e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research Article

CHROMATOGRAPHIC FINGER PRINT ANALYSIS OF HIBISCUS RADIATUS & TERMINALIA ARJUNA OF METHANOLIC EXTRACTS BY HPTLC TECHNIQUE AND EVALUATION FOR THEIR ANTIOXIDANT & ANTIMICROBIAL ACTIVITIES

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ABSTRACT

The present study was aimed to investigate the antimicrobial, antioxidant activity and HPTLCfinger print profile of methanolic extract of Hibiscus radiatus (MEHR) and Terminalia arjuna (META) .HPTLC finger print profile was showed the active compounds present in crude extracts, which may responsible for the antioxidant and antimicrobial prospective. Antioxidant bioautography shows the strong antioxidant activity of one flavonoid band, whereas no activity detected of bitter principles. Bioautography showed that the antimicrobial and antioxidant activity was probably due to flavonoids. These results showed that, the significant antimicrobial properties against pathogen; this work will be helpful to explore the active compound identification in the field of pharmaceutical research and able to produce new drug molecules against pathogens.

Keywords: Antioxidant activity, HPTLC fingerprints, Antimicrobial activity, Hibiscus Radiatus, *Terminalia Arjuna*.

INTRODUCTION

Standardization of plants in terms of establishing a TLC profile and a marker compound for their authentication has become a high priority area in the field of phytopharmaceuticals and is being given considerable attention worldwide. Chromatography techniques, which are used for separating mixtures into individual fractions, are ideal for creating a "fingerprint". HPTLC is well suited to obtain a detailed and definite fingerprint of herbal extract or product. Such a fingerprint comprises of scanning, in UV, fluorescence, ultraviolet spectra and photographic images in ultraviolet light (366 nm) and occasionally using visible light after derivatisation[1].

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The identification of secondary metabolites produced by plants are important steps on the way to use these compounds as active principles in medicinal preparations. One of the most effective and inexpensive techniques of plant extracts analysis is bioautography. Bioautography offers a simple, rapid and inexpensive method for the chemical and biological screening of complex plant extract[3].

Collection and Authentication of Raw Material

Fresh leaves of *Hibiscus radiatus and Terminalia arjuna* were obtained locally. Plant samples were authenticated by Dr. J. Jayaraman Botanist, Thambaram Chennai. The plant voucher No. is BSI/DRC/2019-17/Tech./664/07 dated 30-12- 2019. The leaves of the selected plants were washed thoroughly. The plant materials were air dried and coarse powder was prepared with the help of a blender. The powders were oven dried at 110 ºC for an hour and packed in air tight bottles until further use.

Preparation of Plant Extracts

Fifty grams of air-dried powder was extracted using 300 ml of methanol as solvent in soxhlet apparatus for 18 h. The extracts(MEHR & META) were filtered through four layers of muslin cloth, dried at 50^{o}C and stored at 4^{o}C until further use[4].

SCREENING OF PHYTOCONSTITUENTS BY TLC

The reproducibility obtained with TLC analyses is often not satisfactory because many parameters that influence the chromatographic result are neglected. Consequently, a general trend is observed to consider TLC as an outdated and old-fashioned technique, which should be replaced by the ''more reliable" HPLC or other chromatographic techniques.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

HPTLC is well suited to obtain a detailed and definite fingerprint of herbal extract or product. Such a fingerprint comprises of scanning, in UV, fluorescence, ultraviolet spectra and photographic images in ultraviolet light (366 nm) and occasionally using visible light after derivatisation. The emergence of HPTLC as a powerful tool in TLC has equipped the pharmacognosist with a unique and high precision technology enabling him to work faster and better. The technique has found favor with innumerable scientists all over the globe because of its simplicity and efficiency.

RESULT

The overall investigations can be concluded that the therapeutic activities of *Hibiscus radiatus*and *Terminalia arjuna* have been scientifically validated. HPTLC analysis, were showed accurate results of biological compounds it possesses. Further fractionated compound activities flash more light in the pharmacological efficacy[5-7]. Flavonoid fractions such asβ-sitosterol and Quercetin are having potential biological activity. Flavonoids have also been reported to decrease LDL and increase HDL and it also helps in the removal of cholesterol from peripheral tissues to liver for catabolism and excretion. Presence of these phytoconstituents might be responsible for the bioactivities of these plant extracts[8]. Thus, the present preparations of bioactive natural product recommended for therapeutic uses.

