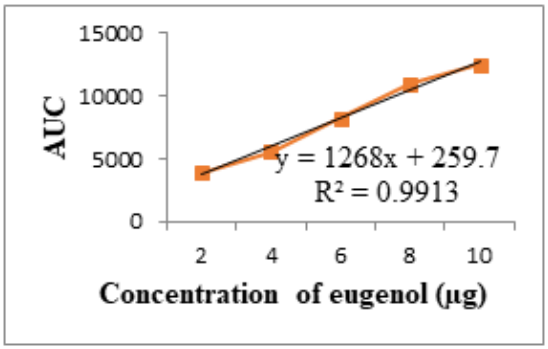

Fig. 3: Calibration Curve of Standard Eugenol

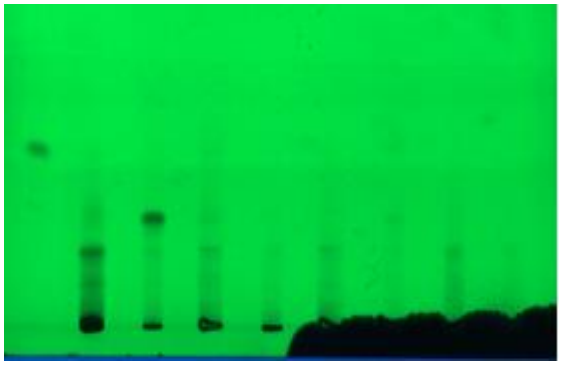

Fig. 4: HPTLC Plate developed at 254 nm

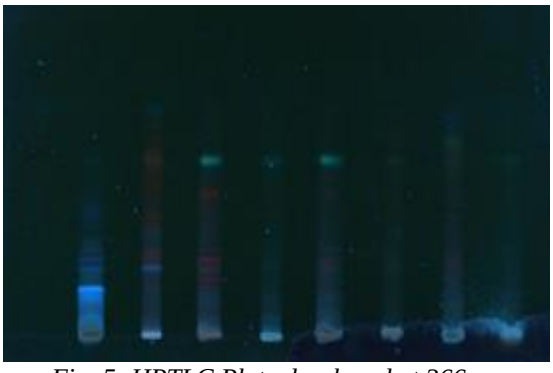

Fig. 5: HPTLC Plate developed at 366 nm

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To ascertain the amount of eugenol in several commercial oral formulations that were gathered from various pharmacy stores in Rohtak, a quantitative estimation using the HPTLC method was carried out. To determine the percentage yield of Himalaya Tulsi tablets, Tulsi Ghanvati, Dabur Honitus hotsip, and Divya Coronil tablets, the extraction procedures (maceration and reflux condensation) were applied. The commercial preparations contain Eugenol, a key ingredient in Tulsi. Following research and a review of the literature, it was determined that Toluene: Ethyl Acetate: Glacial Acetic Acid (7:3:0.2 v/v) can be successfully used as the mobile phase. The method was also found to be quick, easy, accurate, and dependable, with a concentration range of 0.02 to 1.42 ug/g Eugenol.

TABLE II. Percentage Yield of Eugenol(Concentration, R_f value and AUC)

Track no.	Sample ID	R_f Value	AUC	Concentration	Percent Yield (w/w)
1.	Standard eugenol	0.72	1609	0.08	0.16
2.	Ghanvati M	0.70	618.7	0.28	0.56
3.	Honitus M	0.74	338.2	0.06	0.12
4.	Himalaya M	0.70	715.2	0.35	0.70
5.	Coronil M	0.72	298.0	0.03	0.06
6.	Ghanvati R	0.62	821.6	0.44	0.88
7.	Honitus R	0.73	1160.5	0.71	1.42
8.	Himalaya R	0.62	287.6	0.02	0.04
9.	Coronil R	0.62	684.8	0.33	0.66

IV. CONCLUSION

The research work enables the analysis of numerous samples at once as well as the separation of complicated mixtures. In order to increase productivity and efficiency, HPTLC also offers automation options like automatic sample application and multiple development chambers. Eugenol was found in a discernible amount and the peak concentrations at 254 nm were detected after the HPTLC process was applied to the extracts. It is a straightforward, rapid, and reliable approach that can be applied to routine analyses of eugenol.

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