Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Hptlc Method For Isolation, Identification And Quantification Of Luteolin From BiophytumSensitivum Linn.

Archana R Dhole[1]* and Dr. V.C.Yeligar[2]

Rajarambapu College of Pharmacy Kasegaon, Tal- Walva, Dist- Sangli
Pin- 415409. E-Mail-archu_d1008@yahoo.co.in
Principal, Sarojini College of Pharmacy, Kolhapur.Tal-Karvir, Dist-Kolhapur.
E-mail- yveerendra27@gmail.com

ABSTRACT

Biophytumsenssitivumlinn generally known, as lajalu is a medicinal plant widelyused in herbal system of medicine. Plant shows positive tests for chemical constituents like phenolic, flavonoids ,saponin, alkaloids . Plant exhibits a variety of pharmacological action such as hepatoprotective, anti-inflammatory, anti-microbial, antiartherosclerotic and anti-tumor activity. Among the complex mixture of biologically active compounds in the Biophytumsensitivum ,Luteolin a flavonoidalconstituent has been used as an analytical marker indicative of the quality of theplant. Our ultimate aim is there is isolation and estimation of marker compoundfromBiophytumsenssitivumlinn . Presence of isolated luteolin was further confirmed by IRSpectra of isolated and authentic samples of luteolin NMR spectra and GC-MS spectra ofisolatedluteolin. In the present study a simple and, sensitive HPTLC method has been developed forLuteloin. This indicate simple, precise and accurate method gave good resolution from other constituents present in the plant extract and has been fruitfully applied for analysis and quality control of herbal materials.

KEY WORDS- Biophytumsensitivumlinn, luteolin,HPTLC

***Corresponding author**

Archana R Dhole

Rajarambapu College of Pharmacy Kasegaon,

Tal- Walva, Dist- Sangli

Pin- 415409. E-Mail-archu_d1008@yahoo.co.in

1) INTRODUCTION-

Biophytumsensitivumlinn. (Oxalidaceae), is an important and widely used medicinal plant. Biophytumsensitivumlinn used for various conditions such asinflammation, arthritis, wounds, tumors and burns, gonorrhea, stomach ache, asthma, cough.Various constituents were reported from extract such as flavones, cupressuflavone and amentoflavone, three flavonoids, luteol in 7-methyl ether, isoorient in and 3'-methoxyluteol in 7-o-glucoside, as well as two acids, 4 -caffeoylquinicacidand 5- caffeoylquinic acid .

Luteolin, 3′,4′,5,7- tetrahydroxyflavone,belongs to a group flavonoids that are found widely in the plant kingdom. Flavonoids are polyphenols that used in defending plant cells against microorganisms, insects and ultraviolet Irradiation.[1] Evidence from cell culture and animal and human population studies suggested that flavonoids are also beneficial to human and animal health. Flavonoids are found more in foods, e.g., vegetables, fruits and medicinal herbs, flavonoids are common nutrients that are antioxidants, estrogenic regulators and antimicrobial agents.[2] Plants rich in luteolin have been used in Chinese traditional medicine for treating various diseases such as hypertension, inflammatory disorders and cancer. With multiple biological effects such as antiinflamatory, antiallergy and anticancer. Luteolin functions as either an antioxidant or a biochemically prooxidents. Spectral analysis plays an important role in the quality control of complex herbal medicines.for the isolation Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they may appropriately represent the chemical integrities of the herbal medicines and their products, and therefore, be used for authentication and identification of herbal plants. High performance thin layer chromatography (HPTLC) is a more efficient faster method and the results are more reliable and reproducible. Combined with digital scanning profiling, HPTLC also provides accurate and precise retention factor (Rf) values and quantitative analysis of sample by in situ scanning densitometry aided by formation of easily detected derivatives by post chromatographic chemical reactions as required along with a record of the separation in the form of a chromatogram, [8,9,10]with fractions represented as peaks with defined parameters including absorbance (intensity), Rf, height and area. However there is no method available for isolation and estimation of marker compound from Biophytumsensitivumlinn. So our main Aim is to isolate the flavonoid compound and identified with the help of IR, UV, TLC, HPTLC, GC-MS, NMR.

2) MATERIALS AND METHODS

2. 1 Materials And Equipments

All the chemicals and reagent used were of laboratory grade and were procured from Rajarambapu College of Pharmacy, Kasegaon (from manufactures of LobaChemie, Mumbai , Hi Media Lab Mumbai, fine chemicals, Mumbai, Sigma-Aldrich, Mumbai., Finar reagents, Ahmadabad, Merck, Mumbai, Labin, Mumbai ,MolyChem, Mumbai **,**Research lab, Islampur**)**As

2.2 Experimental Methodology

1. Procurement and authentification of selected Plants species.
2. Extraction of bioactive fractions.
3. 2.3Extraction methods: preparation of extract by soxlet apparatus
4. 2.4. Isolation of active principle from Extract
5. 2.5 Identification of active principle from Extract

3 RESULTS AND DISCUSSION

3.1Authentication Plant material

The plant specimens for the proposed study were collected from the Sangli Dist. and authenticated by Botanical Survey of India, Pune.

