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HPTLC Fingerprinting in the Standardization of Arthrum Plus Capsule: A Poly Herbal Formulation

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Abstract: Polyherbal formulations are potential for their safety, cost, effectiveness and better acceptability than allopathic drugs. Quality assurance of herbal products may be ensured by proper quality control of herbal ingredients and by means of good manufacturing practice. Therapeutic efficacy of herbal medicine is exerted due to the bioactive constituents of plants.we have develope simple schem for standardization of(ACP).
Prepared polyherbal formulation was standerdize by TLC and HPTLC fingerprinting for deciding identity,purity and strength of poly herbal formulation. The present study was aimed to standardize a polyherbal formulation (Arthrum plus capsule) including traditional drug extracts such as vitex negundo, Boswellia serata, Pluchea lanceolata, yograj guggul. HPTLC fingerprinting profile showed different band patterns at different wavelength under short UV, at 254nm and 366nm ,after derivatisation with vanillin sulphuric acid spraying reagent unique Rf patterns were recorded. Conclusion: APC was authenticated according to pharmacopeial standards as its analysis was important to ensure the quality of drug.
Keywords: Arthrum plus capsule, Polyherbal formulation, , HPTLC fingerprinting.

I. INTRODUCTION

Polyherbal formulations (PHFs) are now influential drug to traditional medicine practices in India. Quality assurance of Ayurvedic formulations is an important step to study chemical profile and safety of drug, as the standardisation of drug is emerging aspect in Ayurvedic drug preparation.[[1,2]] Leading population of world is now relies on phytotherapy for health care as per World Health Organization (WHO). WHO consider phytotherapy is safe, cost effective and more significant without any side effect [[3,4]]. Phytotherapy is now emerging with issues regarding their quality, safety and efficacy.

WHO has given specific guidelines, for the assessment of safety, effectiveness and quality of herbal drug [[5]]. Ayurvedic Pharmacopoeia of India and AYUSH also provides monograph on the preparation of Ayurvedic PHF, that aided in standardisation of drug. A polyherbal Ayurvedic formulation(Arthrum plus capsule)) was prepared by four ingredient such as vitex negundo extract,Boswellia serata extract, Pluchea lanceolata extract, yograj guggul powder. The formulation will be used in the treatment of Rheumatoid Arthritis condition. polyherbal formulation was subjected for standardisation as per pharmacopeial procedures[6,7,8,9]

II. MATERIALS AND METHODS

Arthrum Plus Capsule consists of four ingredients viz., Boswellia serrata (Shallaki), Yograj Guggula, Pluchea lanceolata (Raasna), Vitex negundo (Nirgundi)[10]

The extract of Boswellia serrata (Shallaki),, Pluchea lanceolata (Raasna), Vitex negundo (Nirgundi) and powder mixture of Yograj guggul, were procured from Prashant Pharmaceuticals, Rajpipala, India. While Finished formulation Arthrum Plus capsule were formulated by Vital care Pvt. Ltd., Vadodara, India. All the extracts used in Arthrum Plus capsule were vaccum dried powder form.

All the reagents and instruments used in standardization and evaluation of Anti- -Rheumatoid Arthritis activity of Arthrum Plus capsule were facilitated by Shri B.M. Shah College of Pharmaceutical Education and Research, Modasa.

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A. HPTLC finger-printing of raw Material and Finished Product[11,12,15]

HPTLC is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). The HPTLC was carried out using a Hemilton 100 µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 60F254(Merck), 0.2 mm thickness. HPTLC finger printing technique is useful to identify and to check the purity of raw herbal extracts as well as finished product. Hence forth it is very useful tool in standardizing process of raw herbal extracts and finished products.

B. Steps Involved in HPTLC Analysis

1) *Selection Of Plate And Adsorbent:* Precoated aluminium plates with Silica Gel 60F254 (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used for the detection. The plates were prewashed by methanol and activated at 60°C for 5 min prior to chromatography.
2) *Sample Solution:* Accurately weighed 50 mg of methanol extract of raw material was taken, dissolved in methanol and transferred to a 10 ml volumetric flask. This solution was further used for HPTLC finger-printing.
3) *Application Of Sample:* Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The automatic application devices are preferable. The most recent automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm width with different concentration of all of extract solution viz. 4,6,8,10,12 µl.
4) *Development:* The plate was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the solvent for 60 min (temperature 25.2 °C, relative humidity 40%). The development distance was 8 cm. Subsequently scanning was done. The mobile phase or solvent system for all the ingredients as well as Arthrum Plus Capsule which is given in the Table 1
5) *Detection:* The plate was scanned at UV 366 nm and 254 nm using CAMAG TLC Scanner-3 and LINOMAT-V. Rf value of each compound which were separated on plate and data of peak area of each band was recorded

Table 1: Solvent system for plant extract, and Arthrum Plus Capsule[13,14,15]

Sample	Solvent system
Boswellia serrata.	Ethyl acetate: Hexane (1:1)
	Toluene: Ethyl acetate (9:1)
Vitex negundo	Toluene: Ethyl acetate (9:1)
	Toluene: Ethyl acetate (7:3)
	Toluene: Ethyl acetate: Formic Acid: Methanol (6:6:1.6:0.4)
	Benzene: Ethyl acetate (9:1)
Plueacha lanceolata	Toluene: Ethyl acetate: Formic Acid (5:5:1)
	Ethyl acetate: Hexane (1:1)
	Toluene: Ethyl acetate (9:1)
Yograj Guggula	Benzene: Ethyl acetate (9:1)
	Ethyl acetate: Hexane (1:1)
	Toluene: Ethyl acetate (9:1)
	Toluene: Ethyl acetate (7:3)
	Toluene: Ethyl acetate: Formic Acid: Methanol (6:6:1.6:0.4)

III. RESULT AND DISCUSSION

A. HPTLC Fingerprinting

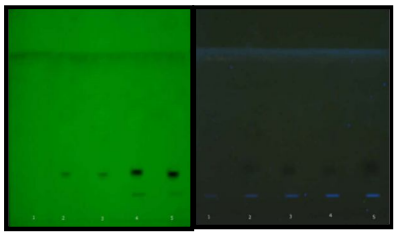
Rf values and colour of the spots in chromatogram developed in diferent mobile system for Arthrum plus capsule were recorded (Table 2). TLC photo-documentation revealed presence of many phytoconstituents with different Rf values and HPTLC densitometric scan of the plates showed numerous bands under short UV. Densitometric scan at 254 nm revealed different peaks corresponding to different compounds in the APC ,Compound with Rf 0.4(0.05 micro gm/ml) 0.24(1.5micro gm/ml) , 0.74(4.45micro gm/ml) , 0.34(0.04 micro gm/ml),0.45(0.03micro gm/ml).lt indicate the concentration of 11-keto- boswelic acid, Quercetin, piprine, gingerol,gallic acid were found in the different extract of Arthrum plus capsule by HPTLC method respectively.

