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Phytochemical Standardization of *Cucumis Melo(L)*. Extract by HPTLC Techniques

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ABSTRACT

To elucidate the terpenoid profile of *Cucumis melo (L)*. fruit extract using high performance thin layer chromatography (HPTLC). The ethanolic extract prepared from the fruit of *Cucumis melo (L)*. using Soxhlet apparatus. n-hexane: ethyl acetate (7.2: 2.9) was employed as mobile phase for terpenoids. 3 μ l of test solution and 2 μ l of standard solution was loaded as 6mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. HPTLC analysis was done using CAMAG HPTLC system equipped with automatic TLC sampler IV, TLC scanner 3, REPROSTAR 3 with 12 bit CCD camera for photo documentation, winCATS Planer Chromatography software. The R_f value of the different compounds present in the extract was found to 0.06, 0.21 and 0.93 of peak 1, 2 and 3 respectively. Among them, peak 1 was found to be terpenoid compounds. *C. melo* fruit extract showed the presence of terpenoids and it was confirmed from the chromatogram after derivatization. HPTLC profile of terpenoids has been chosen here to reveal the diversity existing at biochemical level in *C. melo*. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies.

Keywords: HPTLC, *Cucumis melo (L)*, Secondary metabolites, Terpenoids, Ethanol extract.

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INTRODUCTION

Cucumis melo (Cucurbitaceae) is commonly known as wild melon, cantaloupe, small gourd. Melon is a name given to various members of the Cucurbitaceae family with fleshy fruit. *Cucumis melo* fruit is round in shape, tan to greenish tan with a rough texture and orange pink flesh. It is well known for its sweet taste and fragrance. The fruits can be used as a cooling light cleanser or moisturizer for the skin and has stomachic properties. Seeds are anti-tussive, digestive, febrifuge and vermifuge¹. Many phytochemicals having potential benefits are present in *C. melo*. It is rich in carbohydrates, Proteins, fibre, citric acid, vitamin K, vitamin A and folate. Traditionally, it is used for treatment of Kidney stones, cancer, cardiovascular disorders and stroke². The pulp of the fruit is a powerful diuretic, very beneficial in chronic, and also in acute, eczema. It is widely used as cosmetics such as skin lotions containing melon juice³.

Phytoconstituents are the natural bioactive compounds found in plants. Phytochemicals are basically divided into two groups, i.e. primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acid, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids and tannins⁴. Phytochemicals could prevent diseases (including cancer and cardiovascular diseases) and inhibit pathogenic microorganisms⁵. High Performance Thin Layer Chromatography has emerged as one of the most efficient tools in the last two decades for the separation and quantification of secondary metabolites especially for the evaluation of botanical materials⁶. But there is no report on the HPTLC terpenoid profile of *C.melo* fruit. With this background the present study was aimed to reveal the terpenoid profile of *C.melo* fruit extract using HPTLC.

MATERIALS AND METHODS

Collection of plant material

The fruit sample was collected from the local farmers of Coimbatore district, Tamilnadu, India. The specimen sample was authenticated by Professor Taxonomist Dr. V. S. Ramachandran, Associate Professor, Department of Botany, Bharathiar university Coimbatore, India. Voucher specimen was deposited in the herbarium centre, Department of Botany, Bharathiar University, Coimbatore. The pulp of fresh fruits of *Cucumis melo(L)* was chopped into pieces and dried at room temperature for 24 hours. The air dried pulps were kept at 40⁰C in hot air oven for 24 hours to remove moisture content. The completely dried fruits were ground into powder by using mixer grinder and stored for further uses.

Preparation of extract

20g of the dried fruit powder was successively extracted with ethanol using soxhlet apparatus. The prepared extract was filtered through Whatmann No 1 filter paper (pore size 25 µm) to get the ethanol extract. Then it is stored in refrigerated condition and used for the further study.

