Thin Layer Chromatography (TLC) Separation of Some Medicinal and Aromatic Plants

Reyhan Bahtıyarca Bagdat

Central Research Institute for Field Crops P Box: 24 0670 Yenimahalle/Ankara

ABSTRACT: TLC (Thin layer chromatography) separation of sage (*Salvia officinalis*), lemonbalm (*Melissa officinalis*), thyme (*Thymus vulgaris*) and mountain tea (*Sideritis perfoliata*) was performed in this research. Essential oils and extracts of these drugs were loaded in various quantities to Kiesegel 60 glass plates using several solvents and different doses separately. Plant extracts were condensed under Nitrogen gas flow (3:1). Five different dyeing methods were used after achieving the reference plate. Guaiazulene (8 µl) and Liphophilic (10 µl) were used as references in every treatment. Their lay outs were investigated under visual and 254-366 nm UV light. Toluol:Ethylacetate (93:7) as solvent and Anisaldehyde-H_2SO_4 as dyeing material gave good separation and result.

Keywords: TLC Separation, sage (*Salvia officinalis*), lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*), mountain tea (*Sideritis perfoliata*)

INTRODUCTION

Turkey has the largest flora of any European or Mediterranean country and as a consequence the largest number of medicinal plants species in the Europe and Middle East. Approximately 28,000 tonnes of medicinal and aromatic plants are exported annually, generating nearly 50 million dollars of foreign currency from the trade. This trade has helped to earn 20 times more income compared to one earned through bulbous plants marketed for horticultural purpose. Lange & Schippmann, (1997) and Anonymous (2003) indicate that Turkey is the third largest exporter of medicinal plants of wild origin of any country on earth after China and India.

Sage (*Salvia officinalis*), lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*) are well known medicinal and aromatic plants widely used in folk medicines. Mountain tea (*Sideritis perfoliata*) is, consumed commonly as herbal tea in Aegean and Mediterennean coastal regions of Turkey. The traditional medicinal plants are mostly used for the treatment of wounds (25.3%), cold and influenza (24.6%), stomach disorders (20%), cough (19%), kidney ailments (18.2%) and diabetes (13.4%) (Kultur, 2006).

TLC (thin layer chromatography) is a simple, quick, and inexpensive procedure that gives a quick answer as to how many components are in a mixture. It can be also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate). Once visible, the R_f value (Retention factor), of each spot can be determined by dividing the distance traveled by the product by the total distance traveled by the solvent (the solvent front). These values depend on the solvent used, and the type of TLC plate, and are not physical constants (Harwood *et al.* 1999; Reich and Schibli, 2007).

TLC may be a useful method in medicinal and aromatic plant breeding to make a quick comparison or selection between or among the components of different promising lines to develop new cultivars.

MATERIAL AND METHODS

Extracts and essential oils from sage (*Salvia officinalis*), thyme (*Thymus vulgaris*), lemon balm (*Melissa officinalis*) and mountain tea (*Sideritis perfoliata*) were used as material with the applied

* *To whom correspondence be made:*
E-mail: reyhanbagdat@yahoo.com

dosages of; 2 (essential oil)-20 µl (extract 3:1 N), 25 µl (only extract- 3:1 N), 8-5 µl (3:1 N) and 5-25 µl (3:1 N) in; sage, lemonbalm, thyme, and mountain tea respectively.

Extracts were obtained after maturation of 0.5 g dried drugs in 5 ml n-hexane for 30 minutes. The solution was agitated in every ten minutes, and than filtered. To obtain a clear flow, the exracts were condensed under 3:1 Nitrogene gas flow. Essential oils were obtained using water distillation method with neo-clevenger apparatus, boiling the drug material for three hours.

Reference plate was optimized using Toluol:Ethylacetate (90:10, 95:5, 95:3 and 93:7) and Chloroform:Toluol (75:25) by their flow convenience.

Kiesegel 60 plates are sheets of glasses coated with a thin layer of a solid adsorbent (silica). Small amount of the mixtures to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid (solvent), or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straight forward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. The plate itself contains a fluor which fluoresces everywhere *except* where an organic compound is on the plate (Anonymous, 2010).

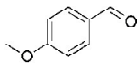
Kiesel 60 (20x20 cm) glasses were divided into two with glass cutter making obtaining 20x10 cm plates for the experiment.