(Table 2). Rf value of the sample

Track		Peak	Max. Rf	Peak Area	Concentration (µg/ml)
Formulation	B. serrata .Ext	1	0.74	2632.9	3.8
	V. negundo extract		0.57	595.5	-
	Vitexin		0.56	555.4	-
	Yograj guggul Piperine	1	0.33	359.5	0.06
	Yograj guggul Gingerol	1	0.33	552.6	1.8
	Yograj guggul Guggulosterol		0.82	438.4	-
	Yograj guggul Gallic acid	1	0.4	423.1	0.05
B. serrata .Ext		1	0.24	6112.6	1.5
P.lanceolata		1	0.74	2956.5	4.4
V. negundo extract			0.56	105.4	-
Vitexin			0.57	796.8	-
Yograj guggul Piperine		1	0.34	333.7	0.04
Yograj guggul Gingerol		1	0.33	929.3	5.6
Yograj guggul Guggulosterol			0.82	602.4	-
Yograj guggul Gallic acid		1	0.45	363	0.03

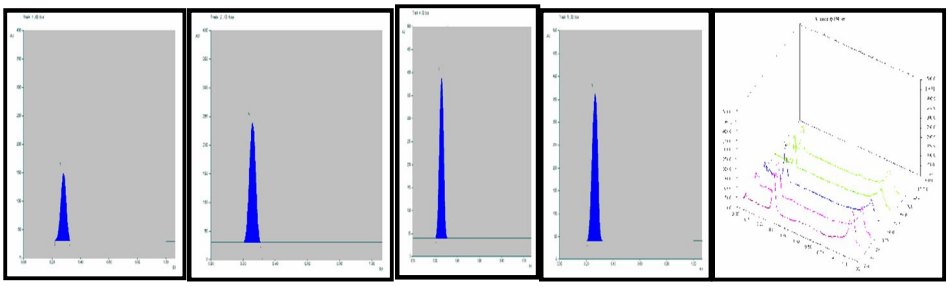
B. Quantitative Estimation of 11-keto-boswellic acid in B. serrata Roxb by HPTLC method

1) Calibration curve for 11-keto-boswellic acid (STANDER)



UV 254 nm UV 366nm

Figure: 1A HPTLC plats of 11-keto-boswellic acid



1 µL 2 µL 3 µL 4 µL 3D-image of 11-keto-b.acid

Figure: 2 HPTLC Chromatogram and 3 Dimage of 11-keto-boswellic acid

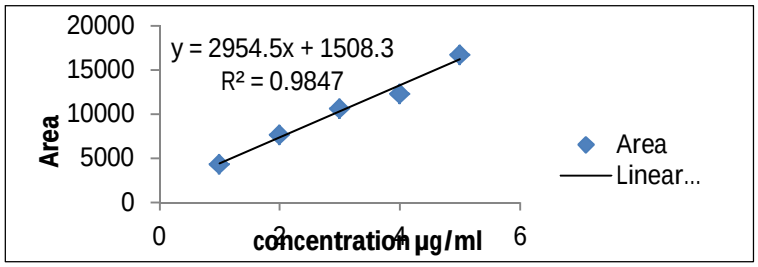
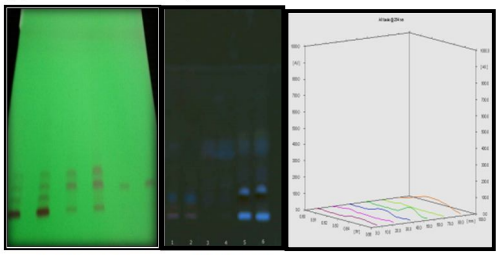


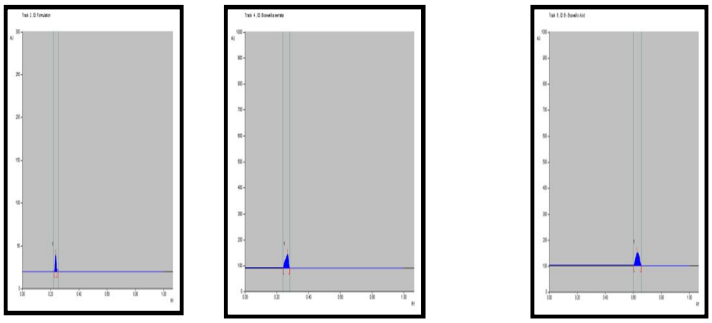
Fig: 3 calibration curve of standard 11-keto-boswellic acid

2) B. serrata Roxb(11-keto-boswellic acid)(in formulation)



UV 254 nm UV 366nm 3D image

Fig.4 HPTLC plats and 3D image of Formulation, B. serrata extract and 11- keto boswellic acid

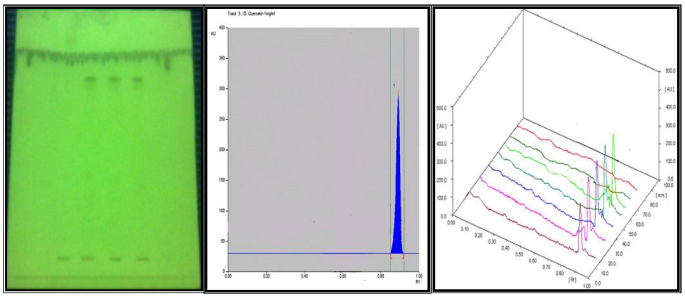


Formulation Extract of *B. Serrat* 11- keto-boswellic acid

Fig: 5 HPTLC Chromatogram of Formulation, *B. Serrata* and 11- keto-boswellic acid

C. Quantitative estimation of quercetinin P.lanceolata by HPTLC method

1) Calibration of Quercetin (Stander)