Table 1: Solvent systems and derivatization reagents used for detection of flavonoids, saponins using TLC

Biomolecules	**Solvent used**	**Proportion**	**Derivatization Reagent**
Flavonoids	Ethyl acetate: formic acid: glacial acetic acid: water	100:11:11:26	1% Ethanolic aluminium chloride reagent
Saponins	Chloroform: Glacial acetic acid: methanol: water	64:32:12:8	Vanillin sulphuric acid reagent
Sterols	Toluene: ethyl acetate: methanol	8.5:1.5:0.5	Anisaldehyde sulphuric acid reagent followed by heating at 110 ºC for 10min

Table 2: TLC analysis of MEHR and META

Name of the Phytochemical	**Observed R_f values at 366nm**	
	MEHR	**META**
Flavonoids	0.046, 0.076,0.120, 0.184 0.230, 0.276 , 0.384	0.076, 0.184 0.384, 0.538
Sterols	0.08, 0.15 , 0.36 0.5 , 0.6 , 0.68, 0.8, 0.89	0.07, 0.22, 0.35, 0.5, 0.9
Saponins	0.15, 0.27, 0.44. 0.49	0.15, 0.27

Table 3: Solvent systems and spray reagents for HPTLC fingerprint analysis for flavonoids

Compound	Solvent system	Proportion	Derivatization reagent
Flavanoids	Ethyl acetate: Formic acid: Glacial acetic acid: Water	10:0.5:0.5:1.3	Anisaldehyde sulphuric acid reagent followed by heating at 110ºC.

Table 5:Solvent systems for quantitative estimation of different standard compounds in plant extracts using HPTLC

Compound	Solvent system	Proportion
Quercetin .	Toluene: Ethylacetate: formic acid: Glacial aceticacid	3:7:1.1:1
β-sitosterol	Toluene: Ethyl acetate: Formic acid	9:1:0.5

Table 4. HPTLC fingerprint of MEHR and META for flavonoids with Rf values and percentage area at 366 nm.

MEHR at 366 nm			META at 366 nm		
Peak	**Max Rf**	**Percentage Area**	**Peak**	**Max Rf**	**Percentage Area**
1	0.01	0.33	1	0.13	5.00
2	0.09	0.86	2	0.27	2.62
3	0.13	4.09	3	0.47	24.81
4	0.30	5.17	4	0.55	9.90
5	0.36	2.79	5	0.74	36.34
6	0.38	3.37	6	0.78	13.81
7	0.50	20.69			
8	0.58	8.98			
9	0.66	8.27			
10	0.75	44.45			

Table 6. HPTLC fingerprint of MEHR and META for quantitative estimation of β-sitosterol at 366 nm.

Track	Sample	Rf	Area	Concentration (Calculated)
1	MEHR	0.29	1028.35	173.86 ng
2	MEHR	0.28	1004.52	169.46 ng
3	β-sitosterol	0.27	608.99	100 ng
4	β-sitosterol	0.29	1180.74	200 ng
5	β-sitosterol	0.27	1718.74	300 ng
6	β-sitosterol	0.27	2283.81	400 ng
7	β-sitosterol	0.27	2765.76	500 ng
10	MEHR	0.28	730.73	118.91 ng
11	MEHR	0.27	754.61	123.32 ng

Table 7: Quantitative estimation of quercetin at 366 nm.