3.2 Preparation of Crude Extract

Samples of*Biophytumsensitivum*were collected. Specimen was stored in air tight container at 25^0 C for further study.The plant material (500 g) was extracted with ethanol using a Soxhlet apparatus.[11]Isolation, identification and quantification of Luteolinwas carried out by HPTLC, IR and NMR spectral studies in the plant species.

3.3 Thin layer chromatographywas performed using silica gel G as adsorbent. Slurry of silica gel was prepared in distilled water. The slurry was applied to get a thin layer of 0.1 mm thickness over a clean and dry glass plate of 10 x 20 cms size by an applicator. The plate was activated at 110 ± 1^oC for one hour. After TLC extract was applied for Column chromatography As shown in **table no-1.** It is noted that RF value was TLC plate was developed in the solvent system and single spot, was observed for ethanol extract at the same $R_f$value 0.72, 0.42, 0.31 with the help of iodine vapours.

Table no – 1 Details of TLC

Adsorbent	: Silica gel G
Thickness	: 0.1 mm
Plate size	: 10 x 20 cms
Activation temperature	: 110 ± 1^{o}C for one hour
Mobile phase	:Toluene : ethyl acetate: formic acid, 6:4:0.5 (v/v/v)

A) 3.4 Column chromatography of Alcoholic extractof*biophytumsensitivumlinn.*

1)Isolation of active principle from Ethanolicextract of *Biophytumsensitum*L

ii) Column chromatography of extract

The following fractions were collected and were used for isolation purposes

Table – 2 Elution scheme for column chromatography of extract of*Biophytumsensitum (L)*

		Rf	Colour developed
Spot 1	**7.2cm/10cm**	**0.72**	**Light orange**
Spot 2	**4.3cm/10cm**	**0.43**	**Orange**
Spot 3	**3.3cm/10cm**	**0.33**	**Orange**

Elution of the extract was done sequentially with solvent systems of gradually increasing polarity using hexane, chloroform, ethylacetate and methanol in the following ratio: Hexane: chloroform 100:0, 80:20, 60:40, 40:60, and 20:80; Chloroform: ethylacetate 100:0, 80:20, 60:40, 40:60, and 20:80; Ethylacetate: methanol 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100. A measured volume (400 ml) of each solvent combination was used in the elution process.Fractions were analyzed by UV and same λ max fractions were collected. Then evaporated to dry at room temperature. After dryingwhite crystals were obtained,which were further used for UV, IR, NMR and GCMS

1) Chartarization of active principle fromextract of *Biophytumsensitum*L

a) Melting point of isolated compound was found to be 227-229^{0}c

b) UV Spectrum

The UV spectrum of fractions obtained by column chromatography is shown below:

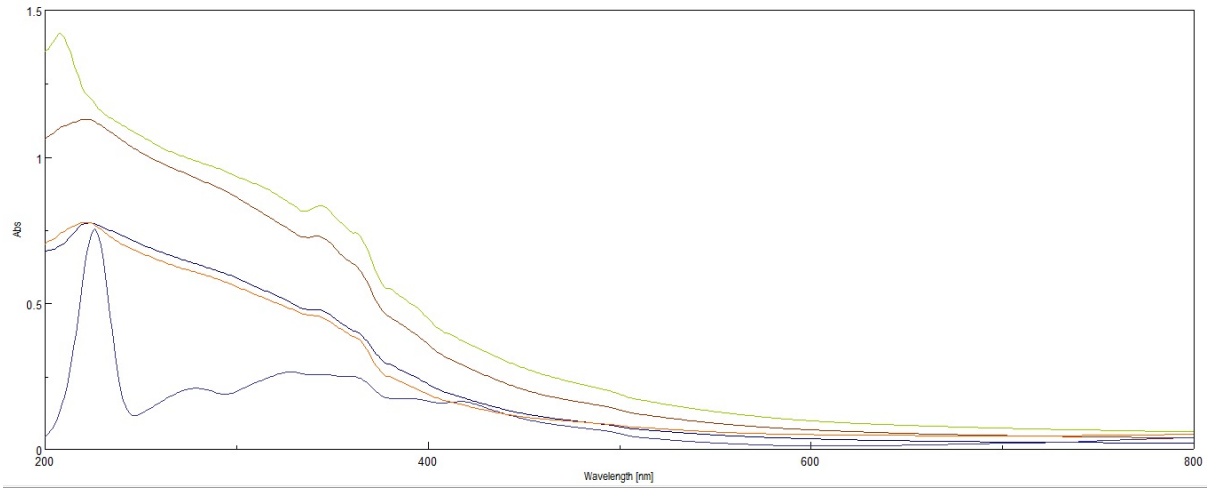


Figure no- 1 UV spectra ofsimilarfractionsisolated by coloumchramatograpy.