Standard quercetin 3 D- image of the quercetin HPTLC chromatogram

Fig. 6 HPTLC plate, chromatogram and 3 D- image of the quercetin

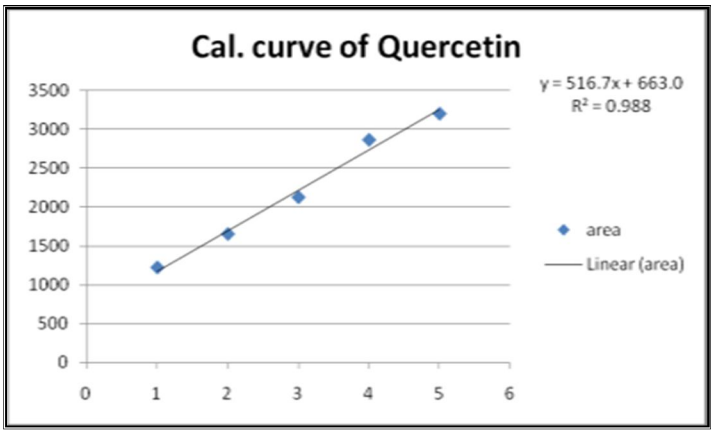


Fig. 7 Calibration curve of Quercetin

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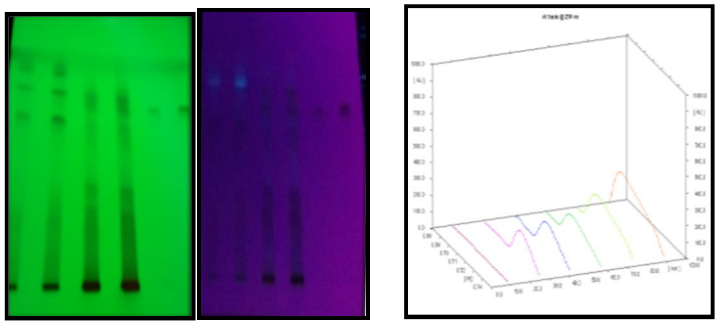
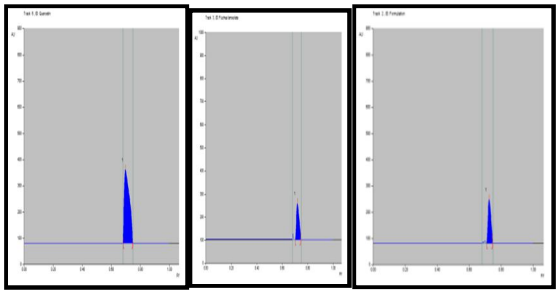
2) P.lanceolata (Quercetin)(formulation)



UV 254nm UV 366nm 3D HPTLC chromatograph

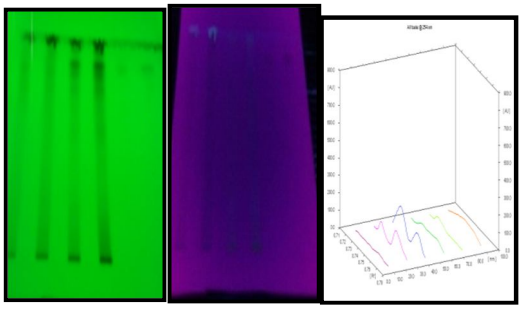
Fig:8 HPTLC plate and 3D chromatogramof formulation, *P. Lanceolata* extracts with quercetin



Formulation Extract of P. lanceolata Quercetin

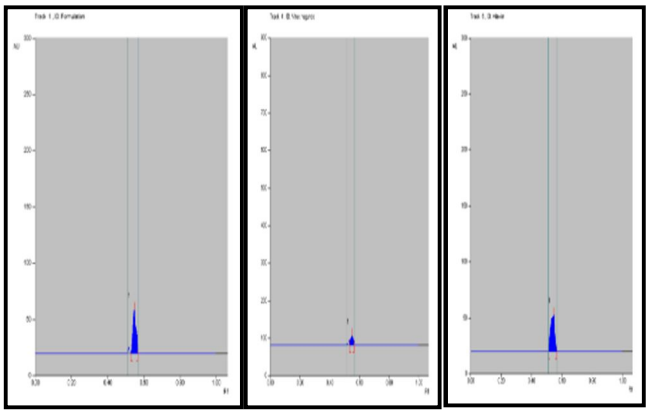
Fig. 9 HPTLC Chromatogramof P. lanceolata extract, formulation and quercetin

D. Estimation of vitexin in vitex negundo extract and Formulation by HPTLC



UV 254nm UV 366nm 3D HPTLC chromatograph

Fig. 10 HPTLC plats and 3D HPTLC chromatogram of *vitex negundo*



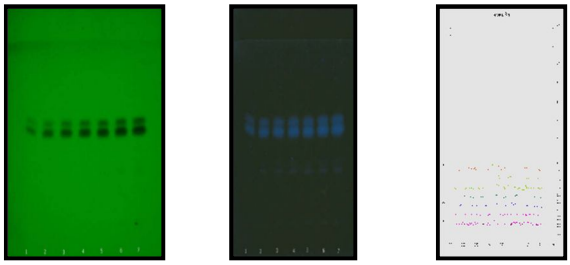
Formulation V. negundo extract vitexin

Fig. 11 HPTLC Chromatogram of v. negundo extract and formulation

E. Quantitative estimation of Yograj Guggul powder by HPTLC method.

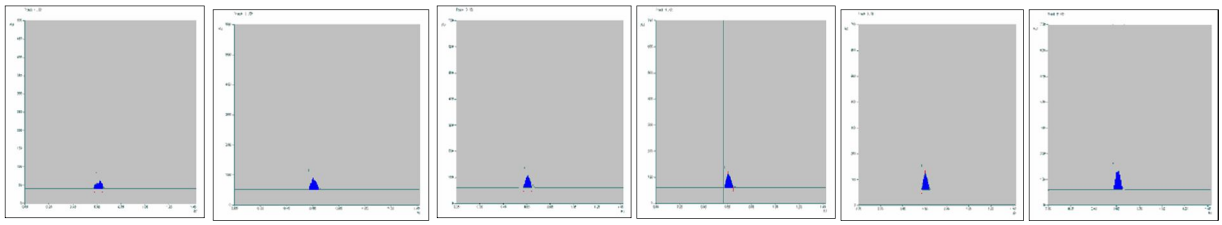
Quantitative estimation of piperin in Yograj Guggul powder by HPTLC method.

1) Calibration of Piperin



UV 254 nm UV 366 nm HPTLC chromatogram

Fig. 12 HPTLC Chromatogram and 3D image of Piperine



1 µL Piperine 2 µL Piperine 3 µL Piperine 4 µL Piperine 5 µL Piperine 6µLPiperine

Figure: 13HPTLC Chromatogram of Piperine

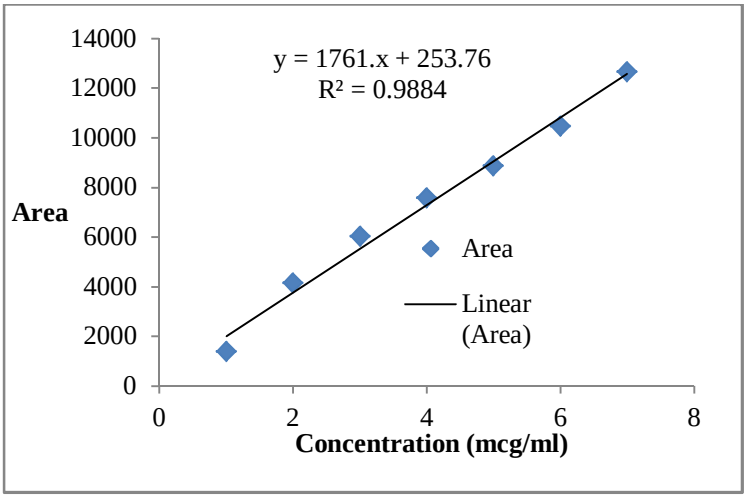
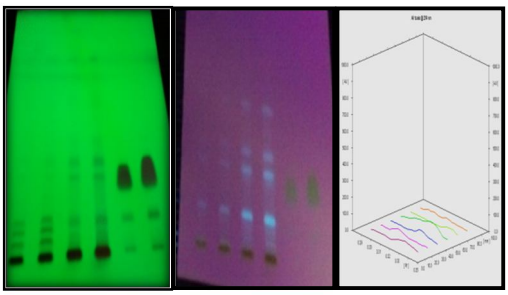