After trying different doses (0,20,30 µl); Guaiazulene (8 µl) and Lipophilic (10 µl) were found most appropriate and loaded to every sample as references.

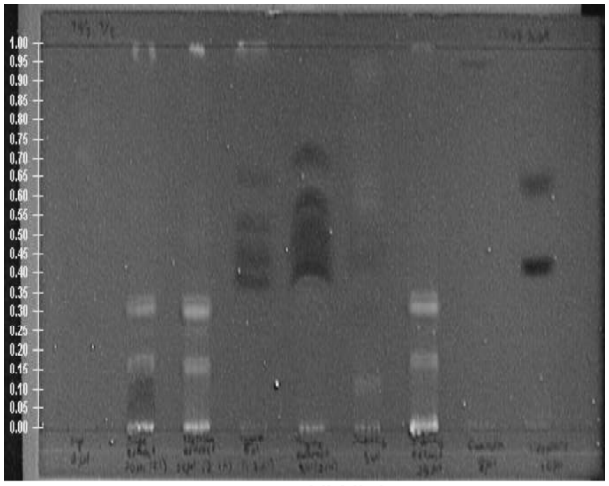
Each lay outs were photographed and recorded under 254-366 nm UV and visual lights. R_f values are also marked on the left side of the sign (Fig. 1,2).

RESULTS AND DISCUSSION

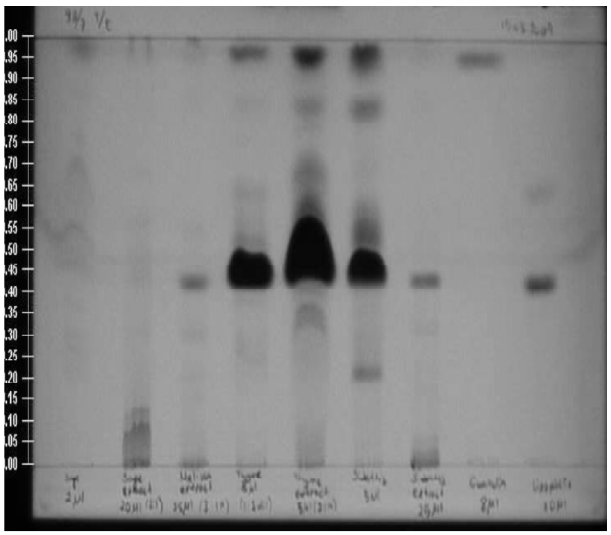
It has been observed that, 93:7 T:E gave simultaneous running with 10 µl dosages of lipophilic and 8 µl Guaiazulene. This treatment was applied to all replicates and assumed as reference. After achieving the reference plate, following dyeing methods were conducted and recorded.

Anisaldehyde  **($C_8H_8O_2$)-H2SO4**

Anisaldehyde, is an organic compound that consists of a benzene ring substituted with an aldehyde and a methoxy group. Different spots on the plate can be stained with different colours allowing easy distinction (Anonymous, 1989). Reference plates were sprayed with Anisaldehyde ($C_8H_8O_2$)-H_2SO_4, UV pictures were taken before spraying and photographing plates using visual light (Fig. 3).



366 nm UV light



254 nm UV light

Figure 1, 2: The Layouts and R_f Values under 366 *nm UV* and 254 *nm UV* Lights

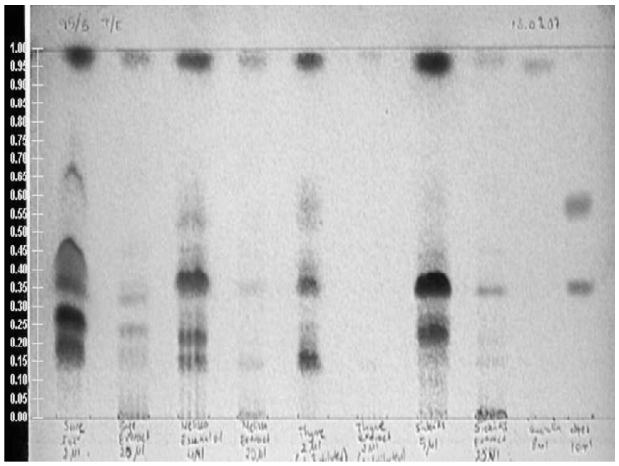


Figure 3: R_f Values of the Reference Plates Dyed with Anisaldehyde under Visual Light

Molybdate  **/Phosphoric Acid**

After dyeing the reference plate with Molybdate/ Phosphoric Acid dark blue spots on the yellow background become visible by reduction of the molybdate (Fig. 4).

