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Angha B Anmod
PG Student, Department of Botany, N.E.S. Science College, Nanded, Maharashtra, India

DM Jadhav
Assistant Professor, Department of Botany, N.E.S. Science College, Nanded, Maharashtra, India

Corresponding Author
Angha B Anmod
PG Student, Department of Botany, N.E.S. Science College, Nanded, Maharashtra, India

HPTLC profiling of fruit rind of some *Citrus* species

Angha B Anmod and DM Jadhav

Abstract
Citrus sinesis, citrus reticulata, Citrus aurantium and Citrus limon was most widely grown citrus species throughout the world. In this prospective study to evaluate the comparative chromatographic analysis 20 gm powdered rind of each species was extracted separately using 80% ethanol in a Soxhlet Extractor for about twelve hours. Extracts were later used for further phytochemical and HPTLC technique analysis. HPTLC chromatogram was developed in ethanolic extract of *Citrus sinesis, citrus reticulata, Citrus aurantium and Citrus limon* by using Toluene-Ethyl acetate-Formic acid-Methanol (8.5:1.5:0.5) as mobile phase. The study revealed that *Citrus sinesis and citrus reticulata* has similar chromatographic pattern showing nine phytochemical compounds. From this it can be concluded that in absence of *Citrus sinesis* rind one can supplement *citrus reticulata* rind or vice versa. Similarly *Citrus limon* and *Citrus aurantium* rind showed eight and seven polyvalent phytochemical compounds.

Keywords: *Citrus*, HPTLC technique, TLC, flavonoids

Introduction
Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom and about 300 varieties of flavonoids are known (Kuhnau J. 1976) [4]. Flavonoids or bio flavonoids are compounds that give vegetables, fruits, grains, leaves, flowers and bark the colour. Citrus plants contain a wide range of flavonoid constituents, some of which, e.g., hesperidin, naringin, and polymethoxylated flavones (PMFs), are characteristic of them and others such as rutin and quercetin are common in the plant kingdom. Attempts have recently been made to find biological activities among citrus flavonoids. For instance, naringin has been found to lower the total cholesterol and low-density lipoprotein cholesterol levels in plasma, (Jung, U.J. *et al.* 2003) [2] while the administration of hesperetin and its metabolites significantly lowered the total cholesterol and triglyceride concentrations in plasma (Kim, H.K., *et al.* 2003) [3]. Hesperidin and diosmin, both alone and in combination, act as a chemopreventive agent against colon carcinogenesis induced by azoxymethan (Tanaka *et al.* 1997) [7]. The polymethoxylated flavone, nobiletin, has been reported to effectively down-regulate the production of promatrix metalloproteinase and to interfere with the proliferation of synovial fibroblasts. Tangeretin has been reported to have a suppressive effect on malignant tumour invasion and metastasis (Brack *et al.* 1994) [1]. The purpose of these present studies is to prevent chronic diseases through the daily intake of citrus fruits and to increase their added value.

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 (Morlock *et al.*, 2010) [5]. No single methods have been reported for the quantitative estimation of ethanolic extract of *Citrus sinesis, citrus reticulata, Citrus aurantium and Citrus limon* by high-performance thin layer chromatography (HPTLC). Therefore the aim of present investigation was to develop as simple, precise and accurate HPTLC densitometric method for determine of presence of various phytochemical such as flavonoid in *citrus* peel.

Materials and Methods
Extract preparation for HPTLC analysis
Rind of four different citrus species namely *Citrus limon, Citrus sinesis, Citrus aurantium, Citrus reticulata* were used for present. The rind were removed from fruit and dried separately. The dried rind made powder using mixture grinder. About 20 gm powdered rind of each species were extracted separately using 80% ethanol in a Soxhlet Extractor for about twelve

hours. After extraction the extracts were evaporated to dryness using hot plate. The dried extracts were redissolved in 5 ml ethanol and filtered using Whatmann filter. The filtered extracts were later used for further phytochemical and HPTLC analysis. HPTLC fingerprinting extracts of *Citrus rind* were carried out as per the method described by (Sheila, 2017). Two micro-litters of the ethanolic extract was applied (band length –8.0 mm) on a precoated TLC aluminium sheets of silica gel G60 F254 of 200 µm thickness plate- 05 x10cm (Merck, Mumbai) using Linomat V TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100-µL syringe. Prior application, the plate was pre-washed with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Toluene: Ethyl acetate: methanol (8.5:1.5:0.5) in a Camag HPTLC twin-trough chamber (10 x10 cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed up to 85.0 mm and dried under stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode (multi wavelength Scanning) operated by Win CATS software (version 1.4.8). After scanning the spectra and tables thus obtained were analyzed to interpret the results.

