ESTIMATION OF URSOLIC ACID AND LUTEOLIN IN *PHYLA NODIFLORA* (L.) GREENE. USING HPTLC

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Why HPTLC?

- Versatile separation technique
- Official in most of the pharmacopoeias
- Simultaneously handle several samples of divergent nature
- Most simple technique for natural products available today
- Extreme flexibility in various components:
  - stationary phase,
  - mobile phase,
  - developing and detection techniques[1].

Why *Phyla nodiflora* Greene.?

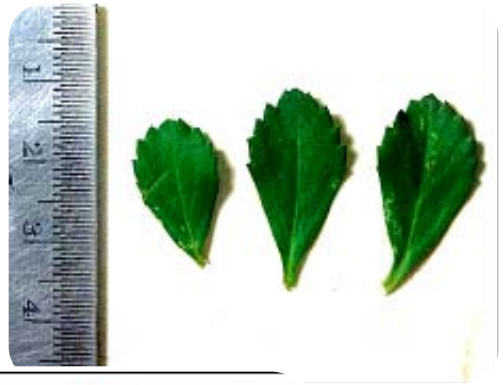
- *Phyla nodiflora* (Verbenaceae) was selected for phytopharmacological studies as it is being used by local community as well as mentioned in the literature as a tonic[2].
- While carrying out preliminary phytochemical screening it was found that the aerial parts contained two important marker compounds and were reported in literature also.
- Thus, it was planned to estimate two marker compounds using HPTLC.

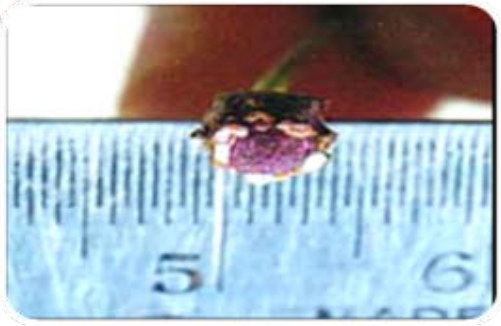
PLANT PROFILE in brief

Phyla nodiflora (L.)Greene.

(Vernacular name “Jal pippali”)

belongs to family verbenaceae.





UTILIZATION of *Phyla nodiflora*[2]

- The plant is bitter
- useful in diseases of the heart, blood and the eye
- good for ulcers
- wounds
- asthma
- diuretic

REVIEW

ethanolic extract of the dried plant.	two glucosidic colouring matters, nodiflorin – A and nodiflorin – B [3]
aqueous extract of the plant.	free lactose, maltose, glucose, fructose and xylose [3]
plant	flavone, nodifloretin and a mixture of glucosides of β-sitosterol and stigmasterol [4]
plant	two flavone glycosides namely lippiflorin A and lippiflorin B, nepetin and batatilfolin[5]

flowers	**flavone glycosides, nepetin and batalifolin[6]**
aerial parts	**15 flavonoids, 3 flavone aglycones and 12 new flavone sulphates[7]**
dried powdered leaves	**halleridone and hallerone [8]**
plant	**Triterpene :3β, 19α - dihydroxy – Urs – 12, 20 – diene - as a minor constituent; glycoside of ursolic acid,pomolic acid, catalpol, loganin and α-ethyl galactose [9]**

- Antispasmodic[10]
- Mild degree of CNS stimulation, hypotensive activity, significant antiinflammatory activity, active against Gram +ve and Gram-ve bacteria[11,12,13]
- Antiepileptic[14]
- Moderate anti Helicobacter pylori activity[15]
- Gastroprotective effect[16]
- Diuretic potential[17]
- Antimalarial, spasmolytic, sedative, hypotensive activity of essential oil[18]
- Immunity boosting activity[19]

OBJECTIVE

- To standardize the plant using the marker compounds ursolic acid and luteolin by validated HPTLC method.
- Method was developed using single solvent system .
- As there was no data found in the literature on estimation of two marker compounds.
- This was the first time estimation of two marker compounds using HPTLC was done for this plant.

