Original Article

HPTLC Analysis of *Mentha piperita* and *Citrus sinensis*

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ABSTRACT

Mentha piperita and *Citrus sinensis* was an aromatic plants well known for its fragrance and distinct aroma. In this prospective study to evaluate the chromatogram detection of ethanol extract of *Mentha piperita* and *Citrus sinensis* with standard flavonoid marker eriocitrin by HPTLC technique. HPTLC chromatogram was developed in ethanolic extract of *Mentha piperita*, *Citrus sinensis* and standard flavonoid marker eriocitrin by using Toluene-Ethyl acetate-Formic acid-Methanol (3:6:1.6:0.4) as mobile phase. The identified bands of compounds 1-9 in the ethanolic extracts of *Mentha piperita* and *Citrus sinensis* was confirmed under the TLC scanner with the standard marker eriocitrin at 254nm.

Keywords: HPTLC, UV absorption spectra, Eriocitrin, Flavonoid, Fragrance.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom and about 300 varieties of flavonoids are known[1]. Flavonoids or bio flavonoids are compounds that give vegetables, fruits, grains, leaves, flowers and bark the colour. These compounds also protect the plants from disease, UV light and from predators. There are many types of flavonoids- flavanols, flavanones, flavones, flavonols, anthocyanins and isoflavanoes- each with health promoting benefits. Flavonoids exhibit several biological effects such as anti-hepatotoxic, anti-inflammatory and anti-ulcer activity. Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality[2]. The flavonoid, eriocitrin, is a flavanone glycoside. Eriocitrin was found to be especially rich in lime fruits and it was abundant in the peel obtained as a byproduct from juice factories. These results suggested that eriocitrin as an anti oxidant can become available by the effective utilization of the peel[3].

Polyphenolic compounds such as eriocitrin, luteolin, diosmin, hesperidin were identified in an aqueous extract obtained from pepper mint leaves and eriocitrin is a powerfull anti-oxidant on the free radical scavenger[4]. The principal citrus flavanones are invariably combined with various sugars,

especially disaccharides and suppresses exercise-induced oxidative damage or stress, prevention of macular degeneration and controls cholesterol.

High-performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurement[5].

No single methods have been reported for the quantitative estimation of eriocitrin by high-performance thin layer chromatography (HPTLC) for *Mentha piperita* and *Citrus sinensis*. Therefore the aim of present investigation was to develop as simple, precise and accurate HPTLC densitometric method for the estimation of eriocitrin.

MATERIALS AND METHODS

Extract preparation for HPTLC analysis

The shade-dried leaves of *Mentha piperita* and peel of *Citrus sinensis* were coarsely powdered and then pulverized. The 10gm of powder was added to 100ml of the ethanol, then sealed with the glass stopper and kept on the rotary shaker for 24 hrs. After 24 hrs, the solution was concentrated under reduced pressure at 45^{o} C using the rotary evaporator to 1/10th of the initial volume. An aliquots of 100mg of ethanolic extract of *Mentha piperita* and *Citrus sinensis* was weighted in an electronic balance and ethanol extract dissolved in 1ml of ethanol. This solution was centrifuged at 300rpm for 5 minutes. The supernatant was used as test solution for HPTLC analysis.

Sample loading

2µl of the above test solution and 2µl of standard solution eriocitrin were loaded as 6mm band length in 10x10cm silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT5 instrument.

Mobile phase

The organic solvents such as Toluene-ethylacetate-Formic acid-Methanol (3:6:1.6:0.4) of analytical grade was purchased and used as a mobile phase. The twin trough developing chamber was saturated with the mobile phase for 10minuters before the development of samples loaded TLC plate.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber with respective mobile phase (flavonoid) and the plate was developed in the mobile phase upto 85mm.

Photo-documentation

The developed plate was dried by hot air to evaporate the solvents from the plate. The plate was kept in the photo documentation chamber (CAMAG REPROSTAR 3) and captured the images at 254nm.

Scanning

The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The peak table, peak display and peak densitogram were noted.

RESULTS AND DISCUSSION

Chromatogram was developed in *Mentha piperita* and *Citrus sinensis* ethanol extract of sample and standard flavonoid marker compound eriocitrin under chamber saturation conditions using Toluene-Ethyl acetate-Formic acid-Methanol (3:6:1.6:0.4) as mobile phase or solvent system. The identity of the bands of compounds 1-9 in

the ethanol extracts were confirmed by TLC scanner with the standard marker eriocitrin at 254nm.The ethanol extract sample Rf 0.17 and 0.15 matched with flavonoid standard marker compound eriocitrin. The ethanol extract of *Mentha piperita* and *Citrus sinensis* bands are identified and confirmed by comparing the chromatogram obtained from the reference standard solution (Table-1, Fig-5) and comparing retention factor (Rf) value from sample and standard solution.