3.4 IR and NMR Spectral studies: Infrared spectra were obtained onJasco FT/IR-4600.As shown in Figure no-2and table no -3

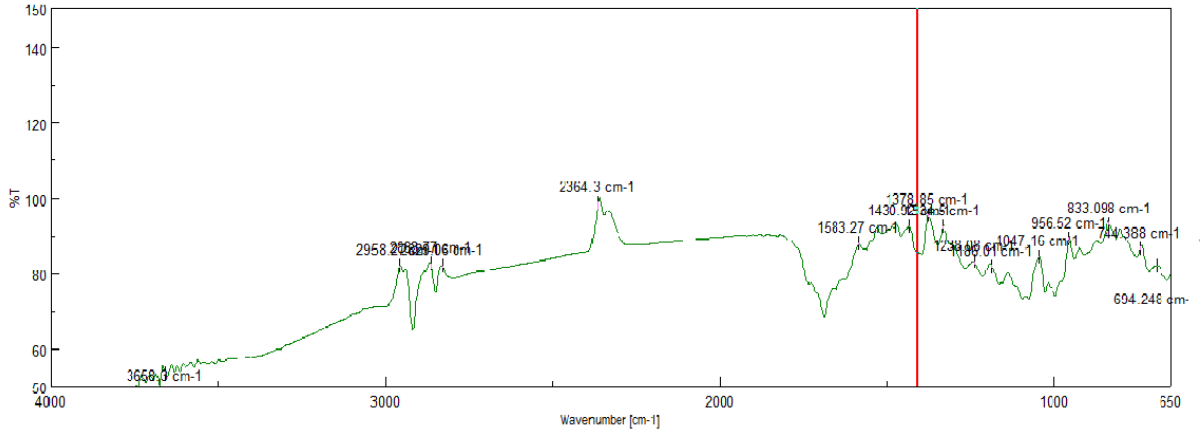
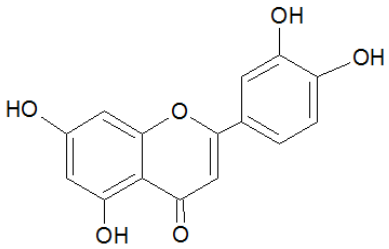


Fig. no. 2IR spectra of isolated compound obtained from *Biophytumsensitum*L

Table – 3IR study of isolated compound obtained from *Biophytumsensitum*L

	Wave number(cm-1)	Functional group
	3656.3 cm-1	Presence of stretching vibration of phenolic groups containinghydrogen bonding.
	2958.26 cm-1	stretching vibration of aromatic (C-H) group
	2364.3	Bending vibration of aliphatic (C-H) group
	1583.27. cm-1	indicates stretching vibration of aromatic (C=C) group
	1430.96 cm-1	stretching vibration of aromatic (C=C) group
	833.098,744.388,694.248	C-H bending of aromatic hydrocarbon

The NMR spectra H1 NMR, ^{13}C NMR were obtained with a Bruker instrument with NMR software .Luteolin had a melting point of 328-330C, molecular weight of 286.24 and yellowish in

