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HPTLC QUANTIFICATION OF THYMOL IN DIFFERENT EXTRACTS AND VOLATILE OIL OF *SAFOOF-E-MUHAZZIL*

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Safoof-e-Muhazzil, Thymol, HPTLC, Obesity, Hypolipidemia

ABSTRACT

Safoof-e-Mohazzil (*SEM*), is a polyherbal formulation used by Unani physicians for treatment of obesity. The formulation consists of *Trachyspermum ammi* L., *Apium graveolens* L., *Nardostachys jatamansi* DC, *Rosa* × *damascena* Mill. *Origanum vulgare* L., and lakh maghsool. Thymol is one of the major active constituents of ajwain (*Trachyspermum ammi*). HPTLC quantification of thymol was done in volatile oil and different extracts of *SEM* for the quality standard purpose. Calibration curve of standard thymol (1mg/ml, 100-2000 ng/spot) was made. The samples were applied (2 - 4 µl) on HPTLC plate and developed using toluene: ethyl acetate, (93:7) as mobile phase. The plate was dried and sprayed with anasaldehyde-sulphuric acid reagent and heated at 105 ºC for 5 min. The plate was scanned at 513 nm in CAMAG HPTLC scanner. A spot of orange colour of R_f value 0.56 was observed in chromatogram of the different extracts of *SEM*. The amount of thymol in different extracts and volatile oil of *SEM* was calculated by using the regression equation (height, 45.37 + 0.3001* x, R^2 = 0.99788, Sdv = 5.14 %; area, 28.54 + 12.55* x, R^2 = 0.99716, Sdv = 6.92 %). The amount of thymol was found to be 4.428 ± 0.21 %, 1.267 ± 0.11 %, 0.303 ± 0.07 %, 0.243 ± 0.04 %, 1.883 ± 0.12 % and 0.889 ± 0.17 %, respectively in volatile oil, hexane, chloroform, acetone, methanol and hydro-alcoholic extracts of *SEM* on dry weight basis (w/w).

INTRODUCTION

The prevalence of obesity is increasing worldwide[1] resulting in an association with major health problems such as type 2 diabetes, ischemic heart diseases (includes angina, myocardial infarction, chronic post-ischemic cardiac failure, and sudden ischemic death), stroke, and cancer. It is necessary to treat obese individuals by both lifestyle interventions and/or pharmacological therapy[2].

Complementary and alternative therapies have long been used in the Eastern world but recently these therapies are being used increasingly worldwide[3]. Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. These plant-based traditional medicine systems continue to play an essential

role in health care[4]. Herbal drugs are gaining popularity again day by day in the World since last decades because of its efficacy and lower toxicity as compared to allopathic drugs.

Safoof-e-Muhazzil or *Sufoof-e-Mohazzil* (*SEM*) is a polyherbal formulation used in the Unani System of Medicine for treatment of obesity since ages. The formulation given in National Formulary of Unani Medicine[5] contains ajwain (Nankhwah: *Trachyspermum ammi* Linn., seeds), ajmoth (Tukhm-e-Karafs: *Apium graveolens* Linn., seeds), jatamansi (Sumbul-ut-Teeb: *Nardostachys jatamansi* DC, rhizomes), red rose (Gul-e-Surkh: *Rosa* × *damascena* Mill., petals), oregano (Marzanjosh: *Origanum vulgare* Linn., whole plant) and lakh maghsool (Natural resin-animal origin *Laccifer lacca* Kerr.).[5,6]

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with carvacrol. It has been reported the many plants which contains thymol as major component likes *Trachyspermum ammi* (Ajwain), *Monarda didyma, Monarda fistulosa, Origanum dictamnus Origanum compactum Origanum dictamnus Origanum ssonites, Origanum vulgare, Thymus glandulosus, Thymus hyemalis, Thymus vulgaris, Thymus zygis, Satureja hortensis*. [7-11]