HPTLC Profile

HPTLC studies were carried out following the method adopted by Reich and Schibili, (2007) ⁷

Test solution preparation

The given ethanol extract 25mg was weighed in an electronic balance (Afcoset) and dissolved in 250µl ml ethanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

Sample application

3µl of test solution and 2µl of standard solution was loaded as 6mm band length in the 3 x 10 Silica gel 60F₂₅₄ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Terpenoid) and the plate was developed in the respective mobile phase up to 90mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured at White light, UV 254 nm and UV366 nm.

Derivatization

The developed plate was sprayed with respective spray reagent (Terpenoid) and dried at 100°C in Hot air oven. The plate was photo-documented in Day light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning Before derivatization, 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. The software used was winCATS 1.3.4 version.

Analysis details for Terpenoid

Mobile phase: n-Hexane-Ethyl acetate(7.2:2.9).Spray reagent Anisaldehyde sulphuric acid reagent.

RESULTS AND DISCUSSION**Detection**

Blue, bluish violet coloured zones at Visible light mode present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the Presence of Terpenoid in the given standard (Lupeol) and sample.

The extract was run along with the standard terpenoid compound and it was observed that the extract showed the presence of terpenoid and it was confirmed from the chromatogram after derivatization. The fruit extract which shows the presence of terpenoids in the chromatograph as well as in UV after derivatization. The Rf value of the different compounds present in the extract was found to 0.06, 0.21 and 0.93 of peak 1, 2 and 3 respectively. Among them, peak 1 was found to be terpenoid compounds. The peak height of respective terpenoids was given in the Table 1.

Table 1: HPTLC terpenoid profile of ethanol extract of *Cucumis melo* (L). fruit

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.06	542.7	18860.6	Terpenoid 1
Sample A	2	0.21	47.7	1382.0	Unknown
Sample A	3	0.93	14.4	438.1	Unknown
STD	1	0.71	143.0	4328.0	Terpenoid standard

The well resolved HPTLC profiles of the ethanolic extract of *C.melo* were presented in Figure 1 and Table 1 to authenticate the presence of terpenoids. HPTLC chromatogram of the standard and the terpenoids profile of *C.melo* was depicted in Figure 2. This confirmed the presence of terpenoids in the fruit extract of *C.melo*. It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers ensures the concentration and ratio of components in the parts of the plant.