HPTLC plate:

- The aluminium plates coated with silica gel GF_{254} (E. Merck) were used in the present study.

SAMPLE PREPARATION FOR ESTIMATION OF URSOLIC ACID IN AERIAL PARTS OF *PHYLA NODIFLORA:*

An accurately weighed 1.0 g quantity of powdered drug was hydrolyzed with 2 N methanolic hydrochloric acid (50 ml) under reflux on water bath at 100° C for 2 h.

The extract was filtered through Whatmann No. 1 filter paper, and the residue left after extraction (marc) was washed with methanol.

The combined filtrates were transferred to a 50 ml volumetric flask and the volume made upto 50 ml with methanol.

This test extract was used for estimation of ursolic acid.

SAMPLE PREPARATION FOR ESTIMATION OF LUTEOLIN IN AERIAL PARTS OF *PHYLA NODIFLORA:*

An accurately weighed 1.0 g quantity of powdered drug was extracted for 15 minutes with methanol (4X 25 ml) under reflux on water bath at 100°C.

The methanolic extract was filtered through Whatmann No. 1 filter paper.

The filtrates were combined, concentrated and transferred to a 50 ml volumetric flask and the volume made upto 50 ml with methanol.

This test extract was used for estimation of luteolin.

Preparation of standard :

Ursolic acid and luteolin :

- 4.0 mg of pure ursolic acid and luteolin(Sigma Aldrich Ltd.) were accurately weighed and dissolved in 10 ml ethanol separately (stock solution).
- Required concentrations of standard solutions were prepared from the respective stock solution.

Application of the sample:

- The automatic application device LINOMAT IV was used for application of the sample and standard solutions.

Plate 1

- 40 µl of test extract along with
- 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 µl from stock solution of standard ursolic acid was applied for its estimation.

Plate 2

- 30 µl of test extract along with
- 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 µl from stock solution of standard luteolin was applied for its estimation.

Development:

- After application of the test and respective standard extracts, the plates were developed in solvent system upto a height of 8 cms.

Solvent system:

- toluene: ethyl acetate: formic acid
- (07:03:0.3 v/v)

Detection and estimation:

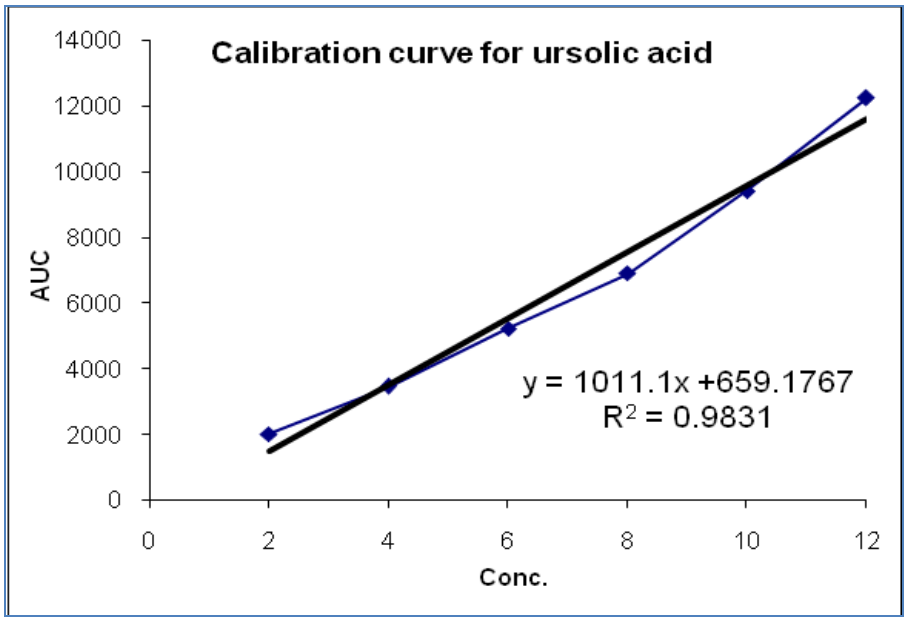
- Detection was carried by scanning the plates using HPTLC scanner (CAMAG)