HPTLC is used for determining the quality and possible adulteration in herbal raw material and herbal products. HPTLC is a type of planar chromatography and presents several advantages as compared to HPLC and other analytical techniques. The advantages of the HPTLC method over other analytical methods are reliability, rapidity and accurate drug analysis, which facilitate accurate sample application and in situ scanning. It also allows simultaneous estimation of several samples utilizing only a small quantity of a mobile phase, hence minimizing the analysis time and the cost of the method[12,13]. HPTLC fingerprints are used by regulatory authorities to compare various samples with standards/reference standard. HPTLC has very powerful separation ability, even for complex chemical components of herbals and products[14]. In this research article thymol is used for quantification of volatile oil of *Safoof-e- Muhazzil* formulation as it is reported to prevent high fat diet induced obesity in murine model[15,16] and also one of the major constituents of ajwain oil[17-19].

MATERIALS AND METHODS

Plant material: All the plant materials were purchased from Shamsi Dawakhana, Ballimaran, Delhi and authenticated by Dr. H. B. Singh, Scientist F and Head, RHMD, NISCAIR, New Delhi, India and a voucher specimen (No. NISCAIR/RHMAD/Consult/-2010-11/1705/05) was deposited in the RHM Division, NISCAIR, New Delhi-110012.

Chemicals: Thymol (purity ≥ 98.6 %), was purchased from Sigma Aldrich, India. Silica gel F_{254} HPTLC plates were purchased from Merck, Mumbai, India. Other analytical grade solvents and reagents were obtained from S.D. Fine Chemicals, Mumbai, India.

Preparation of test samples: About 10 g of the herbal formulation was weighed and extracted in ultrasonicator (Toshniwal, India) separately in different solvent using hexane, chloroform, acetone, methanol and methanol with 10 % water. The extracts were filtered and concentrated under reduced pressure using rotary evaporator (Hanshin, Korea). Samples for HPTLC were prepared (10 mg/ml) from the dried extracts and filtered through 0.45 µm membrane filter and stored at 4 ºC before further analysis.

Preparation of standard solution: Accurately weighed 10 mg of thymol standard and transferred to 10 ml volumetric flask. Methanol was added and sonicated in ultrasonic water bath. Volume was made up with methanol to 10 ml to give a concentration of 1 mg/ml. This solution was used as stock solution for thymol.

Chromatographic conditions: HPTLC was performed on 20 cm × 20 cm aluminium backed plates coated with 0.2-mm layers of silica gel 60 F_{254} (Merck, Mumbai, India). Standard solutions of thymol as well as the sample solutions were applied to the plates as bands; 5.0 mm wide, 10.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by using a CAMAG (Muttenz, Switzerland) Linomat IV sample applicator equipped with a 100-µl Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28 ± 2°C), with toluene : ethyl acetate (9:1, % *v/v*) as mobile phase, in a CAMAG glass twin-trough chamber previously saturated with mobile phase vapour for 30 min.

Table 1: Summary of quantification of thymol

Mobile phase	**Toluene : Ethyl acetate (9:1)**	
Standard Samples	Thymol (1 mg/ ml) Different extracts (10 mg/ml)	100-2000 ng/spot 2-4 µ g/spot
Visualization	Sprayed with Anasaldehyde-sulphuric acid reagent, heated 105 ºC for 5 minutes.	
Scanning	513 nm	
R_f	0.56 (Thymol)	
Height Area	45.37 + 0.3001* x, R^2=0.99788, Sdv=5.14 % 28.54 + 12.55* x, R^2= 0.99716, Sdv = 6.92 %	
Samples	Amount of Thymol (%)	
SEM oil SEM hexane SEM Chloroform SEM Acetone SEM Methanol SEM Hyd-Alc	4.428 ± 0.21 % 1.267 ± 0.11 % 0.303 ± 0.003 % 0.2428 ± 0.04 % 1.8833 ± 0.02 % 0.8895 ± 0.17 %	