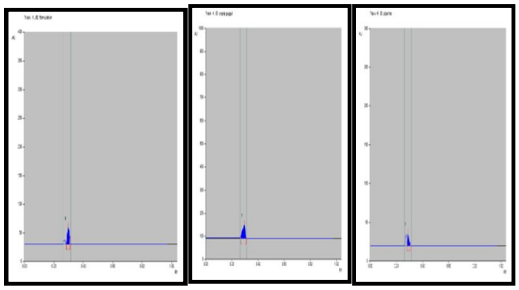
Fig. 14 Calibration curve of piperine

2) Yograj Guggul (Piperine)



UV 254 nm UV 366nm 3D HPTLC chromatograph

Fig. 15 HPTLC and 3D image of formulation, yograj guggul and piperine

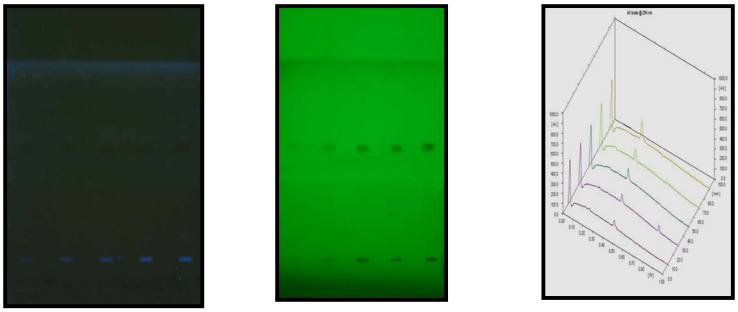


Formulation yograj guggul Piperine

Fig. 16 Chromatogram of Yograj Guggul and formulation

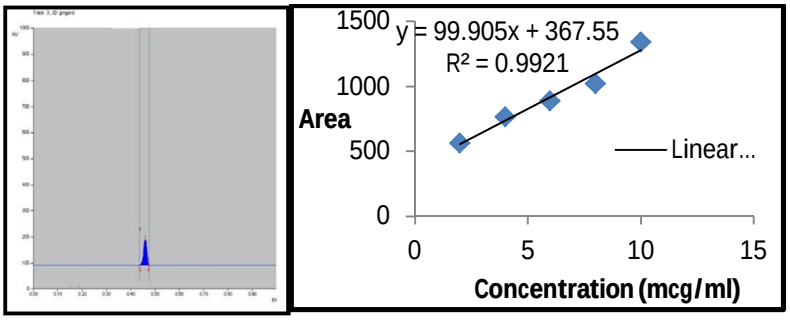
F. Quantitative estimation of Gingerolin Yograj Guggul powder by HPTLC method.

1) Calibration of Gingerol



UV254nm UV366nm 3D image

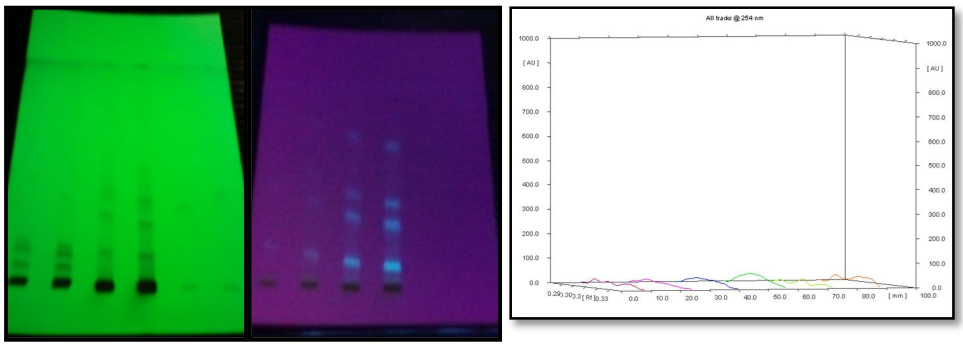
Fig. 17 HPTLC plats and 3D chromatogram of gingerol



HPTLC Chromatograph of gingerol calibration curve of gingerol

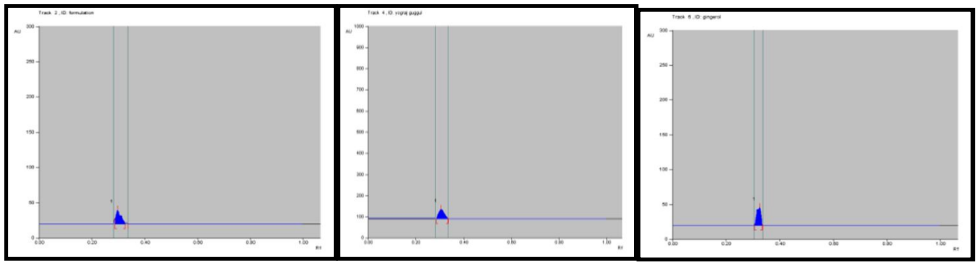
Fig.18. HPTLC Chromatograph and calibration curve of gingerol

2) Zingiber officinales (gingerol)



UV254nm UV366nm HPTLC chromatogram

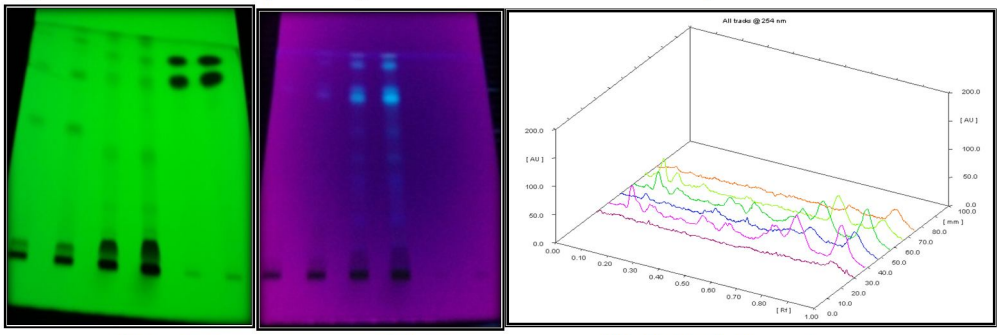
Fig: 19HPTLC plats,3 D chromatogram of Yograj guggul



Formulation Yograj guggul Gingerol

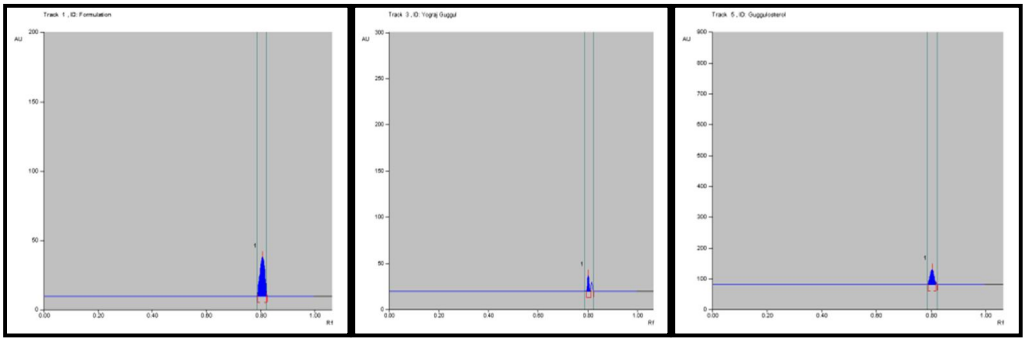
Fig. 20 HPTLC Chromatogram of gingerol, Yograj guggul and formulation

