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Extraction and Quantitative HPLC Analysis of Myricetin in Hydroethanolic extract of *Passiflora incarnata* (Passifloraceae)

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Abstract
Passiflora incarnata has widely been used to treat insomnia, nervous disorders, convulsion, ulcer etc. There has been many flavonoids previously reported in this plant apigenin, luteolin, quercetin, kaempferol, d-allopyranosyl-8-xylopyranosyl-apigenin C-glycosyl flavonoids vitexin, isovitexin, orientin, isoorientin, schaftoside, isoschaftoside, isovitexin-2″-*O*-glucopyranoside, isoorientin-2″-*O*-gluco-pyranoside, 2-glucosylapigenin, isoscoparin-2″-*O*-glucoside, 2″-*O*glucosyl-6-C-glucosylapigenin, 6-β-D-glucopyranosyl-8-d-ribopyranosyl apigenin and swertisin. The percentage of myricetin present in the air dried plant material of HEEPI was calculated as **0.045 %.** This is the first time myricetin has been reported in this plant.

Keywords: Myricetin, tR 11.54, *Passiflora incarnata,* Hydroethanolic extract

Introduction
Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions [1].

Passiflora incarnata Linn is dicotyledonous plant belonging to the family Passifloraceae. It is a perennial, creeping herb, climbing by means of axillary tendrils. Leaves alternate, palmately three to five serrate lobes. Flowers large, solitary, with long peduncles, whitish, with a triple purple and pink crown. Fruits are ovate berries containing numerous ovoid, flattened seeds covered with a yellowish or brownish aril [2].

Flavonoids are reported to be the major phyto-constituents of *P. incarnata*. These include (1) apigenin, (2) luteolin, (3) quercetin, (4) kaempferol [3] 6-d-allopyranosyl-8-xylopyranosyl-apigenin [4]; (5) C-glycosyl flavonoids vitexin, (6) isovitexin, (7)orientin, (8) isoorientin, schaftoside, isoschaftoside, isovitexin-2″-*O*-glucopyranoside, isoorientin-2″-*O*-gluco-pyranoside, 2- glucosylapigenin, isoscoparin-2″-*O*-glucoside, 2 ″-*O*glucosyl-6-C-glucosylapigenin, 6-β-D-glucopyranosyl-8-d-ribopyranosyl apigenin and swertisin [5-9].

Hence the present study was designed to identify the presence of myricetin in the hydroethanolic extract of leaves and stem of *Passiflora incarnata* (HEEPI).

Plant material
The leaves and stem of *Passiflora incarnata* were collected from Salem, Tamilnadu, India. The plant was identified and authenticated by senior plant Taxonomist at Plant Anatomy Research Centre (PARC).

Materials and methods
Extraction of HEEPI
The shade dried and coarsely powdered leaves and stems of *Passiflora incarnata* was defatted with petroleum ether (60-80˚c) for three days by triple maceration. The defatted marc was extracted with 70% ethanol by triple maceration and filtered. The filtrate was concentrated under reduced pressure to obtain a solid residue (HEEPI extract) which was dark green in colour.

HPLC analysis [10-11].
HPLC is one of the most powerful tools in analytical chemistry, with the ability to separate, identify and quantify the target compounds present in any sample that can be dissolved in HPLC compatible liquid.

A Agilent technologies 1220 infinity LC (USA), Zorbax SB – C 18 column, Softwares Open LAB, CDS chem station work station VL was utilized. Separations were done using Solvent A (water) and solvent B (0.02% trifluroacetic acid (TFA) in acetonitrile) with a linear gradient elution: 80% A (5 min), 20 – 40% (8 min), 40 – 50% (12 min), 50 – 40% (17 min), 40 – 20% (21 min) at a flow rate of 1 mL / min; temp 27 ˚C with an injection volume of 10 µL; UV detection was at 280 nm.

Quantitative analysis

Determination of the content of the myricetin in plant material was performed by the external standard method, using pure myricetin (Sigma) as standard. Stock solutions of 1, 5, 10, 15, 20 µg/mL were utilized. Each determination was carried out in triplicate.

Results (Table 1-2, Figure 1-2)

The investigation revealed that the retention time (t_R) of standard myricetin was 11.46 mins (t_R). The retention time of a peak appeared in the chromatogram of HEEPI was found to be 11.54 mins (t_R) and it indicates the presence of myricetin in HEEPI. The calibration curve showed the linearity of the detector over the tested range (1 – 20 µg/mL). The regression equation was y = 11.8873x + 110.5253 R^2 = 0.9980. The percentage of myricetin present in the air dried plant material of HEEPI was calculated as 0.045 % from the slope and the intercept values determined from the linearity graph. These results also showed a low noise level, good sensitivity of the detector at 280 nm, this is the first time myricetin has been reported in this plant

Conclusion

The percentage of myricetin present in the air dried plant material of HEEPI was calculated as 0.045 % by using linearity graph of standard myricetin. Further studies are required to explore and confirm the presence of myricetin and its pharmacological actions.