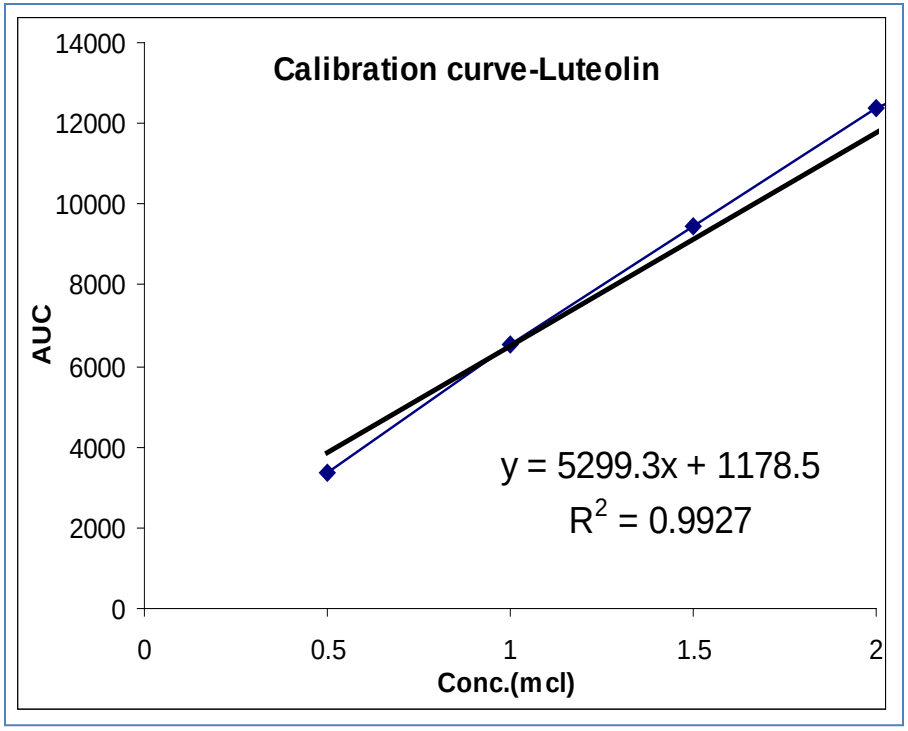
Plate 1

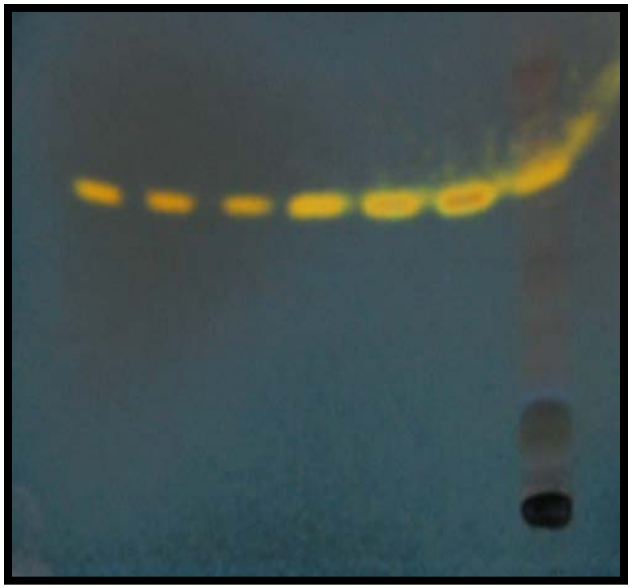
- was scanned at 530 nm and identified after derivatisation with anisaldehyde sulphuric acid reagent, plate was observed in visible light.

Plate 2

- was sprayed with NP-PEG reagent and scanned ain UV light estimation and identification both.