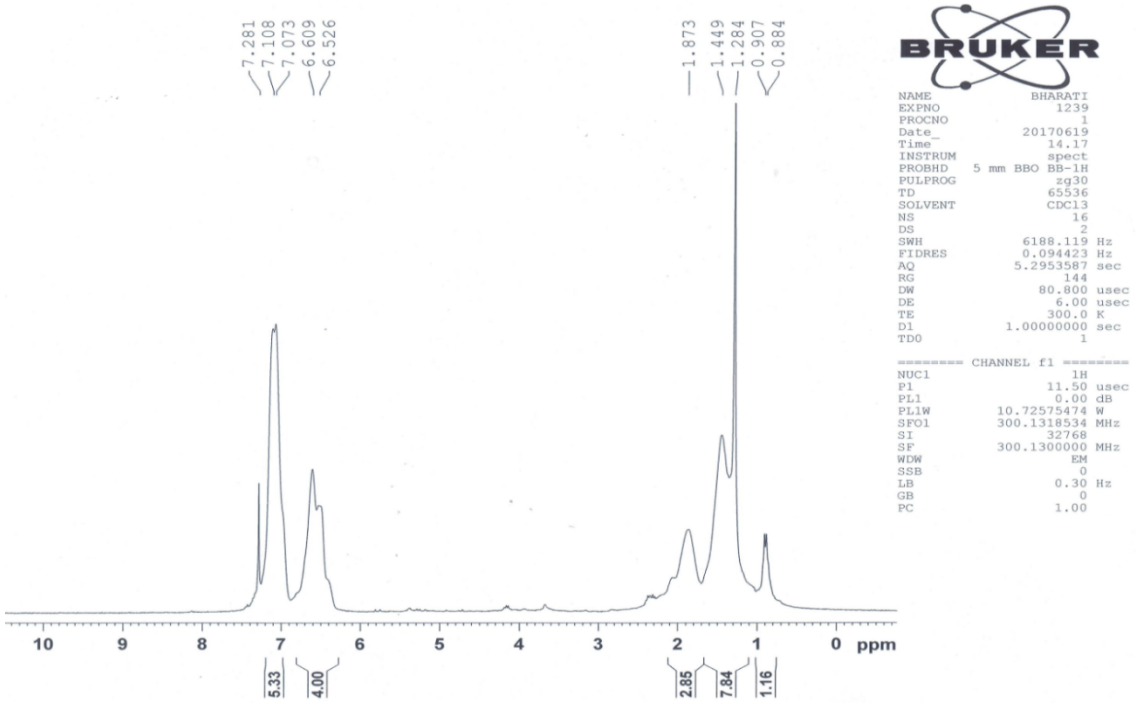
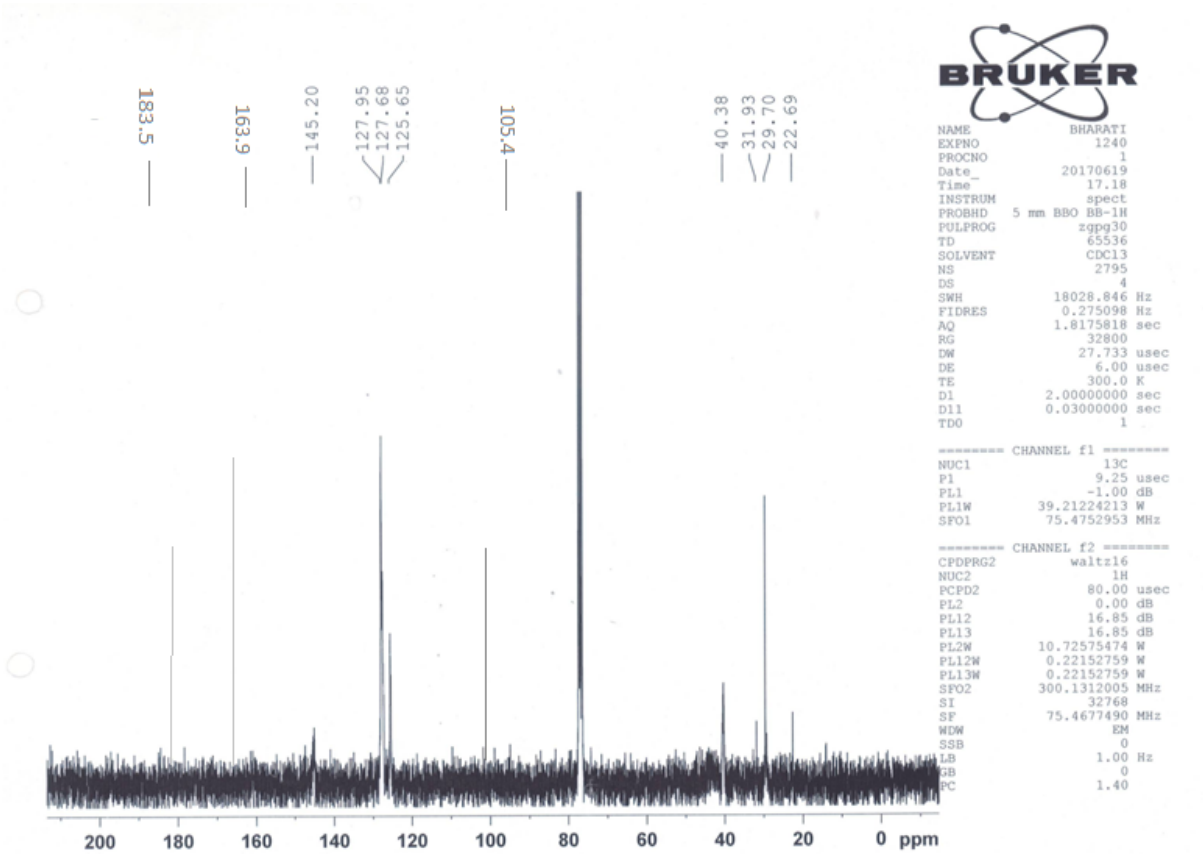


Fig.no. 03 H1-NMR spectra of isolated compound obtained from *Biophytumsensitum*L

Table no.4 H1-NMR study of isolated compound obtained from *Biophytumsensitum*L

Sr.No	1H Nmr values	Interpretation
1	6.60	H3 (1H), H-6(1H)
2	6.52	H8(1H)
3	7.10	H 2'(1H)
4	7.28	H6' (1H)
5	7.06	H-5'(1H)



i) C13-NMR

Fig.no. 04 C13-NMR Spectra of isolated compound obtained from *Biophytumsensitum*L