HPTLC finger printing is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. The developed HPTLC fingerprint will help the manufacture for quality control and standardization of herbal formulations. Such fingerprinting is useful in differentiating the species from the adulterant and plant in the pharmaceutical industry and plant systematic studies[6].

CONCLUSION

From the above procedural workout, it can be clearly concluded that the plant *Mentha piperita* and *Citrus sinensis* does contain the flavonoids. HPTLC analysis further confirms the presence of eriocitrin in the two plants. This procedure, hence therefore though being a very simple process is remarkably very efficient for the purification of compounds from crude extracts of the plants. Also the solvent system, standarised for the separation of flavonoids, found to be suitable for the separation of flavonoids, where the clear separation of bands is one of the outmost tasks to be faced during isolation of pure compounds from plant extracts.

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Table 1. HPTLC analysis of *Mentha piperita* and *Citrus sinensis* extracts correlate with standard flavonoid marker Eriocitrin

S. No	Marker/Samples	Rf	Height	Area
1	Eriocitrin	0.20	301.5	8462.6
2	*Menthapiperita*	0.17	95.3	2730.8
3	*Citrus sinensis*	0.15	79.0	3577.4

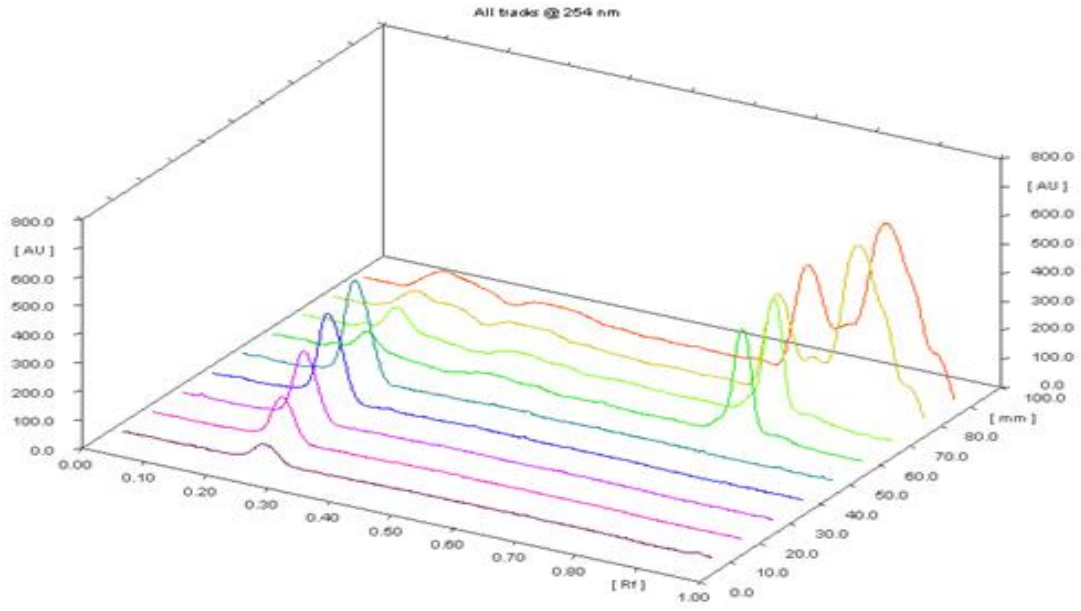

Figure 1. 3D graph of *mentha piperita*, *citrus sinensis* and standard eriocitrin