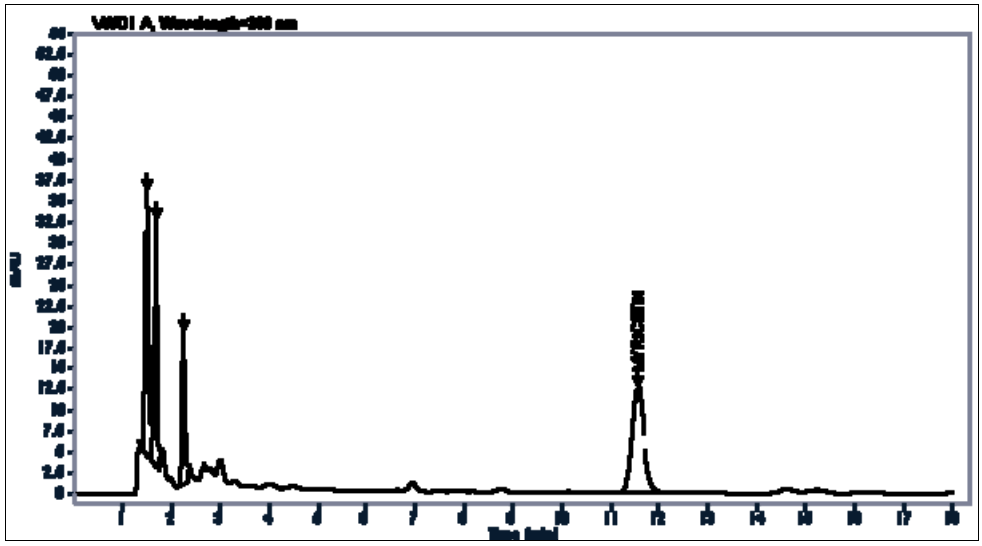

Fig 1: Hplc Chromatogram of Hydroethanolic Extract of *Passiflora incarnata*

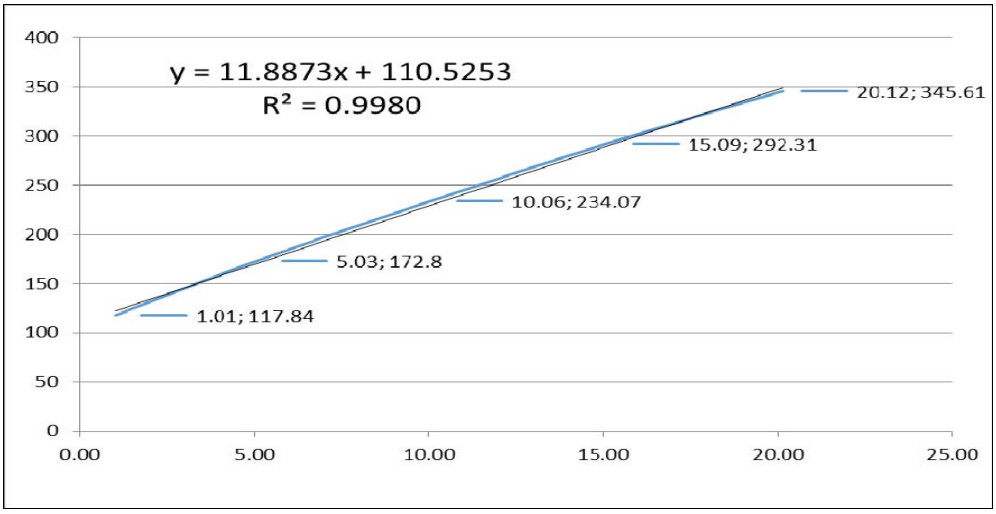

Fig 2: Linearity Graph of Standard

Table 1: Hplc Parameters of Standard Myricetin

Concentration of standard Myricetin	Retention Time (t_R)	Area	Area %	Height	Height %	TP	Tailing Factor
1.01mg/mL	11.42	117.84	100	6.8	100	10070.63	1.07
5.03mg/mL	11.42	172.8	100	9.82	100	9804.03	1.07
10.06mg/mL	11.43	234.07	100	12.93	100	9241.09	1.06
15.09mg/mL	11.44	292.31	100	15.39	100	8417.92	1.06
20.12□g/mL	11.46	345.61	100	17.09	100	7460.97	1.02

Table 2: Hplc Parameters of Heepi

Name	Retention time (t_R)	Area	Area %	Height	Height %	TP	Tailing Factor
	1.49	157.49	27.87	31.74	34.26	2204.76	1.24
	1.68	105.05	18.59	29.58	31.93	6300.21	0.86
	2.23	77.6	13.73	18.52	19.99	7082.49	1.47
HEEPI	11.54	224.97	39.81	12.8	13.82	9995.36	1.07
	Sum	565.11	100				

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