Calibration curve for ursolic acid
AUC
Conc.
y = 1011.1x +659.1767
R2 = 0.9831
14000
12000
10000
8000
6000
4000
2000
0
0
2
4
6
8
10
12


Calibration curve-Luteolin
AUC
Conc.(mcl)
y = 5299.3x + 1178.5
R2 = 0.9927
14000
12000
10000
8000
6000
4000
2000
0
0
0.5
1
1.5
2

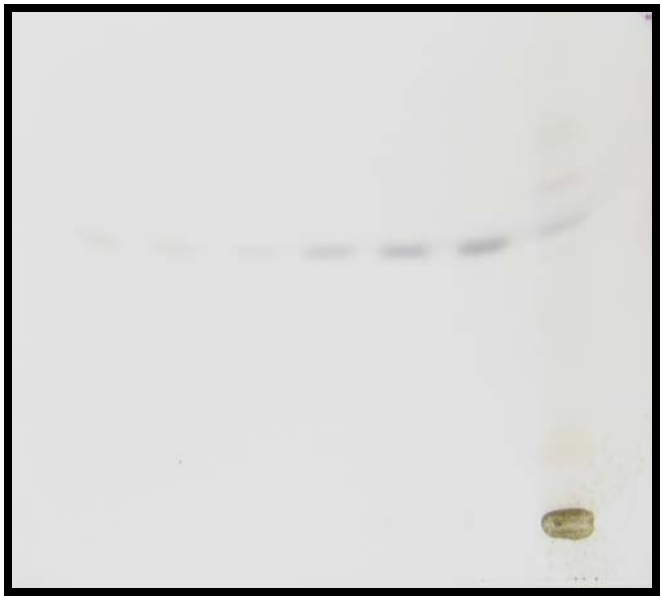


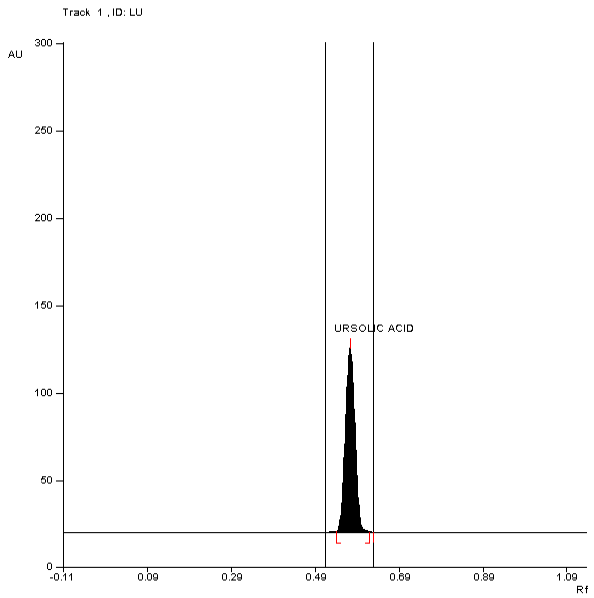
HPTLC chromatograph of ursolic acid standard and test extract sprayed with anisaldehyde-sulphuric acid, observed in

U V light (366 nm)