Track	Sample	Rf	Area	Calculated Concentration
1	MEHR	0.68	4398.78	297.23 ng
2	MEHR	0.66	4395.37	297.97ng
3	MEHR	0.68	4260.33	287.23 ng
4	MEHR	0.68	4239.27	277.23 ng
6	Quercetin	0.66	3080.49	287.23 ng
7	Quercetin	0.68	4478.32	257.23 ng
8	Quercetin	0.68	5774.35	287.23 ng
9	Quercetin	0.68	7041.72	297.23 ng
10	Quercetin	0.66	8287.88	266.23 ng

5. INVITRO ANTIOXIDANT ACTIVITY

Table 8 : **Half maximal inhibitory concentration values of DPPH, Hydroxyl (OH) and Superoxide anion (SO) radical scavenging activities of methanolic extract of Hibiscus radiatus (MEHR) and Terminalia arjuna (META)**

IC50 Values (µg/mL)				
	Standard		Plant sample	
Antioxidant assays	**Ascorbic acid**	**Gallic Acid**	**MEHR**	***META***
DPPH	14.23	ND	139	52.5
SO	ND	119	259	224
OH	166	ND	311	281

6. ANTIMICROBIAL ACTIVITY

Table 9: Antimicrobial activity of Hibiscus radiatus and Terminalia arjuna

Microbial strain (species)	**(Zone of (Inhibition) in mm)**		
	MEHR (4mg/ml)	**META (4mg/ml)**	**Gentamycin**
Bacillus cereus	15.67 ± 1.05	20.36 7± 0.00	15.48 ± 0.23
Klebsiella terrigena	9.04 ± 0.23	11.36 ± 2.32	9.32 ± 0.38
Candida albicans	11.33 ± 0.54	11.23 ± 12.52	14.87 ± 0.00
Pseudomonas aeruginosa	18.33 ± 0.41	17.31 ± 0.00	17.32 ± 32.6
Staphylococcus aureus	17.33 ± 0.23	20.89 ± 0.21	16.53 ± 38.3
Escherichia coli	12 .42 ± 0.54	15.23 ± 0.32	11.23 ± 0.47
Micrococcus mucilaginosus	15.23 ± 0.37	11.2 ± 0.87	15.78 ± 0.32

Screening of Phytoconstituents by TLC

Fig 1: Chromatogram of (1) MEHR and (2) META under UV 366 nm after derivatization. Fluorescence band indicates presence of flavonoids, saponins, sterols.

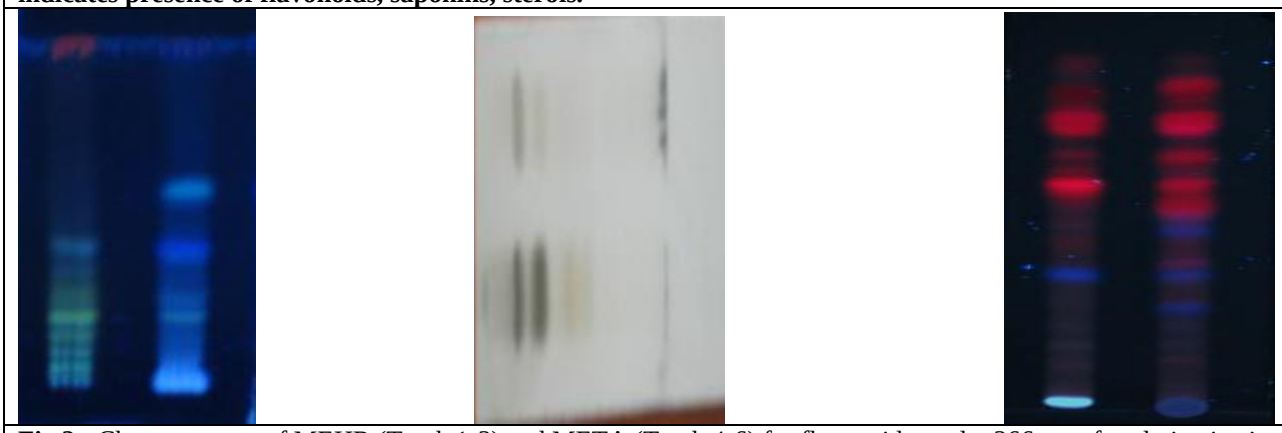


Fig 2 : Chromatogram of MEHR (Track 1-3) and META (Track 4-6) for flavonoids, under 366 nm after derivatization with anisaldehyde sulphuric acid reagent.