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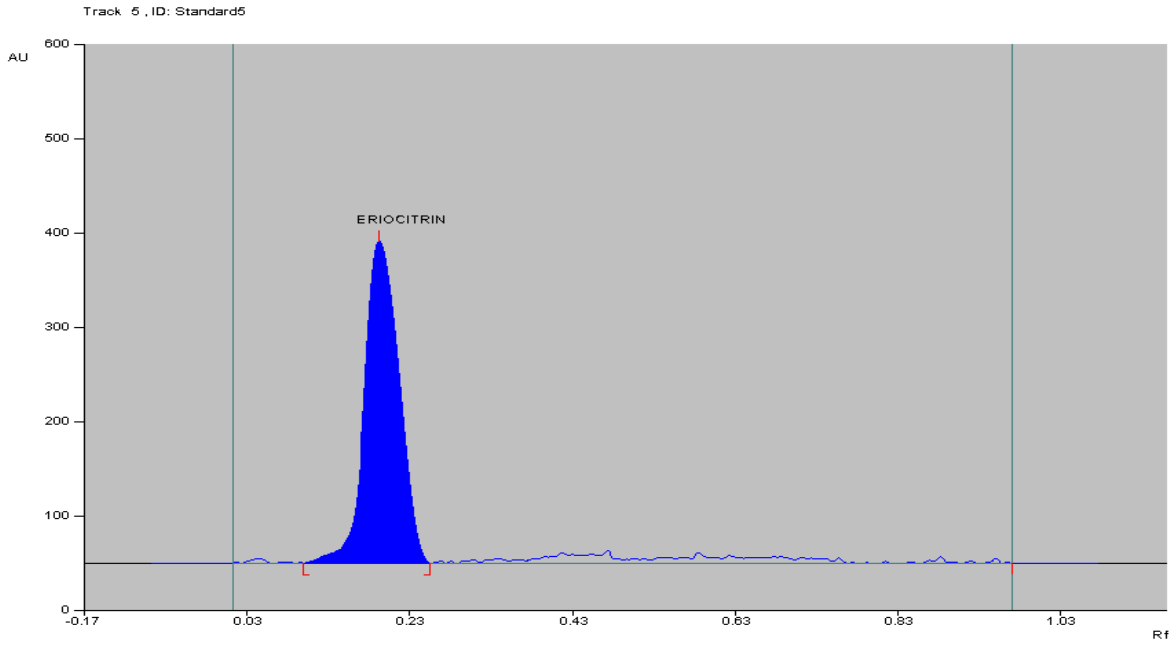