Rf=0.67

visible light

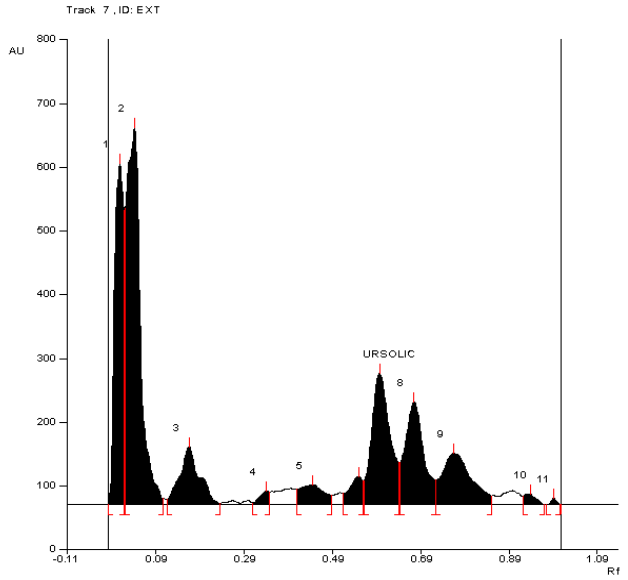


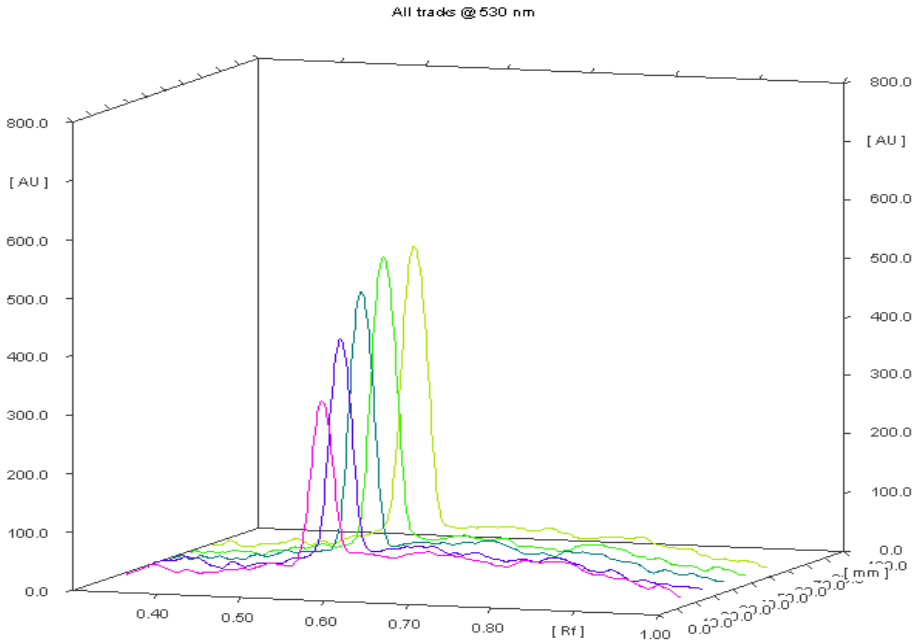


HPTLC chromatogram of

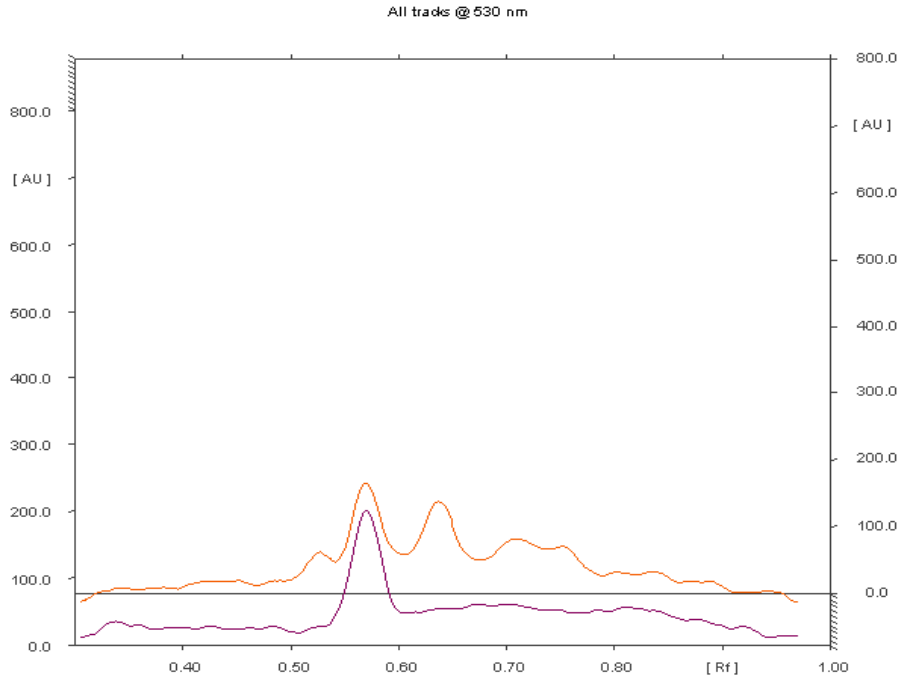
standard ursolic acid

test extract

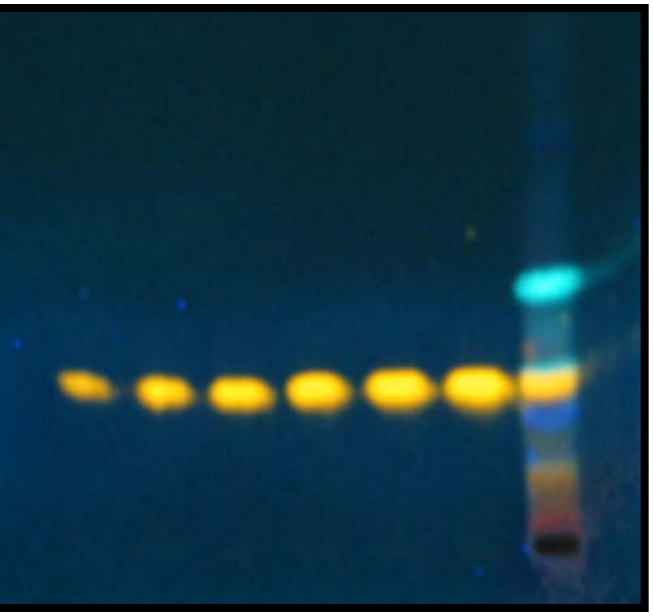




Spectra standard ursolic acid and test extract