Table no. 5 C13-NMR study of isolated compound obtained from *Biophytumsensitum*L

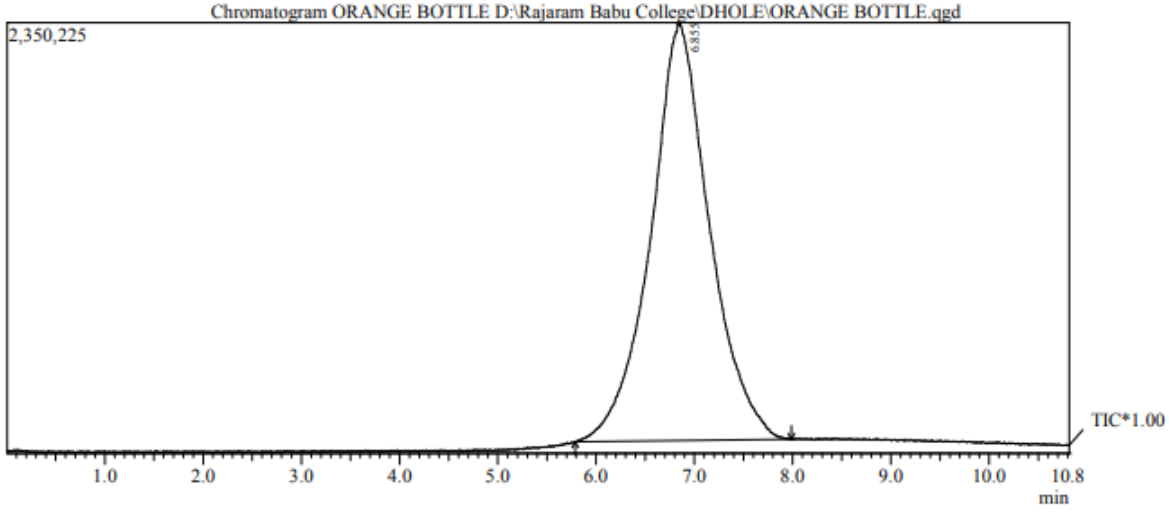
Carbon	C13 NMR Values
2	163.9
3	105.4
4	183.5
6	105.4
7	163.9
8	105.4
9	145.2
10	105.4
1’	105.4
2’	127.95
3’	125.6
4’	163.9
5’	105.4
6’	127.6

Colour. The molecular formula was deduced as C15H10O6. The detailed bond connectivity and assignments of all the 1H and 13C NMR signals were obtained from the shivaji university Kolhapur. As shown in figure no - 3and table no- 4 and figure no 4 and table no-5

3.5GC-MS Spectra- TheGC-MS Spectrawere obtained with GC-MS Single Quadrupole Mass Spectrometer GCMS QP 2010- shimadzu for analysis in shivaji university Kolhapur.

Samples wereinjected in a split mode using a split ratio of 1:1, an inletand transfer line temperature of 250 ^{0}C, and a constantHe flow of 1.0 ml min_1. Separation was achieved with atemperature program with an initial temperature of 80^{0}C, and initial time of 2 min which was then ramped to315 ^{0}C,C at 5^{0}C,min_1 and held at 315^{0}C, for 12 min. TheMS source was maintained at 250 ^{0}C,and the quadrupoleat 150^{0}C,. Mass spectra were recorded while scanning from 40–650 m/z following optimization of MS parameters.As shown in Fig- no-5

Analyzed by : ANIL V MOHITE
Analyzed : 8/1/2017 5:30:44 AM
Sample Name : ORANGE BOTTLE
Sample ID : ORANGE BOTTLE
Data File : D:\Rajaram Babu College\DHOLE\ORANGE BOTTLE.qgd
Method File : D:\DI-method.qgm
Tuning File : D:\Tuning 2017\With Rtx-5 60m_24 March 17.qgt