Citrus limon

Citrus reticulate

Citrus aurantium

Citru sinensis

Results and Discussion

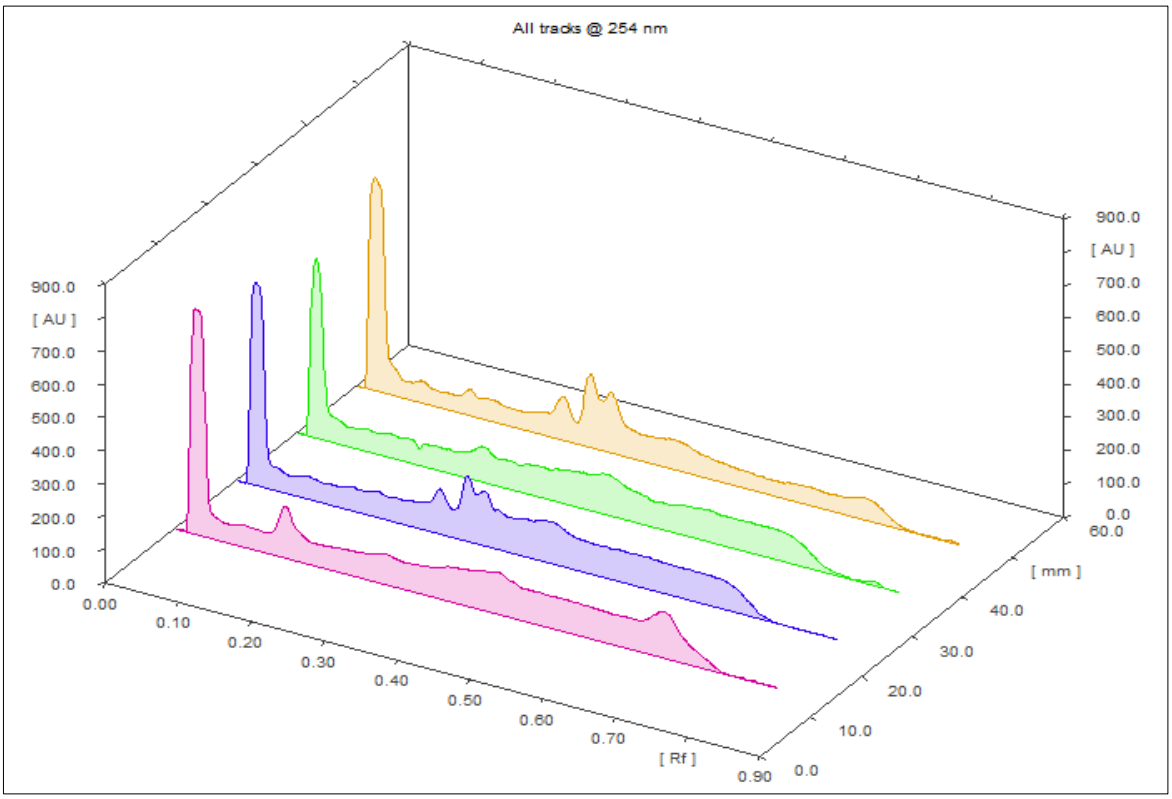
Chromatogram was developed in *Citrus sinesis, citrus reticulata, Citrus aurantium and Citrus limon* ethanol extract of sample under chamber saturation conditions using Toluene-Ethyl acetate-Formic acid-Methanol (8.5:1.5:0.5) as mobile phase or solvent system. The identity of the bands of compounds 1-9 in the ethanol extracts were confirmed by TLC scanner at wavelength 254 nm.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Citrus limon rind* showed seven polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.03 to 0.67 in which highest conc. of the phytoconstituents was found to be 51.10% and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 1.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *citrus reticulata rind* showed nine polyvalent phytoconstituents and corresponding ascending order of Rf values are from 0.03 and 0.65 in which highest conc. of the phytoconstituents was found to be 39.29% and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 2.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Citrus aurantium rind* showed eight polyvalent phytoconstituents and corresponding ascending order of Rf values are from 0.03 to 0.80 in which highest conc. of the phytoconstituents was found to be 48.61% and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 3.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Citrus sinesis rind* showed nine polyvalent phytoconstituents and corresponding ascending order of Rf values are from 0.03 to 0.70 in which highest conc. of the phytoconstituents was found to be 41.26% and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 4.