Comparison of spectra of standard ursolic acid and test extract

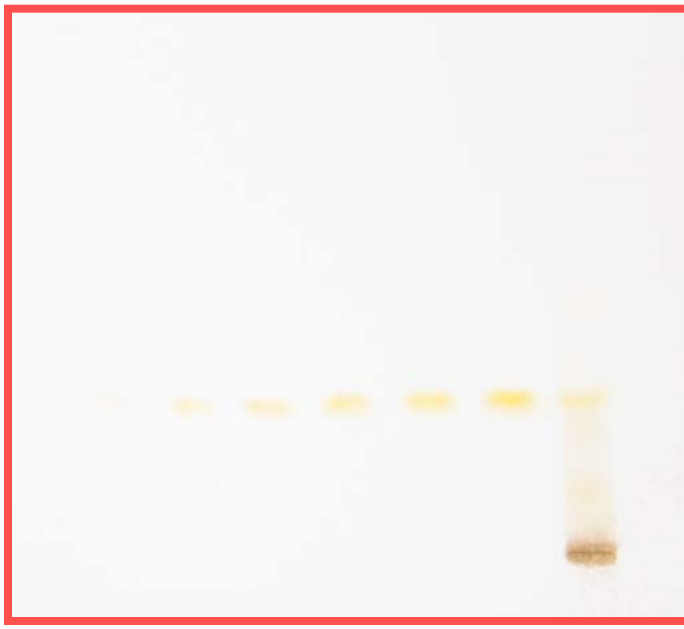


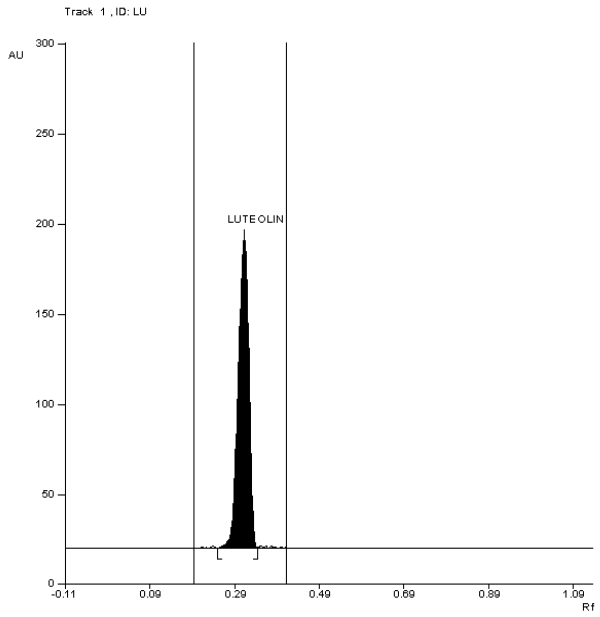
HPTLC chromatograph of luteolin standard and test extract sprayed with NP-PEG reagent, observed in

U V light (366 nm)

