RESEARCH ARTICLE

Phytochemical Screening, High Performance Thin Layer Chromatography Fingerprint Analysis of Leaf Extract of *Citrus limon* Linn.

Vijeta Gupta*, Vijender Singh, Gunjan, Islam Mojahidul, Rini Malhotra

School of Pharmacy, Sharda University, Knowledge Park III, Greater Noida, Uttar Pradesh, India

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ABSTRACT

This research analyzed the major chemical constituents present in the methanolic extract of the leaves of *Citrus limon* Linn. The leaves of *C. limon* Linn. were collected, powdered, de-fatted with n-hexane, and then extracted with 90% methyl alcohol. Preliminary phytochemical screening of the methanolic leaf extract of *C. limon* Linn. showed the presence of chemical constituents like alkaloids, glycosides, phenolic compounds, tannins, and resins. The high performance thin layer chromatography (HPTLC) fingerprint analysis of the extract was carried out using CAMAG HPTLC. The result obtained HPTLC at UV 254, 366 nm, and in visible light for methanolic extract of *C. limon* Linn. TLC plate using toluene:diethyl ether (1:1) as mobile phase in case of bergapten (Br) and umbelliferone (Um) were done. The amount of Br and Um was present 4.227 and 1.451 µg, respectively, in *C. limon* Linn.

Keywords: Bergapten, *Citrus limon*, High performance thin layer chromatography, Umbelliferone.

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INTRODUCTION

Phytotherapeutic agents or phytomedicines are standardized herbal preparations consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases. According to the WHO definition, herbal drugs contain active ingredients of plant parts or plant materials in the crude or processed state plus certain excipients, i.e., solvents, diluents or preservatives.[1,2] Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles.[3] Chromatographic fingerprinting techniques are most significant methods which can be used for routine herbal drug analysis and for quality assurance. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time.[4] *C. limon* Linn. belonging to Rutaceae family is highly reputed plant and has been widely employed in herbal medicine and aromatherapy. *C. limon* Linn. is well known for its nutrition and health-promotion values. Traditional healers have used citrus species for centuries to treat various diseases. Citrus fruits are suggested to be a good source of dietary antioxidants. Different parts of *C. limon* Linn. have been used for the treatment of various human ailments such as itches, cuts, ulcers, swellings, bilious fever, catarrh, eczema, antipsychotic, etc. As the scientific research is less on the leaves of the species *C. limon* Linn., an attempt is made to identify the chemical constituents and HPTLC fingerprint analysis of the methanolic leaf extract of *C. limon* Linn.

MATERIALS AND METHODS

Collection of the Plant Material

The plant specimens for the proposed study were collected during the month of October, from local place of the Allahabad district and dried under shade. Care should be taken for selecting normal and healthy organs. The identity of the plant samples were confirmed by matching with the samples in the LWG herbarium of the National Botanical Research Institute, Lucknow, whose reference no. is 97847.

Preparation and Extraction of the Plant Material

The 200 grams of the coarsely powdered plant material of leaf of *C. limon* was defatted with n-hexane and extracted with 90% methanol using the Soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by Whatman filter paper (no. 1). The extract was evaporated under reduced pressure using rotavapur evaporator.

Preliminary Phytochemical Screening

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile

*Author for Correspondence: vijetavikasgupta@gmail.com

oils, tannins, saponins, flavonoids, etc. The phytochemical investigation of the methanolic leaf extracts of *C. limon* Linn. was carried out with standard protocol. The results were presented in Table 1.

HPTLC Profile

The HPTLC studies were carried out following the method of Harborne and Wagner *et al*.

Sample Preparation

Methanolic extract of leaf of *C. limon* Linn. was prepared through cold percolation by using 2 g of powdered material in 100 mL of methanol.

Development of the Solvent System

The number of solvent system were tried extract, but the satisfactory resolution was obtained in the solvent toluene:diethyl ether in the ratio of (1:1) for the methanolic leaf extract.

The plate was eluted with respective mobile phase in CAMAG twin through chambers. The chamber was saturated with respective mobile phase saturation plate (E. Merck) of uniform thickness 0.2 mm was used for all the HPTLC analysis.

Sample Applicator

The CAMAG Linomate-5 applicator for application of sample in the form of narrow bands, particularly analysis of mixture compound like plant extracts it is advantageous to start with compact, narrow sample application zones as they guarantee optimum resolution for a given planar chromatographic system, the CAMAG Linomat-5 uses the spray-on technique for applying samples on to the chromatogram layer as narrow band this permits the application of larger sample volume than is possible with contact sample transfer, as the solvent almost completely evaporated during the process even when strongly polar solvents are used, e.g., methanolic or aqueous remain contact and narrow. When larger volume requires, especially in preparative applications, a 500 µL syringe can be used instead of the standard 100 µL dosage syringe, another advantage of the Linomat-5 is its self-adjusting plate support. It allows the use of layers differing in thickness without readjusting the spray nozzle. This feature makes it attractive for the preparative application.