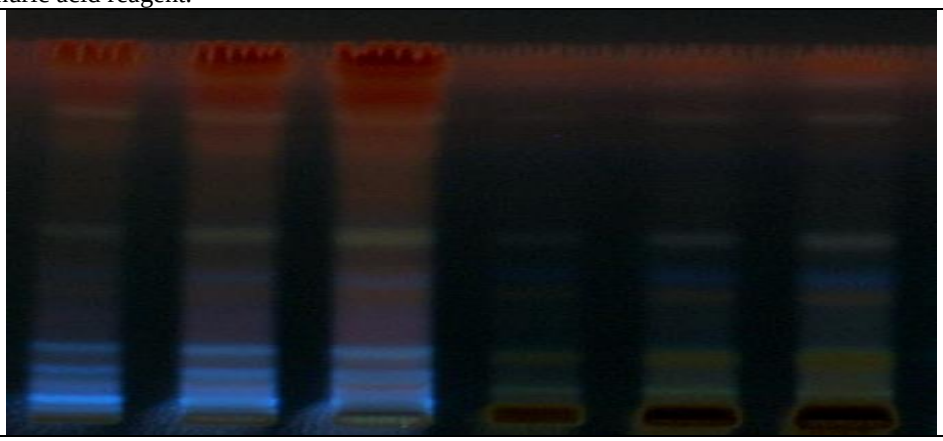


Fig 3: HPTLC peak densitogram display of MEHR for flavonoids at 366 nm

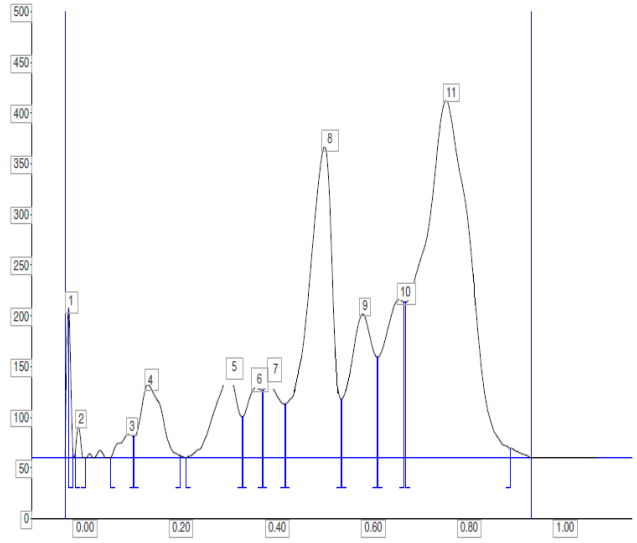


Fig 4: HPTLC peak densitogram display of META for flavonoids at 366 nm

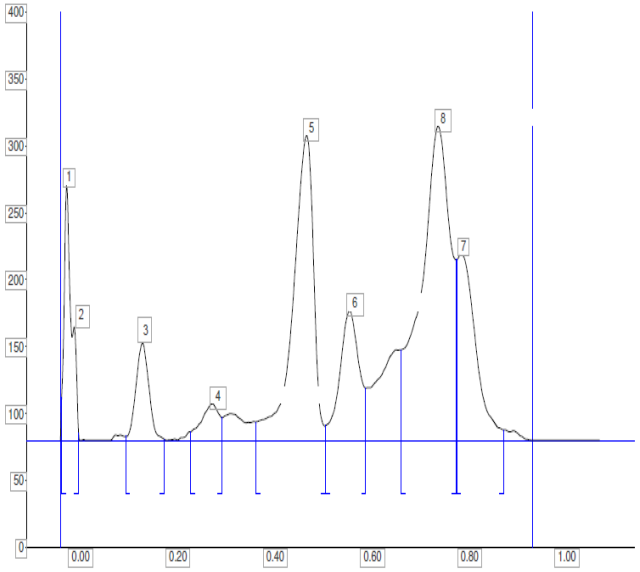


Fig 6 : HPTLC peak densitogram display of MEHR for quantification of β-sitosterol at 366 nm