Rf=0.42

visible light

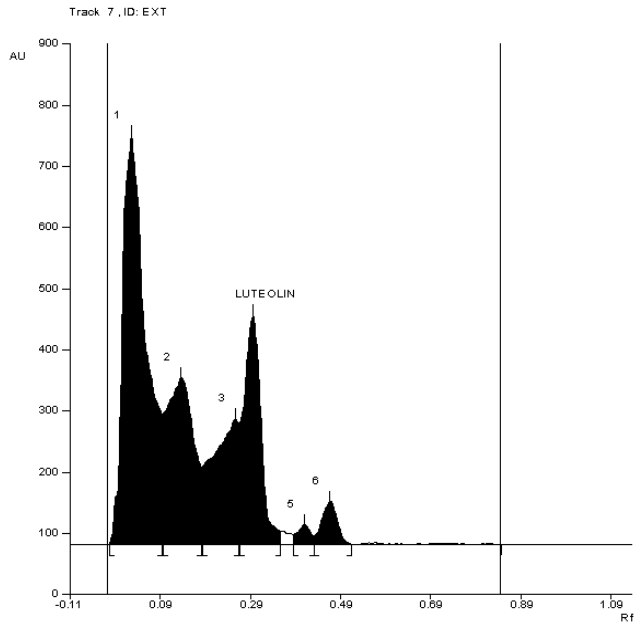


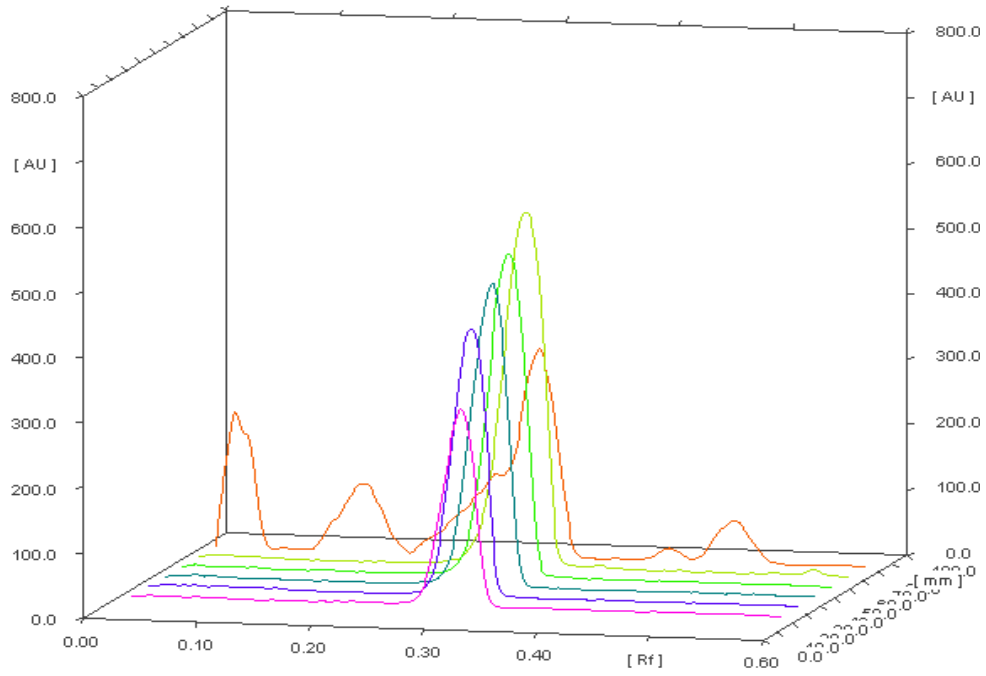


HPTLC chromatogram of

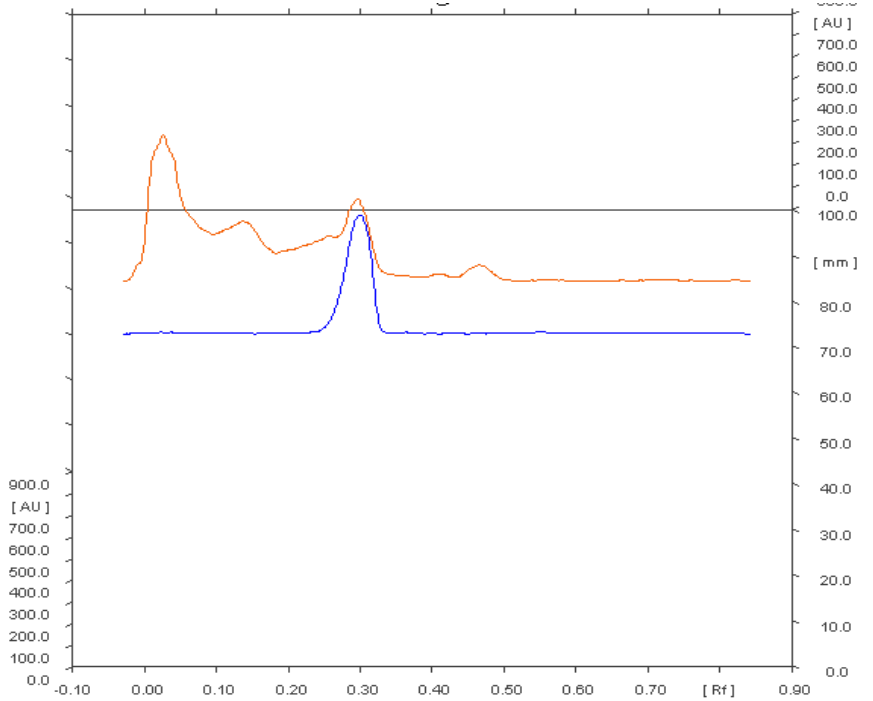
standard luteolin

test extract





Spectra standard luteolin and test extract



Comparison of spectra of standard luteolin and test extract

Sample	Mean peak area ± S.D. (n=5)	Average amount of ursolic acid (ng/spot)	Amount of ursolic acid/g of dried powder	% C.V.
Hydrolysed extract of *Lippia nodiflora* Rich.	4235.9 ± 40.02	1400	**1750 µg**	0.009

Sample	Mean peak area ±S.D. (n=5)	Average amount of luteolin (ng/spot)	Amount of luteolin / gm of dried powder	% C.V.
Extract of *Lippia nodiflora* Rich.	**16697.66 ± 39.93**	**794**	**1323 µg**	**0.002**

SUMMARY

PARAMETERS	URSOLIC ACID	LUTEOLIN
EXTRACT PREPARATION	HYDROLYSIS	DIRECT
SOLVENT SYSTEM	toluene: ethyl acetate: formic acid (07:03:0.3 v/v)	-DO-
DETECTION IDENTIFICATION	AT 530 nm DERIVATISATION	AT UV FLU. MODE DIRECT /DERIVATISATION
Rf	0.67	0.42
AMOUNT/ GM OF DRIED POWDER	1750 μg	1323 μg

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