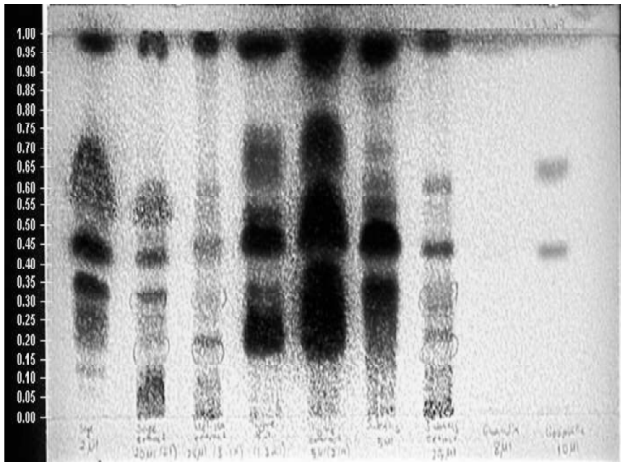


Figure 4: R_f Values of the Reference Plates Dyed with Molybdate/phosphoric Acid under Visual Light

2.4 Dinitrophenylhydrazine

2.4 Dinitrophenylhydrazine is the chemical compounds of $C_6H_3(NO_2)_2NHNH_2$. It is relatively sensitive to shock and friction. The solid changed red to orange, usually supplied wet to reduce its explosive hazard. It is a substituted hydrazine, and is often used to qualitatively test for carbonyl groups associated with aldehydes and ketones. The hydrazone derivatives can also be used as evidence toward the identity of the original compounds.

A positive test is signaled by a yellow or red precipitate (known as a dinitrophenylhydrazone). If the carbonyl compound is aromatic, then the precipitate will be red; if aliphatic, then the precipitate will have a yellow color (Brady and Elsmie, 1926; Allen, 1943).

Predominantly ketones are detected by forming the hydrazones (Fig. 5).

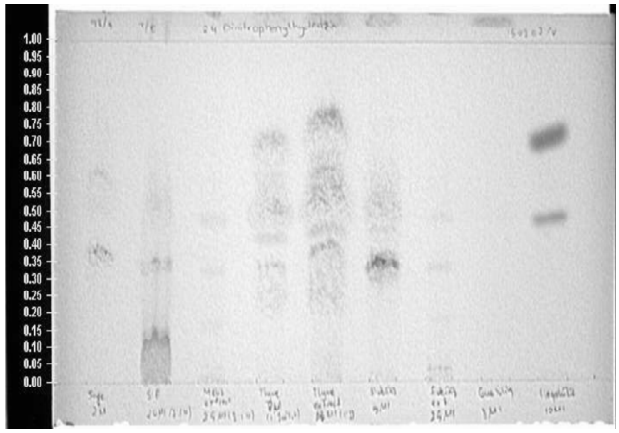


Figure 5: R_f Values of the Reference Plates Dyed with 2.4 Dinitrophenylhydrazine under Visual Light

10% H_2SO_4/ Methanol

In this method, the resultant plate is dried and activated by heating in an oven at 110 °C followed by obtaining dark brown to black spots after heating. The spots weren't clear and visible enough (Fig. 6).

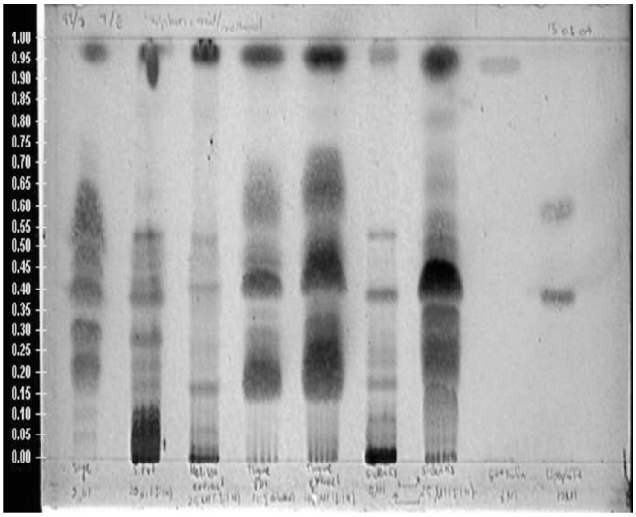


Figure 6: R_f Values of the Reference Plates Dyed with 10% H2SO4/ Methanole under Visual Light

Iodine (I)

Chemically, iodine is the least reactive of the halogens and the most electropositive halogen after astatine. Iodine and its compounds are primarily used in medicine, photography, and dyes (Hille, 2002; Lyday, 2005).