G. Estimationof Guggulosterol in Extract of Commiphora mukul and Formulation by HPTLC



UV 254nm UV 366nm 3D HPTLC chromatogram

Fig. 21 HPTLC plats and 3D chromatogram of Guggulosterol

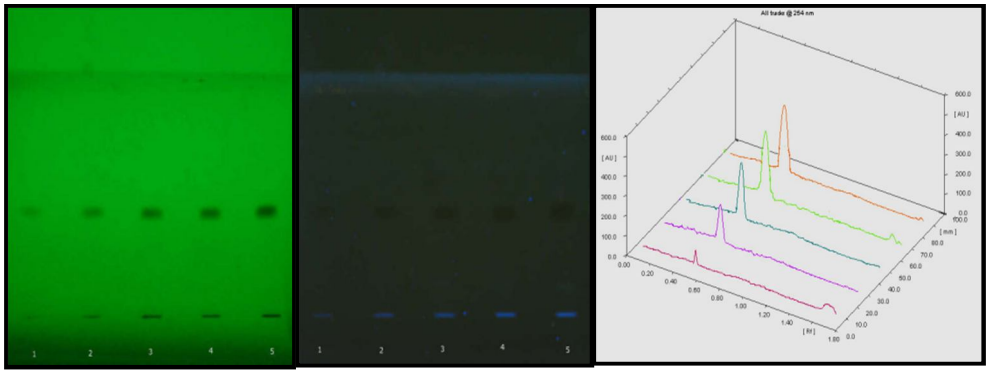


Formulation Yograj guggul guggulosterol

Fig 22 HPTLC chromatogram of Yograj Guggul extract, guggulosterol and formulation

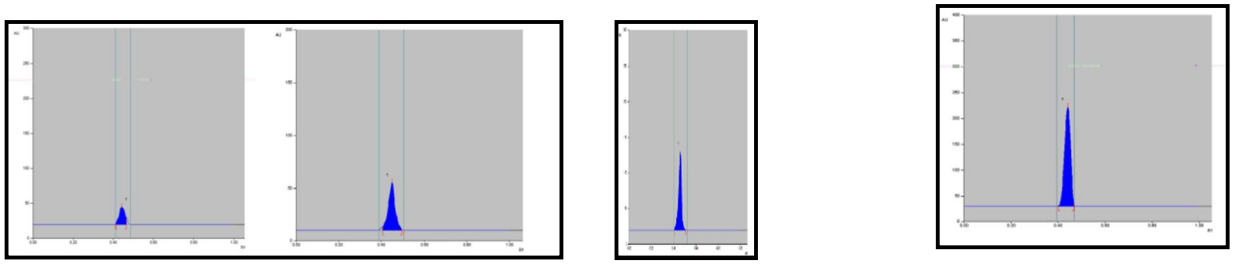
H. Quantitative estimation of gallic acid in Yograj guggul powder by HPTLC method.

1) Calibration of Gallic acid



UV 254nm UV366nm 3-D HPTLC Chromatograph

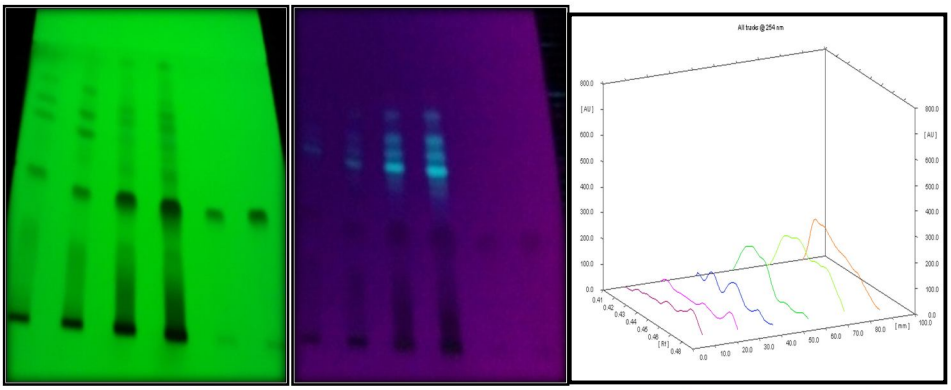
Fig. 23 HPTLC plates and 3-D Chromatograph of gallic acid



3 µL Gallic acid 4 µL Gallic acid 5 µL Gallic acid Calibation curve of Gallic acid

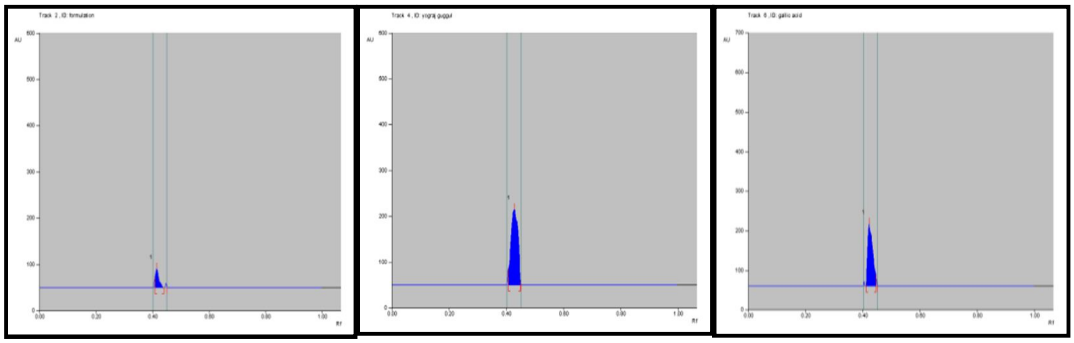
Fig. 24 HPTLC Chromatogram and calibration curve of standard Gallic acid

2) Amla, Baheda and Harde (Gallic acid)



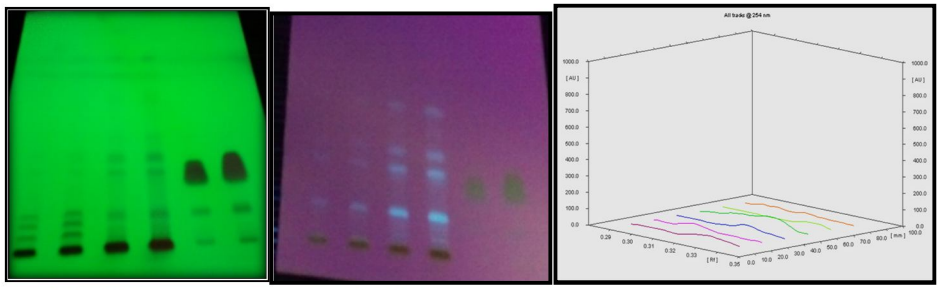
UV 254nm UV 366nm 3D HPTLC chromatograph