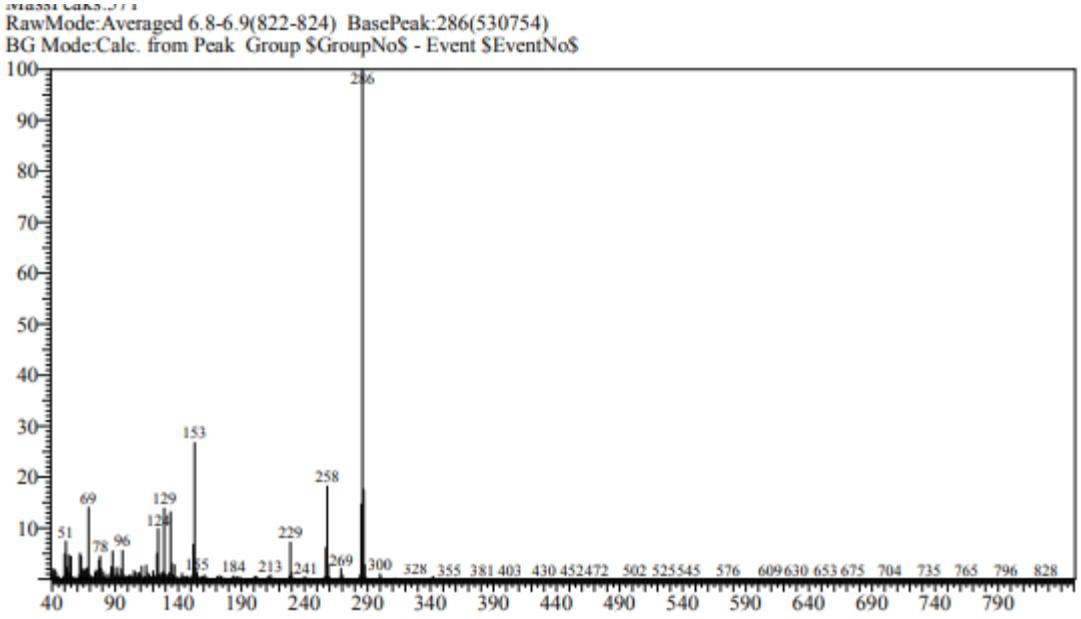
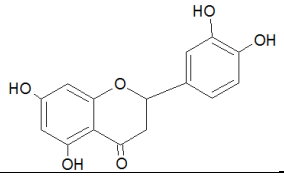
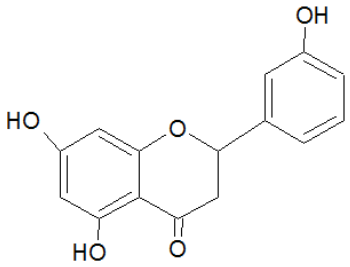
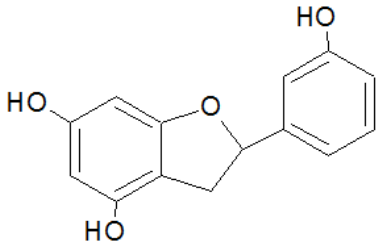
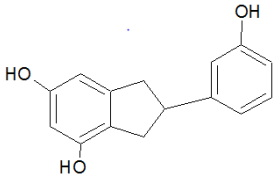
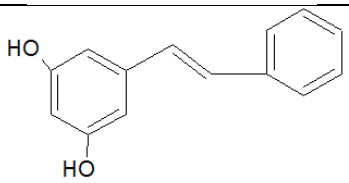
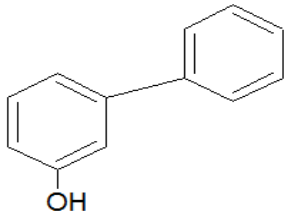
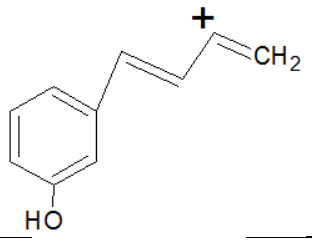
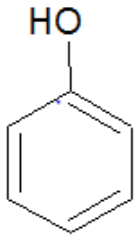


Fig.no.05 GCMS spectra of isolated compound obtained from *Biophytumsensitum (L)*

Table –06 Fragmentation pattern of isolated compound obtained from *Biophytumsensitum (L)*

M/E	Justification for fragment
	269 eliminate HO group
	258 eliminate CHO group
	229 eliminate CHO group
	213eliminate O group
	184C-OH group
	1512C- OH

	129 ring opining
	96 eliminate side chain

3.6 HPTLC Chromatographic conditions

Stationary Phase: Precoated silica gel plates Merck 60 F254 (200 x 100 mm)

Mobile Phase : Toluene : ethyl acetate: formic acid, 6:4:0.5 (v/v/v)

Spotting device :Linomat V Automatic sample spotter, CAMAG (Switzerland).

Development Mode: CAMAG twin trough chamber, CAMAG Densitometer: TLC Scanner III, CATS software, CAMAG.

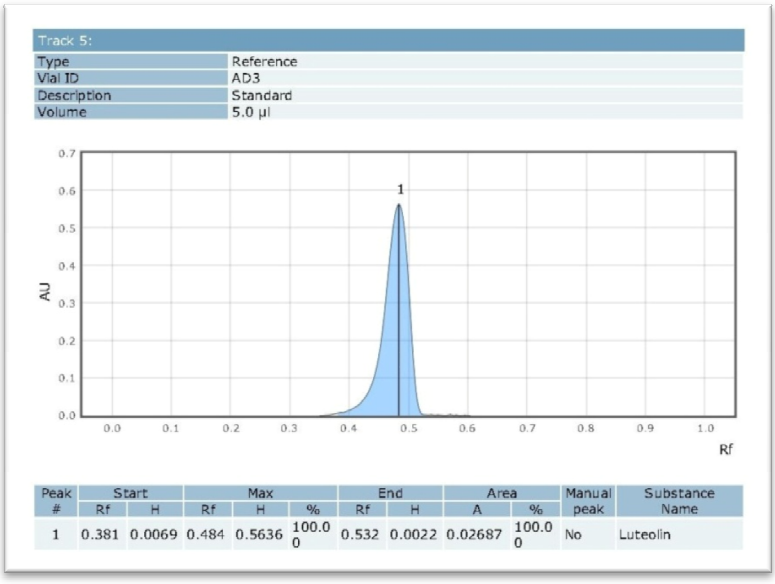
Instrumentation and chromatographic conditions