3 D Spectral display of rind of different citrus species.

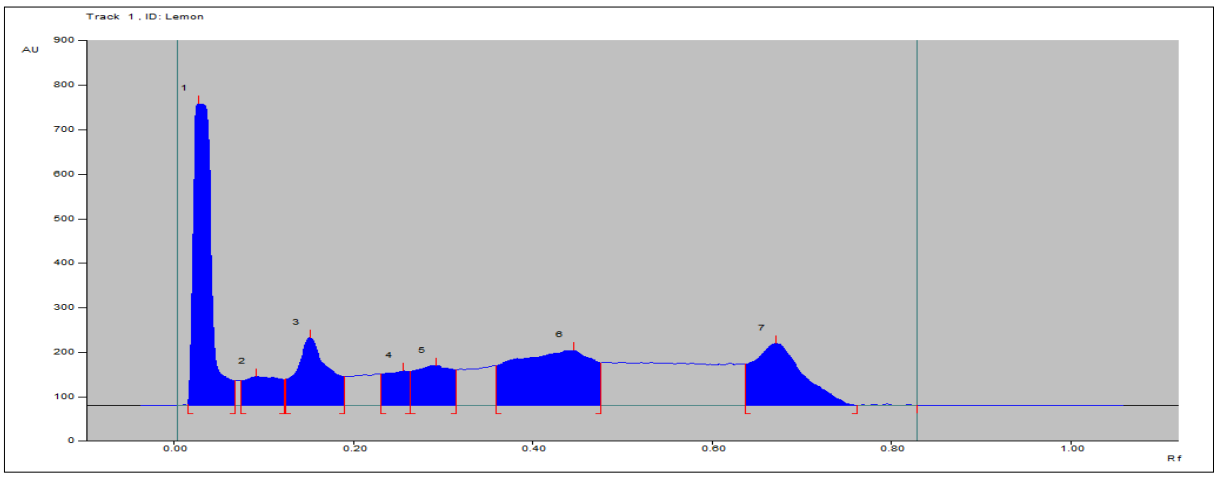


Fig 1: HPTLC profile (Peak Display) of *Citrus lemon rind*

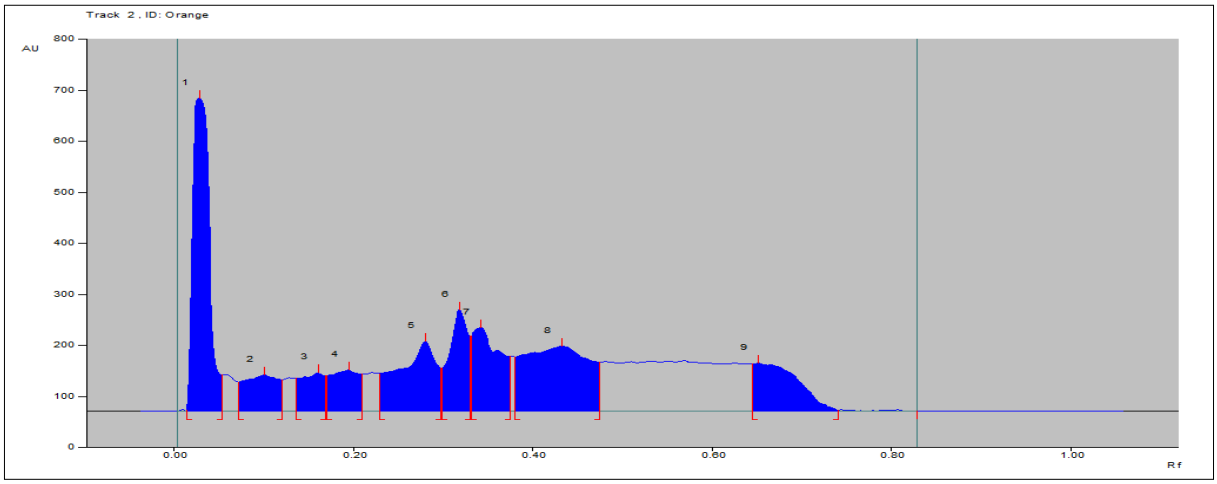


Fig 2: HPTLC profile (Peak Display) of *Citrus reticulata rind*

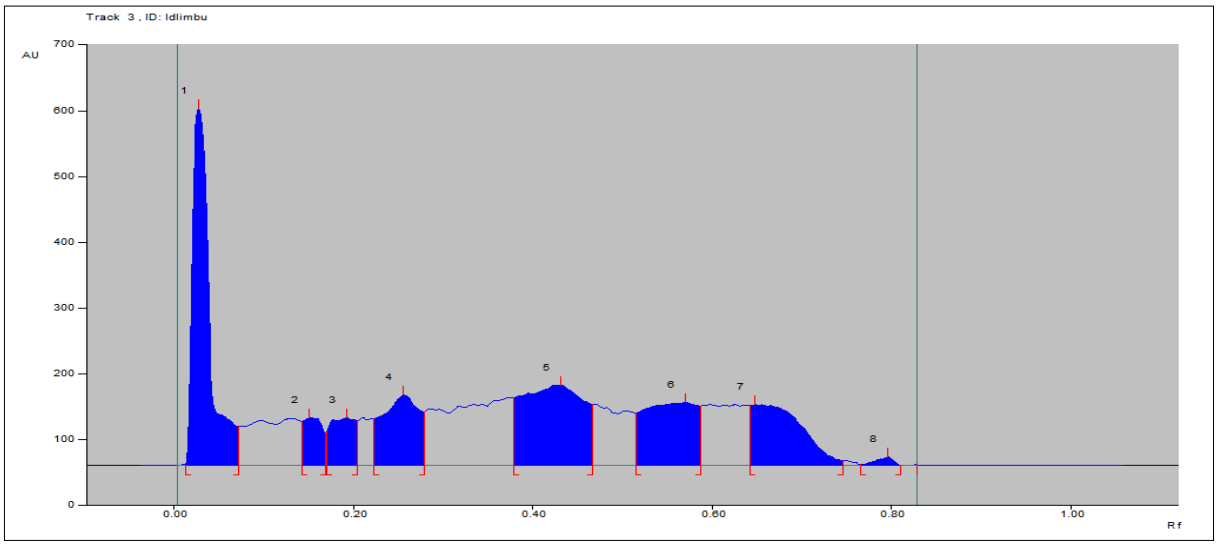


Fig 3: HPTLC profile (Peak Display) of *Citrus aurantium rind*

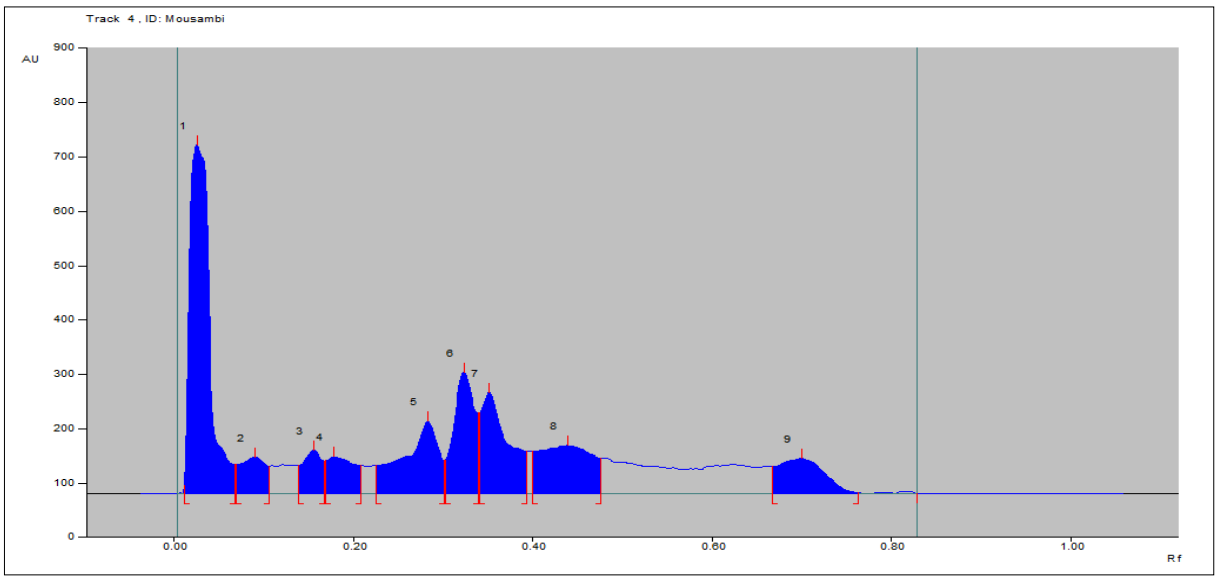


Fig 4: HPTLC profile (Peak Display) of *Citrus sinensis rind*

Conclusion

In this prospective study to evaluate the comparative chromatographic analysis 20 gm powdered rind of each species was extracted separately using 80% ethanol in a Soxhlet Extractor for about twelve hours. Extracts were later used for further phytochemical and HPTLC technique analysis. HPTLC chromatogram was developed in ethanolic extract of *Citrus sinesis, citrus reticulata, Citrus aurantium and Citrus limon* by using Toluene-Ethyl acetate-Formic acid-Methanol (8.5:1.5:0.5) as mobile phase. The study revealed

that *Citrus sinesis and citrus reticulata* has similar chromatographic pattern showing nine phytochemical compounds. From this it can be concluded that in absence of *Citrus sinesis* rind one can supplement *citrus reticulata* rind or vice versa. Similarly *Citrus limon* and *Citrus aurantium* rind showed eight and seven polyvalent phytochemical compounds.

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