Figure 2. HPTLC chromatogram of standard Eriocitrin

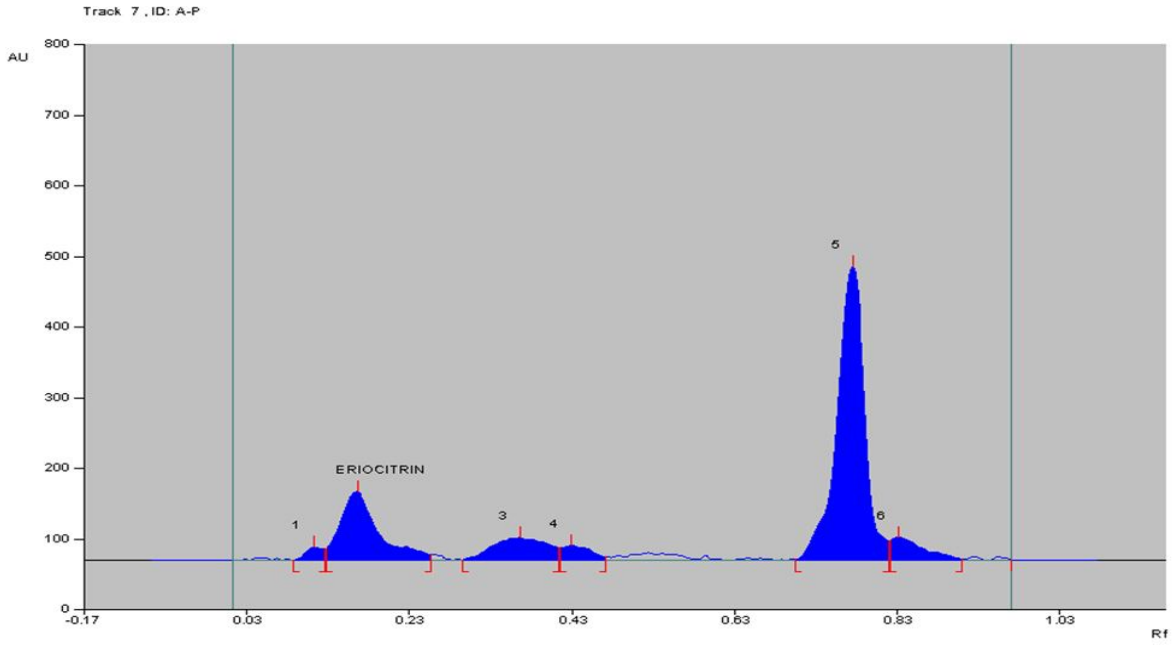

Figure 3. HPTLC of ethanol extract of *Mentha piperita*

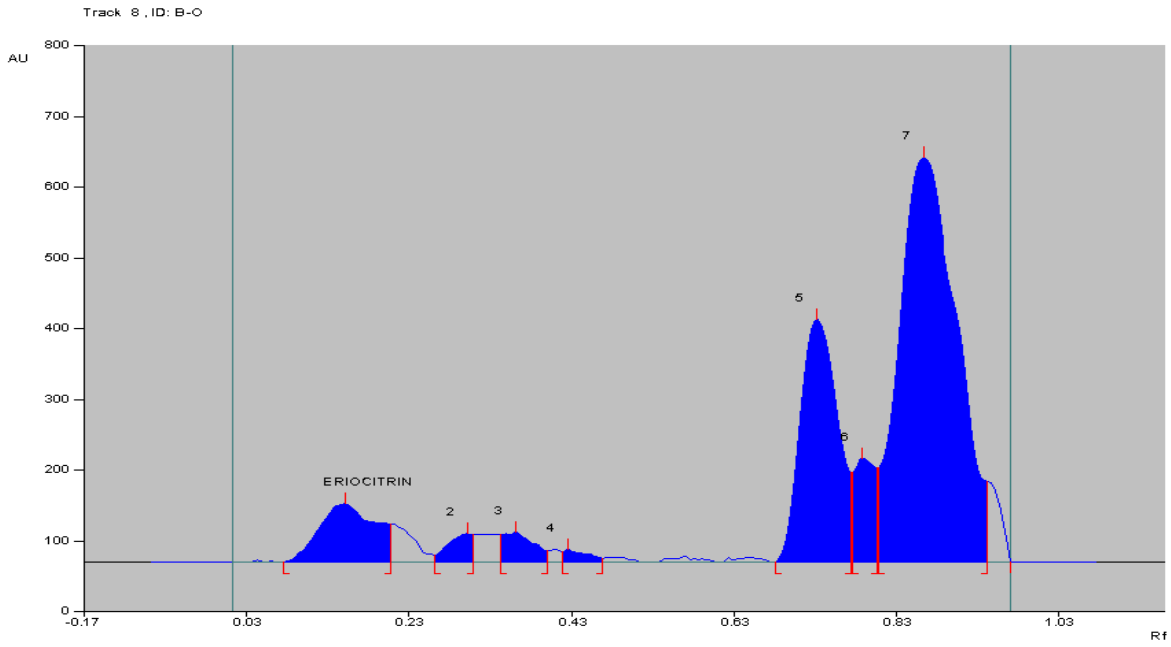

Figure 4. HPTLC of ethanol extract of *Citrus sinensis*

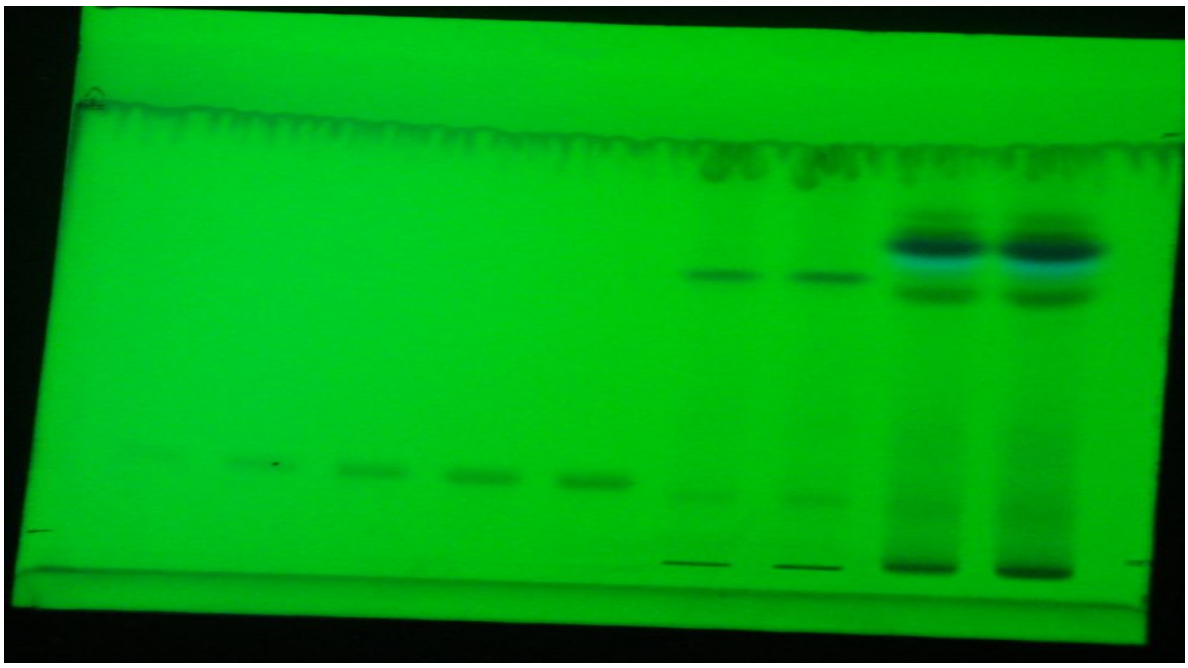

Figure 5. HPTLC Chromatogram of Ethanol extract of *Mentha piperita*, *Citrus sinensis* and Eriocitrin