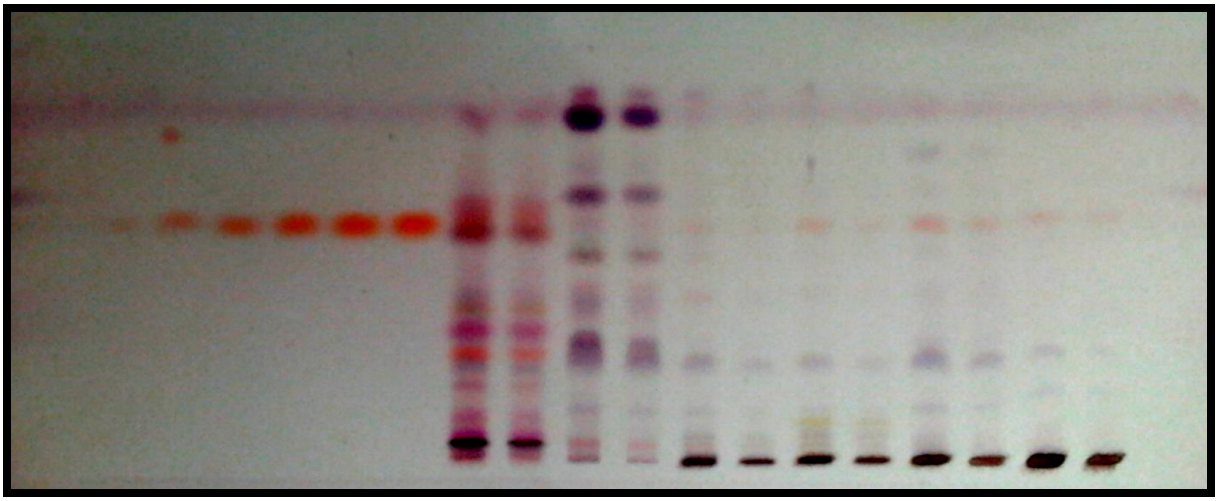


Plate 1: HPTLC quantification of Thymol in volatile oil and different extracts

B. Blank; **1-6.** Thymol; **7.** Volatile oil; **8.** Hexane extract; **9.** Chloroform extract; **10.** Acetone extract; **11.** Methanol extract; **12.** Hydro-alcoholic extract.

UV spectra measured for the band showed maximum absorbance at about 513 nm, and therefore it was chosen as the wavelength for UV densitometry. UV-overlay spectra of thymol in standard and test samples were shown in Fig. 1.

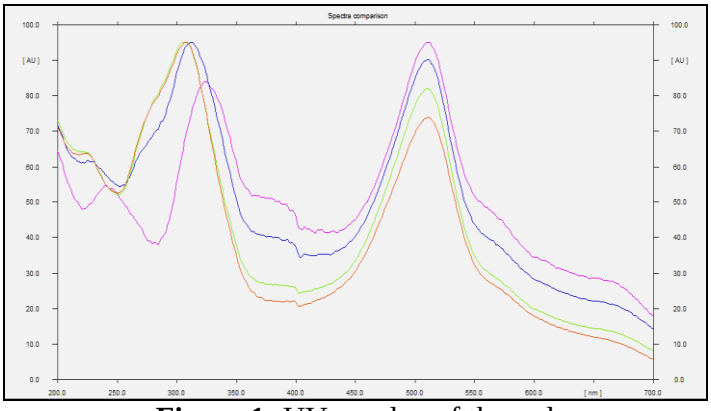


Figure 1: UV overlay of thymol

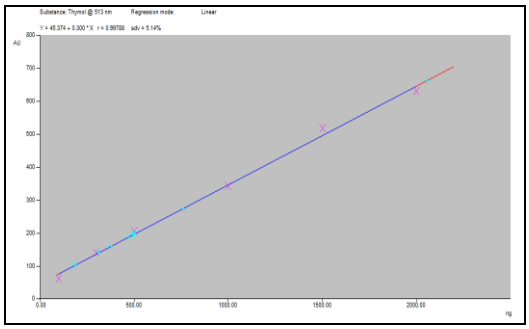


Figure 2. Calibration curve (Height)