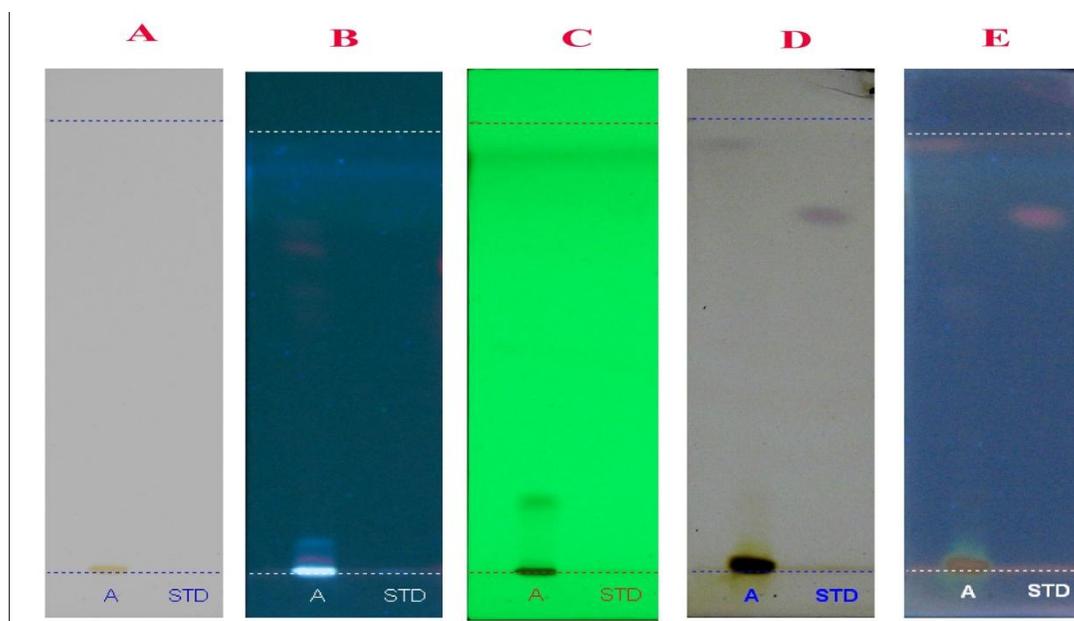


Figure 1: HPTLC studies on the terpenoid of *C.melo*(L)

A: HPTLC of the ethanolic fruit extract of *C.melo* under daylight. **B:** HPTLC of the ethanolic fruit extract of *C.melo* under UV 366 nm **C:** HPTLC of the ethanolic fruit extract of *C.melo* under UV 254 nm. **D:** HPTLC of the ethanolic fruit extract of *C.melo* under daylight – after derivatization. **E:** HPTLC of the ethanolic fruit extract of *C.melo* under UV 366 nm after derivatization.