Figure: 25 HPTLC plats and chromatogram of Yograj Guggul



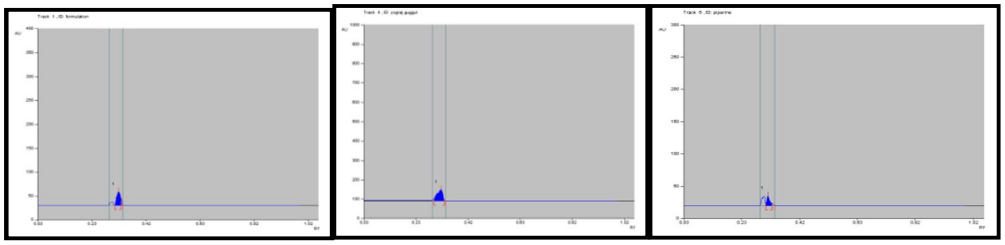
Formulation Triphala Gallic acid

Fig. 26 HPTLC Chromatogram of Gallic acid, Formulation, Extract of Yograj Guggul



UV 254 nm UV 366nm 3D HPTLC chromatograph

Fig. 27 HPTLC and 3D image of formulation, yograj guggul and piperine

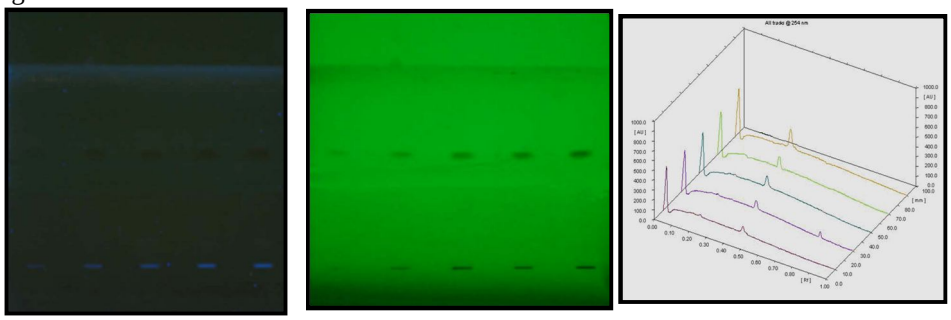


Formulation yograj guggul Piperine

Fig .28 Chromatogram of Yograj Guggul and formulation

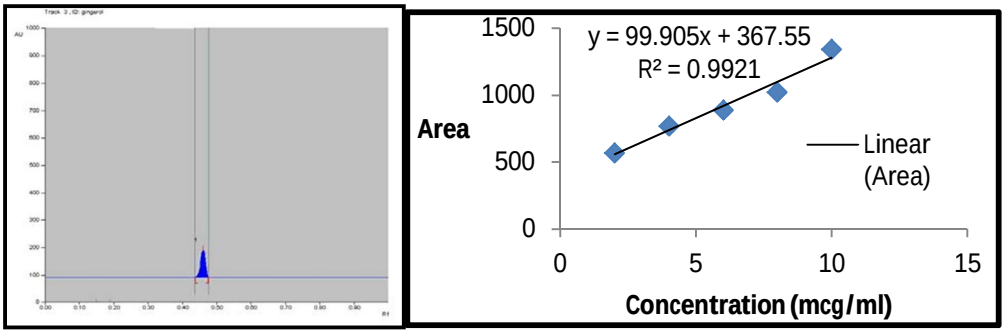
I. Quantitative Estimation of Gingerolin Yograj Guggul powder by HPTLC method.

1) Calibration of gingerol



UV254nm UV366nm 3D image

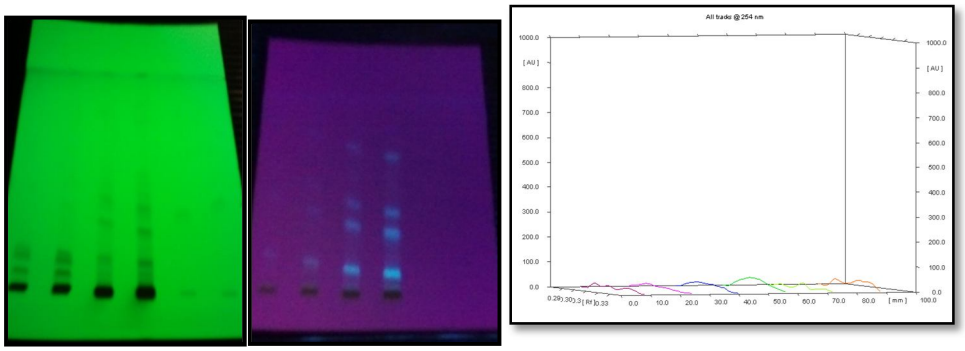
Fig.29 HPTLC plats and 3D chromatogram of gingerol



HPTLC Chromatograph of gingerol calibration curve of gingerol

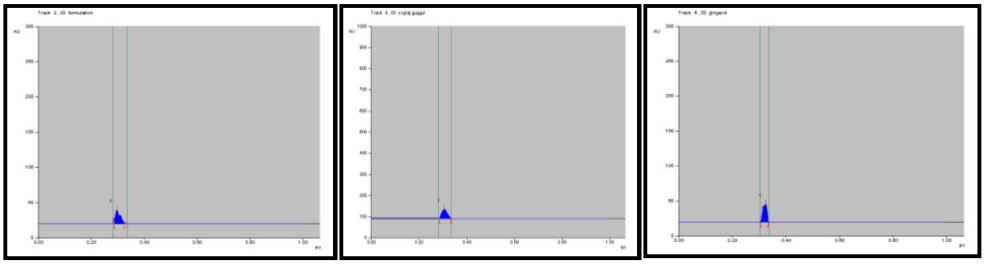
Fig. 30 HPTLC Chromatograph and calibration curve of gingerol

2) Zingiber Officinales (gingerol)



UV254nm UV366nm HPTLC chromatogram

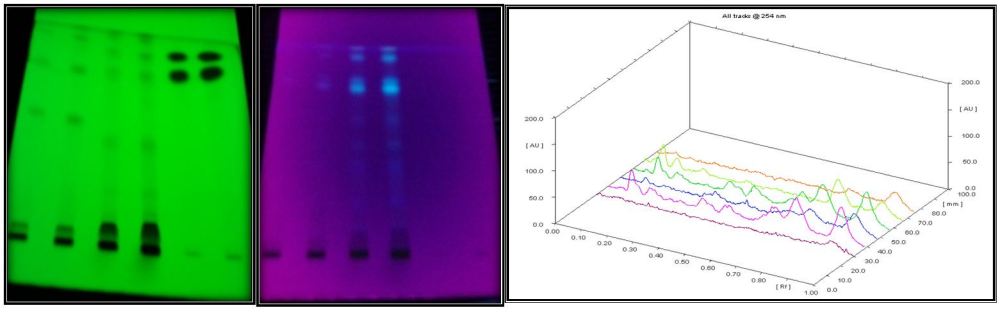
Fig: 31 HPTLC plats 3 D chromatogram of Yograj guggul