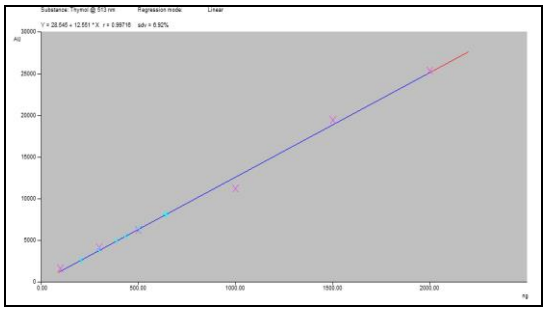


Figure 3. Calibration curve (Area)

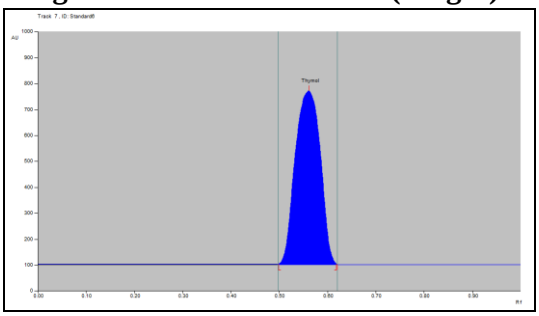


Figure 4: Thymol Standard

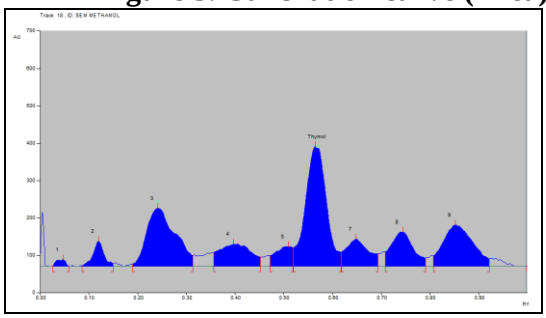
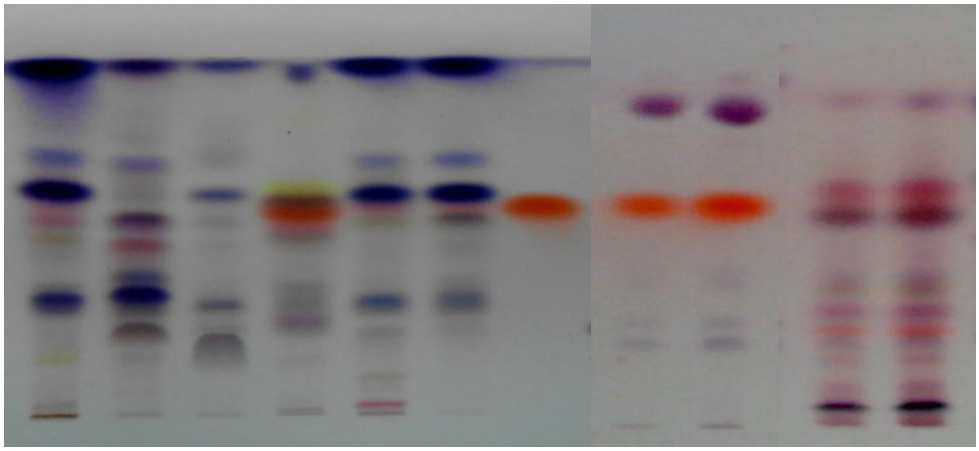


Figure 5: Thymol in methanolic extract of formulation

Table 2: TLC Details of volatile oil samples after Derivatization

Oil Samples	R_f (Colour)
Apium graveolens L. (AG)	0.18 (Yellow), 0.33 (Blue), 0.5 (Yellow), 0.56 (Orange), 0.63 (Blue), 0.73 (Blue)
Nardostachys jatamansi DC. (NJ)	0.17 (Brown), 0.24 (Brown), 0.31 (Brown), 0.35 (Blue), 0.39 (Blue), 0.48 (Pink), 0.54 (Purple), 0.61 (Gray), 0.71 (Violet)
Rosa damascena Mill. (RD)	0.21 (Brown), 0.30 (Gray), 0.48 (Blue), 0.56 (yellow), 0.61 (Blue), 0.71 (Light yellow), 0.81 (Light yellow)
Origanum vulgare L. (OV)	0.17 (Gray), 0.21 (Gray), 0.26 (Violet), 0.31 (Gray), 0.36 (Gray), 0.51 (Violet), 0.56 (Orange), 0.60 (Violet), 0.62 (Yellow)
Trachyspermum ammi L. (TA)	0.23 (Violet), 0.29 (Violet), 0.39 (Light pink), 0.43 (Light pink), 0.58 (Orange), 0.88 (Purple)
Safoof-e-Muhazzil (SM)	0.12 (Pink), 0.19 (Violet), 0.24 (Violet), 0.26 (Orange), 0.33 (Pink), 0.38 (Light yellow), 0.42 (Violet), 0.57 (Orange), 0.67 (Violet), 0.93 (Purple)
Thymol	0.56