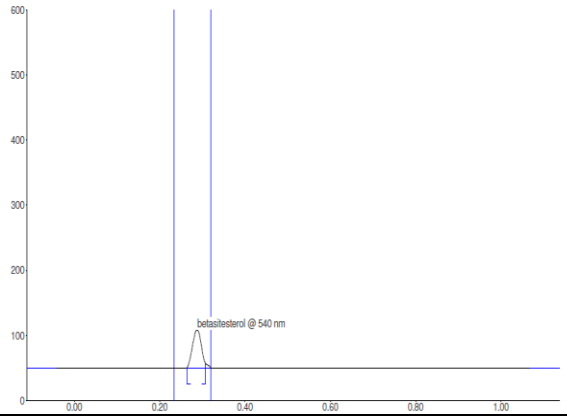


Fig 7:HPTLC peak densitogram display of META for quantification of β-sitosterol at 366 nm

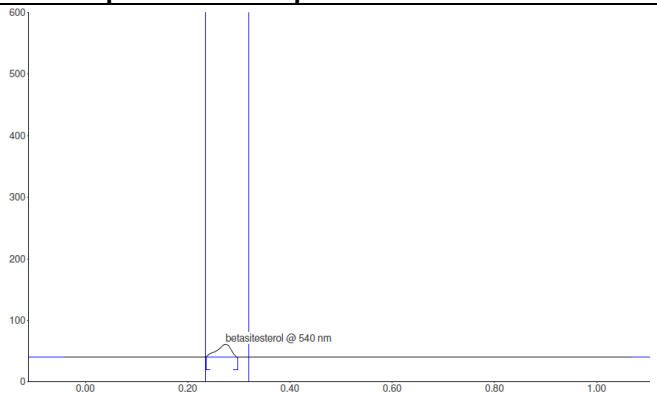


Fig 5: Chromatograms of MEHR, β-sitosterol and META under 366 nm after derivatization with anisaldehyde sulphuric acid reagent.