Formulation Yograj guggul Gingerol

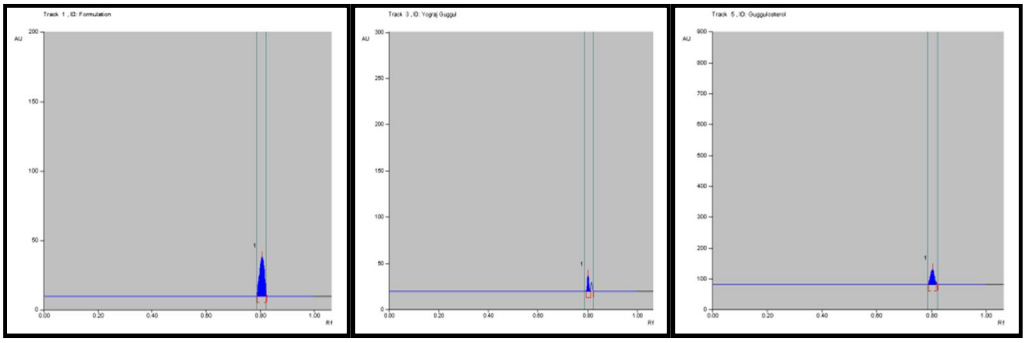
Fig. 32 HPTLC Chromatogram of gingerol, Yograj guggul and formulation

J. Estimationof Guggulosterol in Extract of Commiphora mukul and Formulation by HPTLC



UV 254nm UV 366nm 3D HPTLC chromatogram

Fig.33 HPTLC plats and 3D chromatogram of Guggulosterol

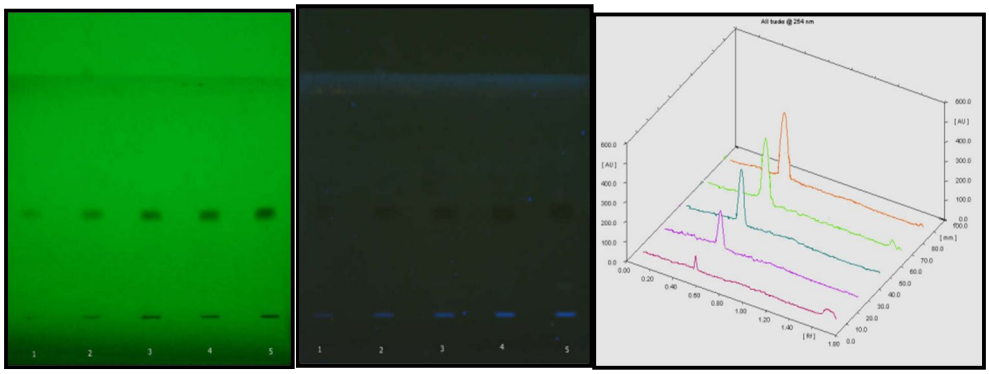


Formulation Yograj guggul guggulosterol

Fig: 34 HPTLC chromatogram of Yograj Guggul extract, guggulosterol and formulation

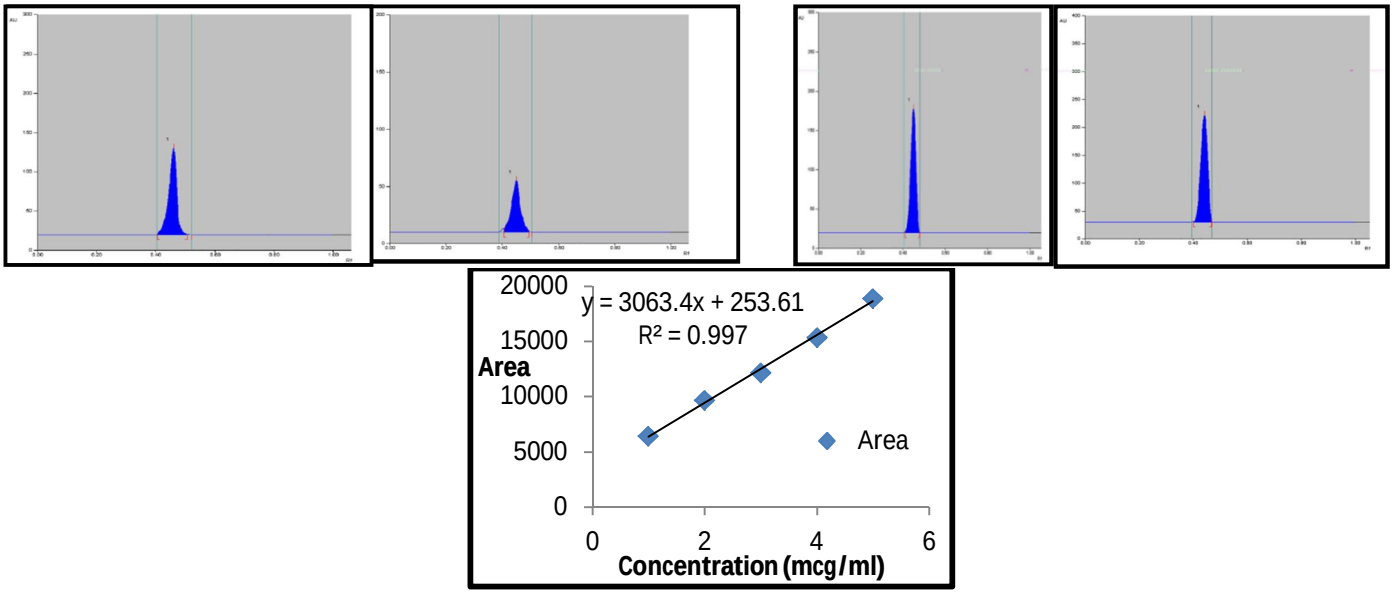
K. Quantitative Estimation of gallic acid in Yograj guggul powder by HPTLC method.

1) Calibration of Gallic acid



UV 254nm UV366nm 3-D HPTLC Chromatograph

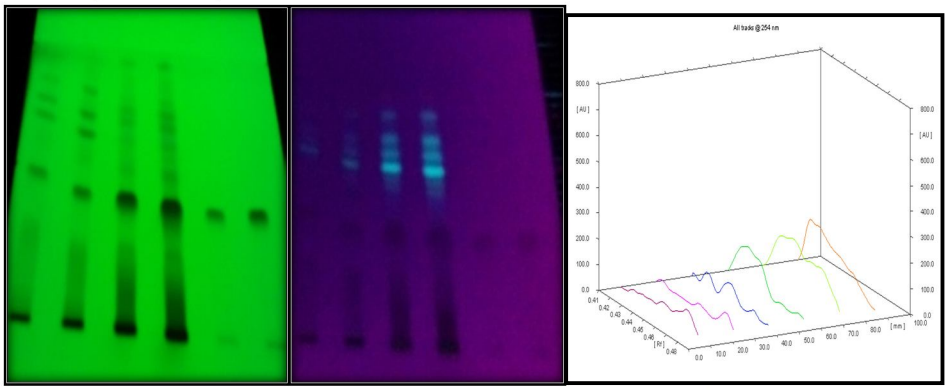
Fig.35HPTLC plates and 3-D Chromatograph of gallic acid