AG NJ RD OV AG AG T TA TA SEM OIL

Plate 2: HPTLC fingerprinting analysis of volatile oils of different ingredients and formulation (*Safoof-e-Muhazzil*).

After development, the plates were dried in air and scanned at 513 nm with a CAMAG TLC scanner with WinCat software and using a deuterium lamp. The slit dimensions were 4 mm × 0.2 mm and scanning speed was 20 mm/S.

HPTLC fingerprinting profile of volatile oil of *Safoof-e-Muhazzil* and its herbal drugs

HPTLC fingerprinting profile of volatile oils of *Safoof-e-Muhazzil* and its herbal drugs were also developed for the quality standard purpose. The volatile oils of different drugs and *Safoof-Muhazzil* formulation were separated by using Clevenger apparatus. The samples were prepared by dissolving 0.1 ml of each volatile oil in 10 ml of toluene in volumetric flask of 10-ml. The samples were applied (2 µl) on HPTLC plate and developed using toluene: ethyl acetate, (93:7) as mobile phase. The plate was dried and sprayed with anisaldehyde-sulphuric acid reagent and heated at 105 ºC for 5 min. The plate was scanned at 522 nm in CAMAG HPTLC scanner.

RESULTS AND DISCUSSIONS

HPTLC Quantification of Thymol in different extracts and volatile oil of *Safoof-e-Muhazzil*

The quantification of thymol in different extracts and volatile oil of *Safoof-e-Muhazzil* was carried out as per the method given in material and methods. A spot of orange colour of R_f value 0.56 was observed in chromatogram of the standards, different extracts and volatile oil of *Safoof-e-Muhazzil* (Plate 1, figure 4). The amount of thymol in different extracts and volatile oil of *Safoof-e-Muhazzil* was calculated by using the regression equation (given in table 1). The content of thymol in volatile oil of *Safoof-e-Muhazzil* was found higher (4.428 ± 0.21 %). In different extracts, the thymol was found highest in methanolic extract (1.8833 % ± 0.02, figure 5) followed by hexane, hydro-alcoholic, chloroform and acetone. The details of results are given in table below:

3.1.2. Calibration curve

The calibration curve area versus concentration (ng/spot) was found to be linear in the range of 100-1000 ng/spot. The linear regression data for the calibration curve showed a good linear relationship over the concentration ranges of 100-1000 ng/spot with respect to peak area, as shown in Table 1 and figure 2 and figure 3. The R_f was found to be 0.56 ± 0.02.

HPTLC fingerprinting profile of volatile oil of *Safoof-e-Muhazzil* and its herbal drugs

HPTLC fingerprinting profile of volatile oils of *Safoof-e-Muhazzil* and its herbal drugs were also developed as the method given in materials and methods. The HPTLC chromatogram of these volatile oils shows different coloured bands on their respective R_f. The details of colour and R_f were given in the table 2 and Plate 2.

CONCLUSION

The HPTLC method developed for thymol is simple, rapid, selective, sensitive and economical. Therefore, this method can be successfully used for the routine analysis of thymol in crude drugs, extracts and finished formulations without any interference that can be explored for standardisation and quality control of raw materials and marketed herbal products of Indian traditional system of medicine and Unani System of medicine.