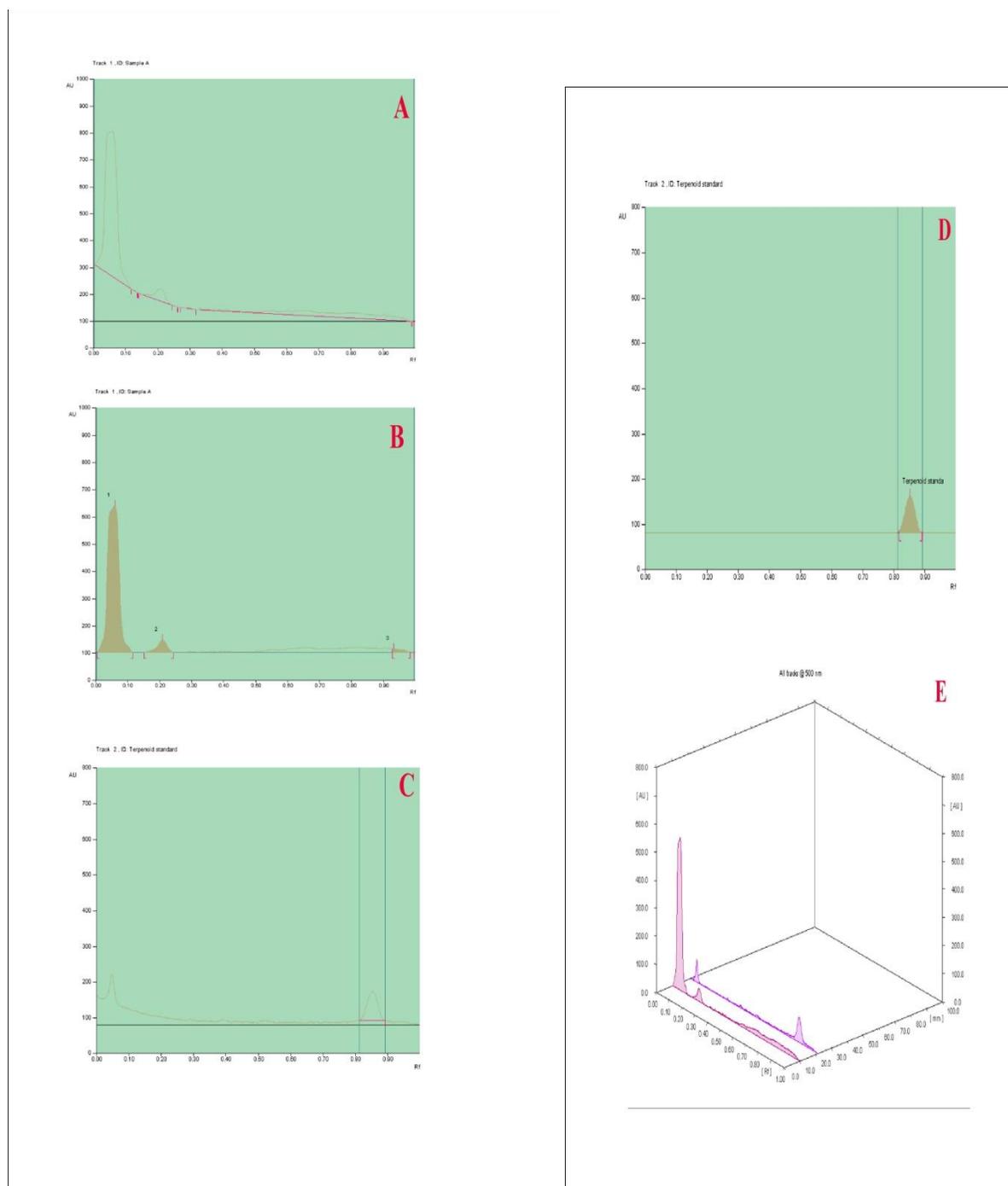


Figure 2: HPTLC chromatogram of ethanolic fruit extract of *C.melo* (L).

A: HPTLC chromatogram of Track STD – Terpenoid standard Baseline display (Scanned at 366nm), **B:** HPTLC chromatogram of Track STD – Terpenoid standard Peak densitogram display

(Scanned at 366nm), **C:** HPTLC chromatogram of Track A – Sample A ethanolic fruit extract of *C.melo* - Baseline display (Scanned at 366nm), **D:** HPTLC chromatogram of Track A – Sample A ethanolic fruit extract *C.melo*- Peak densitogram display (Scanned at 500 nm), **E:** HPTLC chromatogram of 3D display of all Tracks.

Phytoconstituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds in the plants serve a defense mechanism against predation by many microorganisms, insects and other herbivores⁷. Currently, there is an increased interest in natural substances with valuable medicinal properties, such as terpenoids (hydrocarbon composition) and multiple C₅H₈. Terpenoids are secondary metabolites present in most organisms, particularly plants. In every year, more than 40,000 individual terpenoids are identified in nature with new compounds being discovered. From the largest group of phytochemicals, terpenoids are traditionally used for the treatments of various ailments in India and China, are currently being enquired as anticancer agents in clinical trials⁸. Terpenoids are synthesized from two five-carbon building blocks, i.e., the isoprenoid units. Based on the number of building blocks, terpenoids are classified into several types, such as monoterpenes, diterpenes, triterpenes and tetraterpenes⁹. Most of the terpenoids are present in vegetables and fruits and play an important role in traditional herbal remedies. They are currently under investigation by numerous groups for antitumor, antibiotic, anticancer, antineoplastic, antibacterial, anti-inflammatory and other therapeutic properties¹⁰.

HPTLC is an inexpensive method for separation, qualitative identification, or semi-quantitative analysis of samples and it can be used to solve many qualitative and quantitative analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis¹¹. HPTLC plays a vital role for identifying the bioactive components in the medicinal plants. In the present study also we established the HPTLC profile of terpenoids for *Cucumis melo* (L). It has been widely used for the phytochemical evaluation of the herbal drugs, due to its simplicity and minimum sample clean up requirement. HPTLC results are not only reported as peak data but can also be presented and communicated as images¹².

CONCLUSION

The present study reported that ethanol extract of *Cucumis melo* (L) contains the terpenoid profile using HPTLC. The method was validated by determining linearity, peak purity, and limit of detection and repeatability of terpenoids from fruit extract *C.melo* (L). The developed HPTLC

method for terpenoid profile is simple, precise and accurate and can be used for the identification and commercial application. For developing analytical method pure active chemical constituents should be isolated in further study and identification on the basis of reference standard shall be made. HPTLC fingerprinting of *C.melo* fruit extract has been performed which may be used as markers for quality evaluation and standardization of the drug.

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