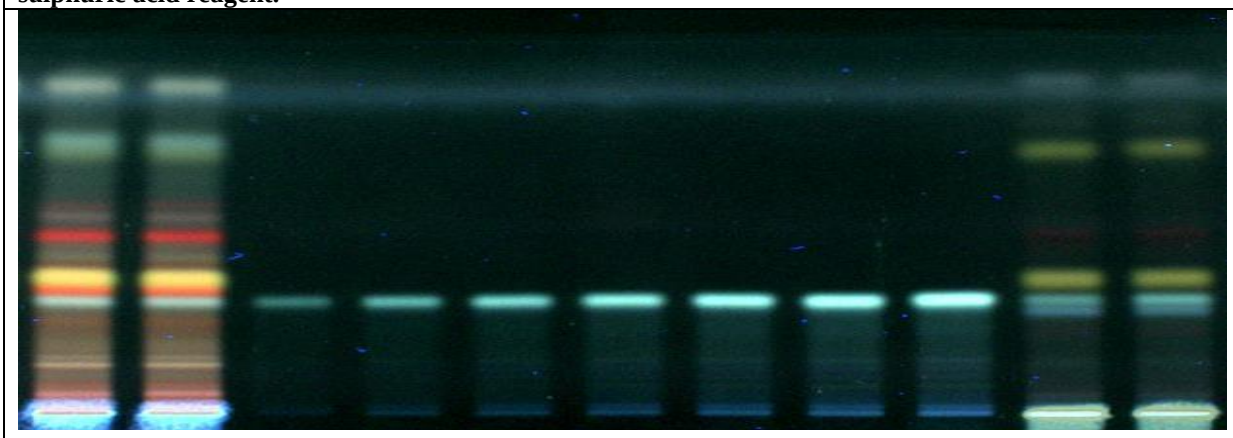


Fig 8: Quantification of quercetin under 366 nm

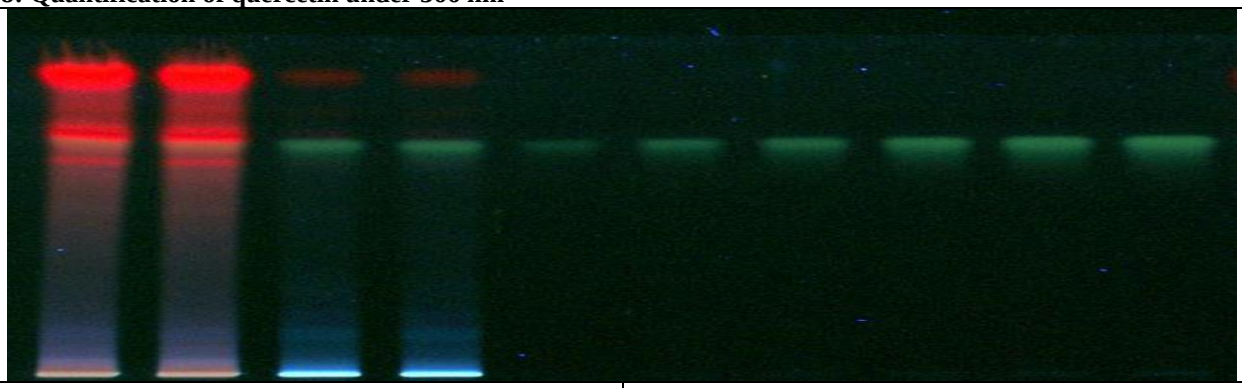


Fig 9 : HPTLC peak densitogram display of MEHR for quantification of quercetin at 366 nm

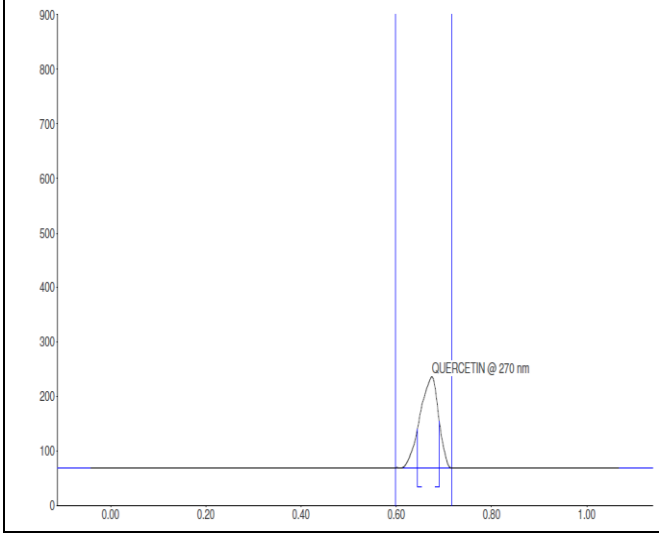
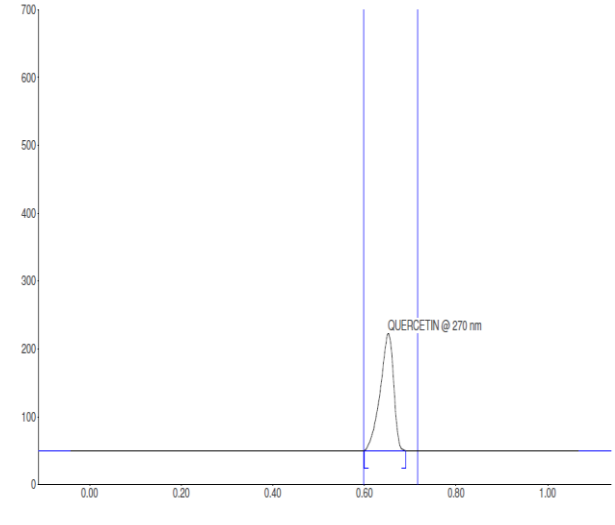


Fig 10 : HPTLC peak densitogram display of META for quantification of quercetin at 366 nm



RESULT

The antioxidant activities of the methanolic extracts of *Hibiscus radiatus and Terminalia arjuna* were measured as the ability of the extracts to scavenge 2-diphenyl-1-picrylhydrazyl superoxide radical and hydroxyl radicals. The methanolic extracts had concentration-dependent antioxidant properties[9]. Thus, MEHR and META extracts had the greatest potential antioxidant activity, which was possibly because they had higher phenylpropanoids levels[10]. reported that phenylpropanoids, the largest group of plant secondary metabolites, are considered to be naturally occurring antioxidant and antimicrobial agents[2].

CONCLUSION

In conclusion, *Hibiscus radiatus and Terminalia arjuna* contain potential antimicrobial and phytochemical components that may be of great use for the development of pharmaceutics as a therapy against various diseases. This plants crude extract could serve as potential sources of antioxidant agents.

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