Kiesegel plates were exposed to iodine crystals (particles) in a covered glass chamber for 30 minutes without touching the plate. It's vapour made the spots visible (Fig. 7). As a consequence most organic compounds accumulate iodine, giving a yellow colour, it has been obtained from yellow to light brown spots on the plate.

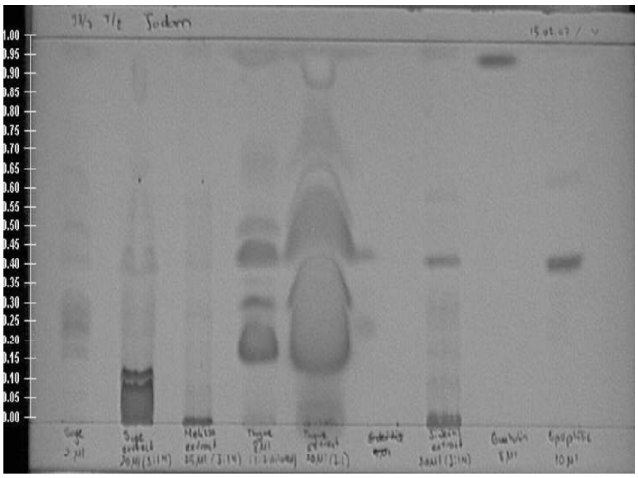


Figure 7: R_f Values of the Reference Plates Dyed with Iodine under Visual Light

Different dyeing methods can be used for screening and observing organic compounds by TLC. In this research, the chemical composition of several medicine and aromatic plants were screened and optimized. Using various solvent doses, the flow convenience of 93:7 Toluol:Ethylacetate was found the best and applied to all treatments as reference.

It is suggested that use of this method may help medicinal and aromatic plant breeders, working with too much lines, to select or restrict dissimilar in a short time in cheap way.

Acknowledgement

Thanks to *Assoc. Prof. Dr. Wulf Schultze* and Dr. *Kai Haacker*, Institute of Pharmacy, Pharmaceutical Biology and Microbiology Dept., Hamburg, for their guidance and valuable technical advice.

References

[1] Allen, C. F. H. **(1943)**, '2,4 Dinitrophenylhyd razine" *Org. Synth.*; Coll. Vol. 2: 228.

[2] Anonymous, **(1989)**, The Merck Index: An Encyclopedia of chemicals, Drugs and Biologicals. 11th Edition, 693.

[3] Anonymous, **(2003)**, *http://www. fao.org/docrep/005/y4496e/Y449E44.htm*

[4] Anonymous, **(2010)**, *http://orgchem.colarado.edu/Hndbksupport/TLC/TLC.html*

[5] Brady, O. L.; Elsmie, G. V. **(1926)**, "The use of 2:4-dinitrophenylhydrazine as a reagent for aldehydes and ketones". *Analyst* 51: 77–78.

[6] Harwood, C. S., Burchhardt, G., Herrmann, H. & Fuchs, G. **(1999)**, Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. *FEMS Microbiol Rev.* 22, 439–458.

[7] Kultur, S., **(2006)**, Medicinal Plants used in Kırklareli Province (Turkey). *Journal of Ethnopharmacology*, 111(2), 4 May 2007, p. 341-364.

[8] Lange, D. J. and Schippmann, U., **(1997)**, Trade Survey of Medicinal Plants in Germany: A contribution to International Plant Species Conservation. Bundes amt für Naturschutz, Bonn, Germany.

[9] Lyday, P. A. **(2005)**, 'Iodine and Iodine Compounds' in Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim.

[10] Reich, E. and Schibli, A. **(2007)**, High-performence thin-layer chromatography for the analysis of medicinal plants (illustrated edition). New York:Thieme. ISBN 3-13-141601-7.

[11] Hile, R. **(2002)**, "Molybdenum and Tungsten in Biology". Trends in Biochemical Sciences 27: 360.