HPTLC was performed on 200 ×100 mm aluminum packed plates coated with silica gel 60 F254 (Merck, Mumbai, India).Standard solution of quercetin and sample solution were applied to the plates as bands 8.0 mm wide, 30.0 mm apart and 10.0mm from the bottom edge of the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28±20c), with Toluene : ethyl acetate: formic acid, 6:4:0.5 (v/v/v) as mobile phase in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 minutes. After development,. These plates were scanned and visualized under visible light at 525 nm and UV light at 254 nm and 374 nm absorbance/reflection mode using reflection mode by CAMAG Scanner III and Atomatic TLC Sampler Camag ATS 4 and deuterium lamp was used to analyze the plates.

3.7 HPTLC Quantification of the extracts

The Luteolin content of various extracts was determined by comparing the area of chromatogram with the calibration curve of concentration of standards. The Rf value of standard **Luteolin** (0.527) was compared with the Rf value of the extracts. Quantitative estimation of the plate was performed in the remission/absorption mode at 374 nm, with the following conditions slit width 6.00x0.30mm, micro scanning speed 20mm/s and data resolution 100 µm step. Calibration parameters were as follows: calibration mode- single level, statistics mode-cv, evolution mode- peak height. The average content of the luteolin in different extracts was expressed in percentage results are shown in fig no-7,8,9,10

i) HPTLC of Standard



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.381	0.0069	0.484	0.5636	100.00	0.532	0.0022	0.02687	100.00	No	Luteolin

Fig.no.07 HPTLC chromatogram of Standard Luteolin

Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.350	0.0000	0.402	0.4425	45.34	0.426	0.1011	0.01481	37.02	No	
2	0.426	0.1011	0.481	0.5335	54.66	0.521	0.0000	0.02520	62.98	No	Luteolin

Fig.no.08 HPTLCchromatogram of isolated compound from *Biophytumsensitum L*

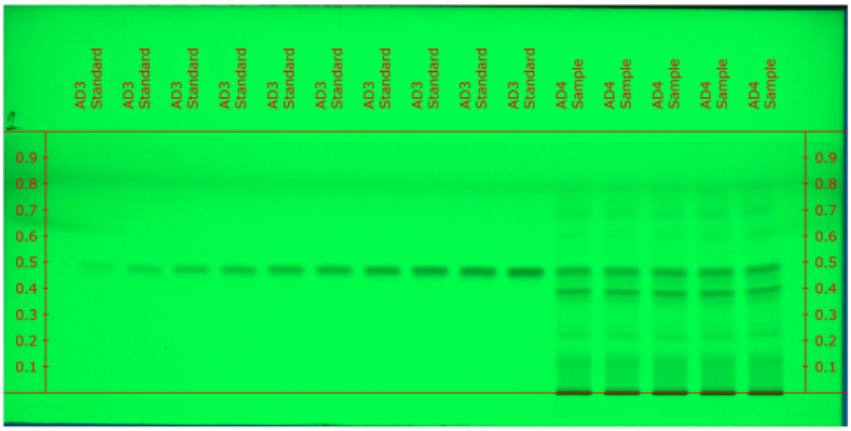


Fig.no.09 HPTLC of isolated compoundat 254 nm obtained from *Biophytumsensitum L*