Sample Application

A 10 mg/mL of plant methanolic extract was prepared 10 µL of this solution was application on the plate, and 1 mg/mL

Table 1: Phytochemical analysis of extracts of all the solvent used of *C. limon* Linn.

Phytoconstituents	*Test*	*Methanol*	*Water*
Carbohydrates	Molisch's test	+	+
	Benedict's test	+	+
	Fehling's test	+	+
Tannins	Ferric chloride test	+	+
	Vanillin hydrochloride test	+	+
	Alkaline reagent test	+	+
Alkaloids	Dragendroff's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
	Mayer's test	+	+
Sterols and triterpenoids	Libermann-Burchard	-	-
	Salkowaski	-	-
Flavonoids and phenolic	Shinoda test	+	+
	Zinc HCl reduction test	+	+
	Alkaline reagent test	+	+
Saponins	Froth test	+	+
Coumarins	Fluorescence test	+	+
Proteins	Heat test	+	-
	Biuret test	-	-
	Xanthoproteic test	-	-
Anthraquinone glycoside	Bontrager's test	+	+
	Modified Bontrager test	+	+
Cardiac glycoside	Keller Killiani test	-	+
	Baljet's test	+	+

(+) present; (-) absent

Table 2: Quantitative analysis of HPLC data

Citrus limon Linn.	*Amount applicated from 10 mg/mL sample solution (µL)*	*Amount present for bergapten (µg)*	*Bergapten %*	*Amount present for umbelliferone (µg)*	*Umbelliferone %*
Leaf	10	4.227	0.303%	1.451	0.052%

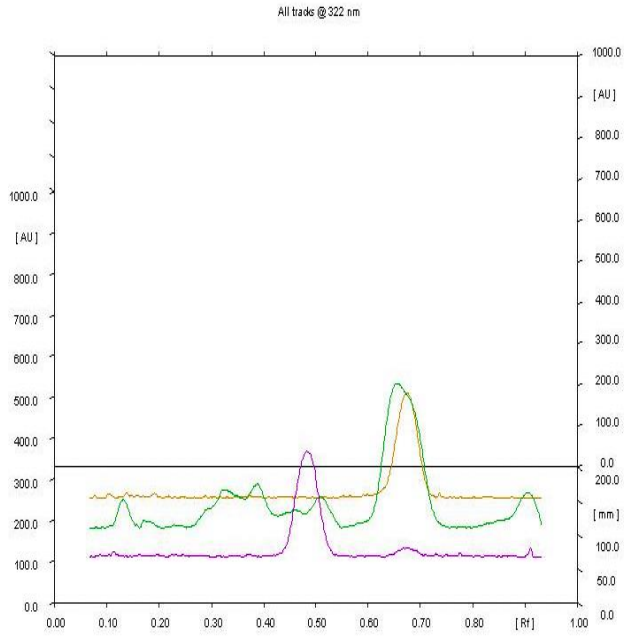


Figure 1: Chromatographic profile of std. and sample *C. limon*; UM: umbelliferone; BR: bergapten; LV: leaf

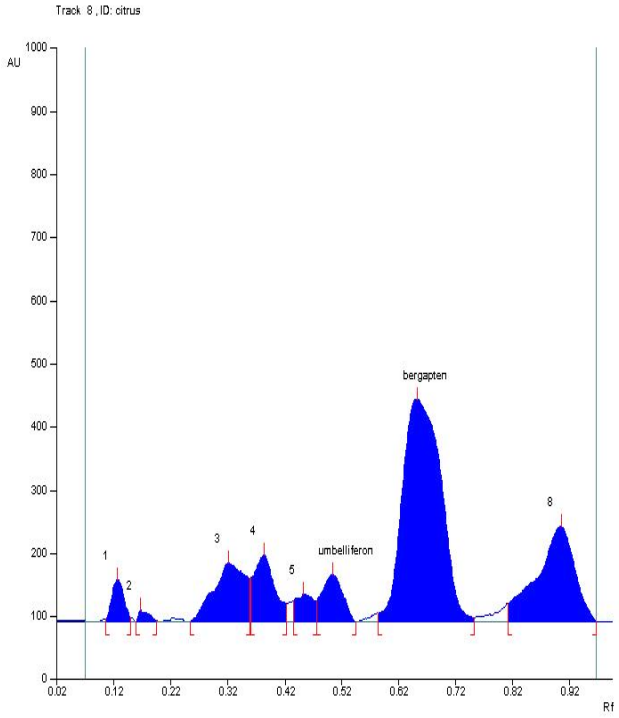


Figure 2: Chromatographic profile of *C. limon*

standard marker solution was prepared and 10 µL of both the standard was applicated.