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Conflict of interest: -None-

REFERENCES

1. Anonymous. Quality control methods for medicinal plant material, World Health Organization, Geneva, 1998, p. 8-78.
2. Hardeman W, Griffin S, Johnston M, Kinmonth AL, Wareham NJ. Interventions to prevent weight gain: a systematic review of psychological models and behaviour change methods. Int J Obes Relat Metab Disord, 2000; 24: 131-143.
3. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of Iranian medicinal plants useful in diabetes mellitus. Arch Med Sci, 2008; 4: 285–292.
4. Liu JP, Zhang M, Wang WY, Grimsgaard S. Chinese herbal medicines for type 2 diabetes mellitus.

Cochrane Database Syst Rev, 2004; 4: CD003642.

5. Anonymous, National Formulary of Unani Medicine, Government of India, Ministry of Health and Family Welfare (Department of AYUSH), New Delhi, Part I, 1981, p. 239.
6. Naquvi KJ, Ansari SH, Ali M, Najmi AK, Haque MR. Volatile oil composition of an anti-obesity Unani formulation *Safoof-e-Muhazzil.* J Pharm Res, 2012; 5(1):12-15.
7. Karami-Osboo R, Khodaverdi M, Ali-Akbari F. Antibacterial effect of effective compounds of *Satureja hortensis* and *Thymus vulgaris* essential oils against *Erwinia amylovora*. J Agr Sci Tech 2010; 12: 35-45.
8. Evans WC, Treas & Evans Pharmacognosy, 16th edn. Saunders Elsevier, New York, 2009, pp 265-266.
9. Nagalakshmi S, Shankaracharya NB, Naik JP, Rao LJM. Studies on chemical and technological aspects of ajowan (*Trachyspermum ammi*) syn (*Carum copticum* Hiren) seeds. J Food Sci Tech, 2000; 37: 277- 281.
10. Liang H, Bao F, Dong X, Tan R, Zhang C, Lu Q, Chen Y. Antibacterial thymol derivatives isolated from *Centipeda minima.* Molecules, 2007; 12: 1606–1613.
11. Rafiul H, Ansari S.H., Najmi AK, Naquvi KJ. Validated HPTLC analysis method for quantification of thymol content in *Trachyspermum ammi* and polyherbal Unani formulation *Arq Zeera*. Int J Pharm Pharm Sci, 2012; 4(3): 478-482.
12. Sethi PD. High performance thin layer chromatography: quantitative analysis of pharmaceutical formulations.CBS Publishers and Distributers, New Delhi, 1996, 1st edn., 10-60.
13. Naquvi KJ, Ansari SH, Ali M, Najmi AK, Ahamad J. Development and validation of HPTLC method for estimation of imperatorin in anti-obesity polyherbal formulation, *Safoof-e-Muhazzil* and some of its ingredients. Asian J Trad Med, 2014; 9(2): 34-44.
14. Joshi DD. HPTLC: Herbal Drugs and Fingerprints. In: Herbal Drugs and Fingerprints. Springer, India, 2012, 49-59. https://doi.org/10.1007/978-81-322-0804-4_3.
15. Haque MR, Ansari HS. Anti-obesity effect of Arq Zeera and its main components thymol and cuminaldehyde in high fat diet induced obese rats. Drug Res (Stuttg), 2018; 68(11): 637-647. doi: 10.1055/a-0590-1956.
16. Haque MR, Ansari SH, Najmi AK, Ahmad MA. Monoterpene phenolic compound thymol prevents high fat diet induced obesity in murine model. Toxicol Mech Methods. 2014; 24(2): 116-23. doi: 10.3109/15376516.2013.861888.
17. Mohagheghzadeh A, Faridi P, Ghasemi Y. *Carum copticum* Benth. & Hook., essential oil chemotypes. Food Chem, 2007; 100: 1217-1219.
18. Khajeh M, Yamini Y, Sefidkon F, Bahramifar N. Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydro-distillation methods. Food Chem, 2004; 86(4): 587-591.
19. Nagalakshmi S, Shankaracharya NB, Naik JP, Rao LJM. Studies on chemical and technological aspects of ajowan (*Trachyspermum ammi* (L.) syn. *Carum copticum* Hiern) seeds. J Food Sci Tech, 2000; 37(3): 277-281.