4 µL Gallic acid 5 µL Gallic acid Calibration curve of Gallic acid

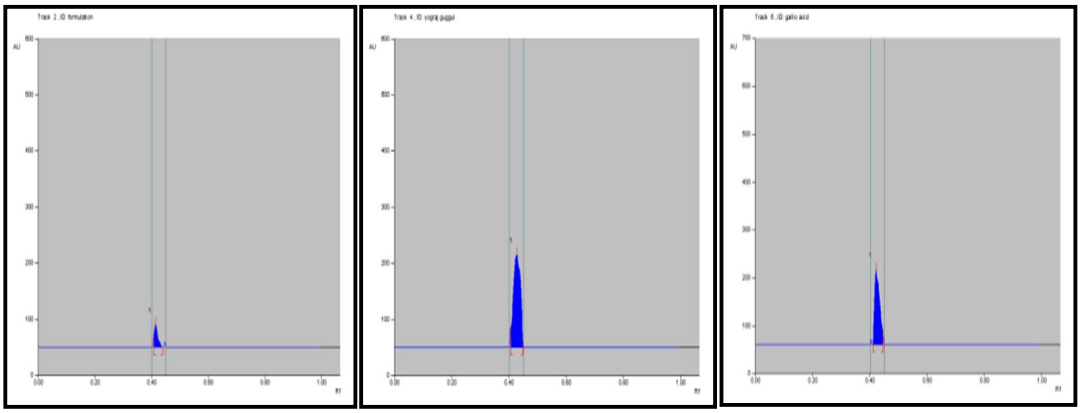
Fig. 36 HPTLC Chromatogram and calibration curve of standard Gallic acid

2) Amla, Baheda and Harde (Gallic acid)



UV 254nm UV 366nm 3D HPTLC chromatograph

Figure: 37 HPTLC plats and chromatogram of Yograj Guggul



Formulation Triphala Gallic acid

Fig. 38 HPTLC Chromatogram of Gallic acid, Formulation, Extract of Yograj Guggul

IV. SUMMARY AND CONCLUSION

Standardization of polyherbal formulation is specified level to assure the quality and efficacy of drug. HPTLC fingerprinting also revealed active constituents at different Rf values**.** Result indicate the concentration of 11-keto- boswellic acid content were found in the extract of *B.serrata* and Arthrum plus capsule by HPTLC method was 0.33mcg/ml and 1.5 mcg/ml with max Rf value 0.24, in extract of p.lanceolata concentration of Quercetin was 3.8,4.4 mcg/ml with 0.74 Rf ,in extract of yograj guggul concentration of piprine 0.06 and 0,04 mcg/mlwith Rf 0.3, gingerol 1.8 and 5.6mcg/ml with Rf 0.33,gallic acid 0.05 and 0.03mcg/ml wirh 0.45 Rf respectively.

A. Conclusion

in conclusion Data suggested that Arthrum plus capsule and its ingredient were consistent with various quality and purity parameter such like HPTLC Analysis.

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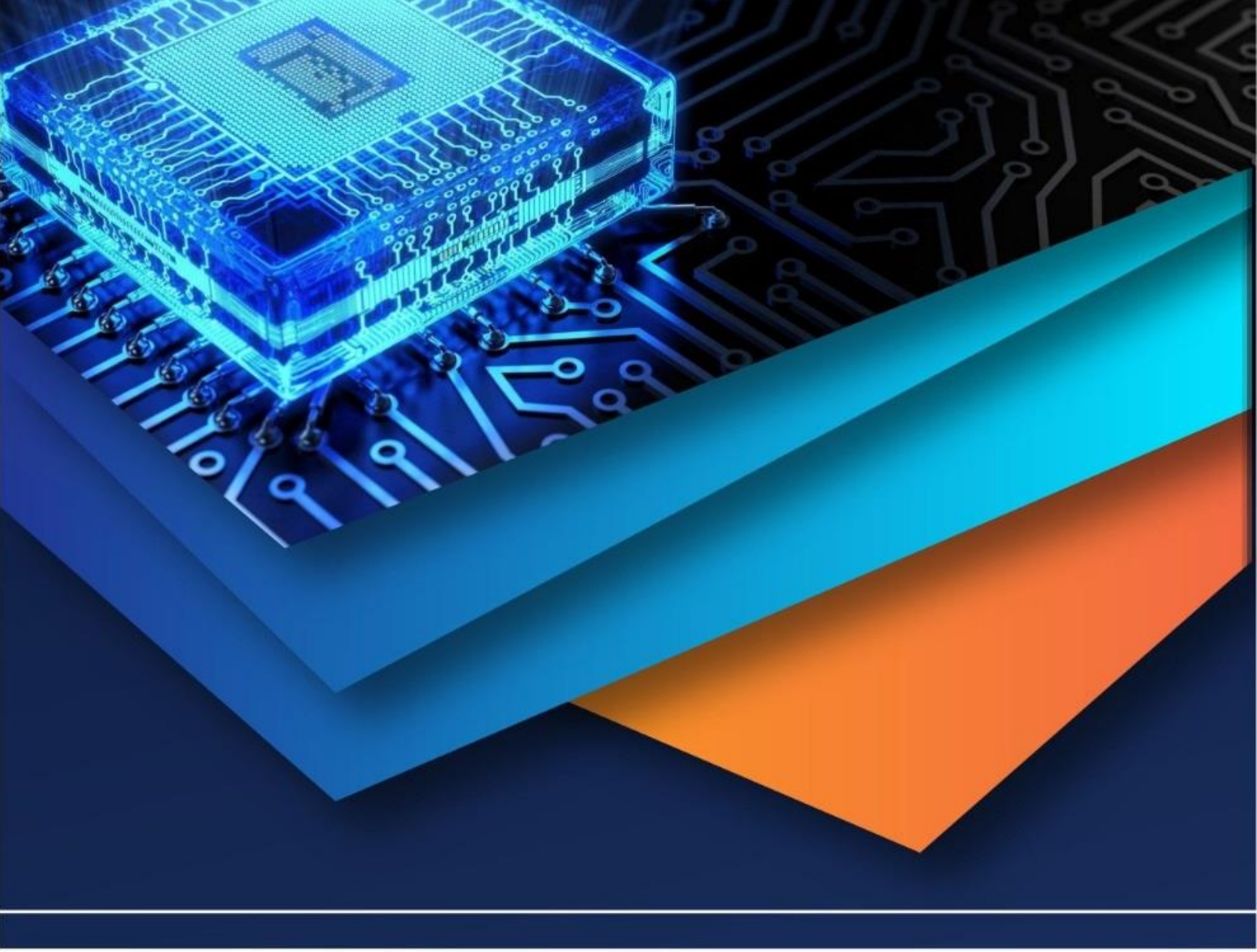

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