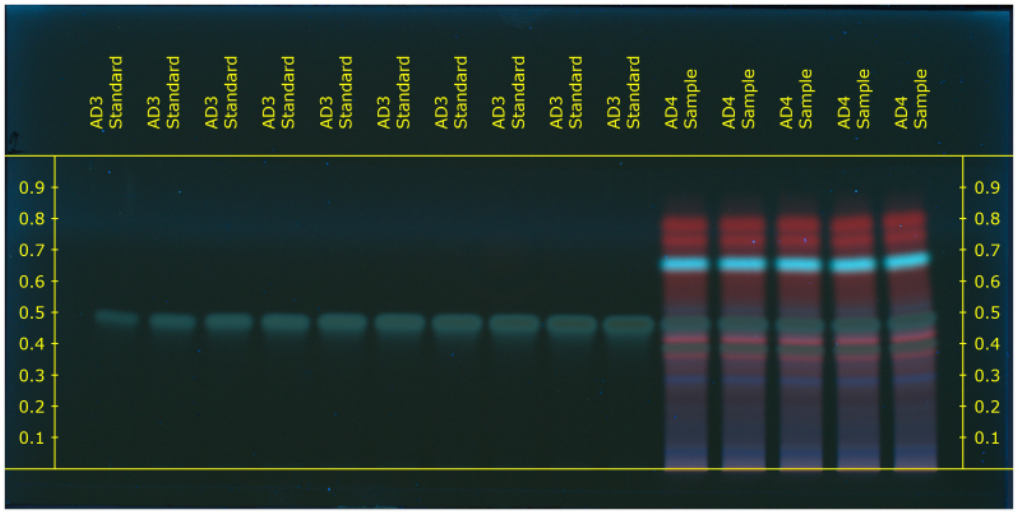


Fig.no.10 HPTLC of isolated compoundat 366 nm obtained from *Biophytumsensitum L*

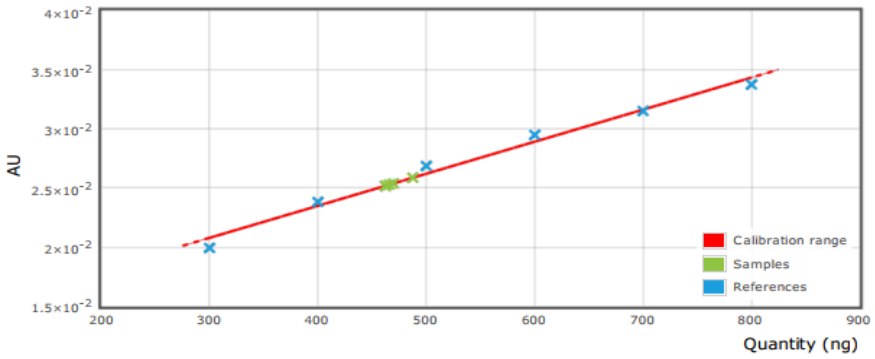


Fig no.11 HPTLC Calibration curve for isolated compound obtained from *Biophytumsensitum L*

4. CONCLUSION-

The isolatedluteolin was subjected to various analytical tests likeTLC, UV spectroscopy, IR spectroscopy, MS spectroscopy, NMRspectroscopy, and percent determination by HPTLC forluteolin confirmation. TLC was performed on silica gel plates using Toluene : ethyl acetate: formic acid, 6:4:0.5 (v/v/v)as the mobile phase The absorption maximaof the isolated luteolin were recorded on shimadzu UV/Vis spectrophotometer using ethanol as a solvent. IR spectrumwasrecorded on FTIR spectrometer. Mass spectrumof isolated compound was recorded on Micromass, Q-TOFMS. NMR spectrum was recorded on Brukerspectrometerusing DMSO as a solvent. The percentage purity of isolatedcompound was determined using HPTLC instrument.Therefore HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal formulation and can be further used in quality control of not established herbals. This study is of practical importance because compound Luteolin was firstly reported to be isolated from plant biophytumsensitivumlinn.

5. REFERENCES:

1. Harborne JB, Williams CA. Advances in flavonoid research since 1992.Phytochemistry 2000;55:48/504.
2. Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention: Flavonoids and isoflavonoids. PharmacolTher 2001;90:15/77.
3. Knekt P, Jarvinen R, Seppanen R, Hellovaara M, Teppo L, Pukkala E, *et al*. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 1997;146:223/30.
4. Neuhouser ML. Dietary flavonoids and cancer risk: Evidence from human population studies. Nutr Cancer 2004;50:1-7.
5. Liang YZ, Xie P, Chan K. Quality control of herbal medicines. J Chromatogr B 2004;812:53/70.
6. ICH. Guidance on analytical method validation, Proceedings of International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada; 2002.
7. Sethi PD. High Performance Thin Layer Chromatography Quality Analysis of Pharmaceutical Formulations, CBS, India; 1996.

8. PankajNariya et al. Analytical Study & HPTLC Profile of Panchagavya – A Traditional AyurvedicPreparation.Asian Journal of Biochemical and Pharmaceutical Research2012;2(2): 198-208.
2. François Eya'aneMeva et al. Simple disclosure of plasmon resonance band maxima by ultraviolet spectroscopycoupled to centrifugation, determination of percentage recovery: a case study of Gnetumbucholzianum Engl. leaf mediated silver nanoparticles. Asian Journal of Biochemical and Pharmaceutical Research. 2017; 1(7):62-74
3. SanjeevKumaret al. Phytoconstituents and Pharmacological activities of Musa paradisiaca Linn. Asian Journal of Biochemical and Pharmaceutical Research Issue 2012; 4 (2): 199
4. John Peter Paul J et al .Preliminary phytochemical analysis of SargassummyriocystumJ.Ag.andTurbinariaornata (Turner) Journal of Biochemical and Pharmaceutical Research 2013 ;2(3): 37-42.