Development of Chromatogram

After the application of sample, the chromatogram was developed in twin trough glass chamber 10 × 10 cm saturated with solvent:diethyl ether in ratio of (1:1) for 15 minutes. The length of chromatogram run was HPTLC plate was dried in a current of air with the help of an air dryer. The slit dimensions of 5 × 0.45 mm and scanning speed of 20 mm/sec were employed in analysis.[5-8]

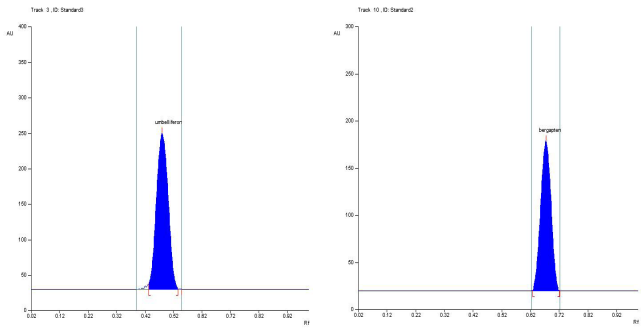
Detection of Spots

The chromatograms were scanned by densitometer plate was observed in the daylight under UV light (254 and 366 nm) (Figure 1). After each observation, the central points of spots appeared on chromatogram were marked with needle.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The phytochemical test on methanolic extract of *C. limon* Linn. leaves showed the presence of various phytoconstituents like carbohydrates, alkaloids, flavonoids, anthraquinone, cardiac glycoside, tannin, and phenolic compounds (Table 1).



(a) Bergapten (b) Umbelliferone

Figure 3: Chromatogram of standard (a) Bergapten and (b) Umbelliferone

HPTLC

The HPTLC fingerprinting analysis was performed according to the aforesaid procedure and the bands were observed on the HPTLC plates (Table 2).

The result obtained HPTLC at UV 254, 366 nm, and in visible light for methanolic extract of *C. limon* Linn. TLC plate using toluene:diethyl ether (1:1) as mobile phase in case of Br and Um were done. The amount of Br and Um was present 4.227 and 1.451 µg, respectively, in *C. limon* Linn. (Figure 2-4).

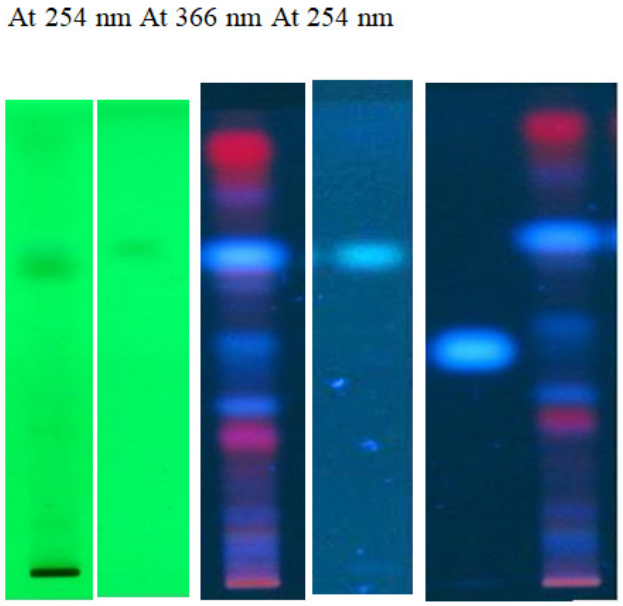


Figure 4: HPTLC fingerprinting profile of *C. limon* Linn. at 254, 366 nm; LV: leaf; UM: umbelliferone; BR: bergapten

CONCLUSION

The standard preliminary phytochemical investigation of the methanolic extract of *C. limon* leaves showed the presence of some secondary metabolites like carbohydrates, alkaloids, tannins, phenolic, and flavonoids compounds, and cardiac and anthraquinones glycosides. The result obtained HPTLC at UV 254, 366 nm, and in visible light for methanolic extract of *C. limon* Linn. TLC plate using toluene:diethyl ether (1:1) as mobile phase in case of bergapten and umbelliferone were done. The amount of bergapten and umbelliferone was present 4.227 and 1.451 µg, respectively, in *C. limon* Linn. These chromatographic profiles can be used for the identification and evaluation of the quality of the plant. Phytochemical studies, HPTLC fingerprint profile, has been useful for fixing standardization for this plant. As a preliminary basic work, in this, we have presented herein all the above-mentioned data's more work is required for the identification of extract core components, their formulas and chemical structure to establish the docking ability. Finally, for the development of better therapeutic agents for clinical assessment, detailed pharmacology and toxicology, including genotoxicity and reproductive toxicology studies need to be performed in order to generate data on the potential short and long term toxicities as well as affirmed pharmacological action.

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